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An Investigation of Minerals, Vitamin C, and Antioxidant Capacity of Four *Bougainvillea* spp. Cultivars

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Abstract

The *Bougainvillea* spp. is an ornamental, medicinal, and edible flower whose edibility has been less addressed in previous studies. This research explored the edible application of four bougainvillea cultivars (*B. glabra, B. glabra* 'Snow White', *B. glabra* 'Scarlett O'Hara', and *B. glabra* 'Louis Wathen'). The bougainvillea were purchased at the full blooming stage from a commercial producer in Talesh County, Guilan province, and their petals were used to determine the contents of minerals, vitamin C, anthocyanin, and antioxidant activity. The results showed that *B. glabra* 'Scarlett O'Hara' had the highest P (32.10 mg/100 g FW), Ca (98 mg/100 g FW), Fe (3.79 mg/100 g FW), and anthocyanin content (31.14 mg/100 g FW). The highest K contents (181.81 and 181.52 mg/100 g FW) were obtained from *B. glabra* and *B. glabra* 'Scarlett O'Hara', and the highest Zn content (0.35 mg/100 g FW) was recorded by *B. glabra*. Also, *B. glabra* 'Snow White' was the weakest in Zn, Fe, P, and anthocyanin contents. *B. glabra* 'Snow White' and *B. glabra* 'Louis Wathen' were the best in vitamin C content, and *B. glabra* 'Snow White' and *B. glabra* were the best in antioxidant capacity. In general, it was found that all four cultivars had nutritional value. However, the best were *B. glabra* 'Scarlett O'Hara' due to its mineral content and *B. glabra* 'Snow White' due to its vitamin C content and antioxidant capacity.

Keywords: Anthocyanin, Antioxidants, Edible flower, Nutritional value, Ornamental plants.

INTRODUCTION

Flowers have traditionally been used to beautify and scent the environment and treat many diseases. Flower application in cuisine for improving the organoleptic properties and visual beauty of foods and beverages has increased in recent years. The flowers of medicinal and aromatic plants, fruit trees, and vegetables are the main categories of edible flowers, which are rich in nutrients, vitamins, and antioxidants and have numerous health benefits, such as anti-cancer, anti-diabetics, antibacterial, antiviral, antifungal, and anti-inflammatory activities (Pensamiento-Niño *et al*., 2024; Pires *et al*., 2023). Although edible flowers are abundant as natural sources of nutrients and biologically active compounds, naturally occurring flowers need to be tested for their chemical and biological features to avoid the likely risks, especially their toxicity. So far, 180 species, 100 genera, and 97 families of edible flowers have been detected (Saurabh and Barman, 2020; Pensamiento-Niño *et al*., 2024), consumed fresh as salad. In addition, edible flowers are used as a raw material in preparing foods, such as stews, cakes, beverages, seasonings, and desserts (Zhao *et al*., 2019).

An essential reason for using flowers in cuisine is the appealing color of their petals. In addition to beautification and attraction of pollinators, pigments endow the flowers with antioxidant properties. Therefore, pigments, especially anthocyanins, are a crucial category of chemicals in flowers that can increase their nutritional and medicinal value due to their vigorous antioxidant activity and other beneficial physic-chemical and biological properties (Benvenuti *et al*., 2016).

In addition to pigments, edible flowers contain water, fiber, proteins, fats, carbohydrates, vitamins, minerals, sugars, free amino acids, alkaloids, organic acids, phenols, and antioxidants in abundant amounts (Fenanndes *et al*., 2019; Barani *et al*., 2022). Although the nutritional value and many health benefits of edible flowers have been reported, they are generally consumed at very low levels due to the lack of their detection and the fear of their toxicity. So, applied research is required to increase public awareness and introduce new edible flowers.

A research study on the nutritional and medicinal value of 23 rose cultivars revealed that the flowers, especially red cultivars, were an excellent edible source of phenolic compounds, vitamin C, and anthocyanins (Kalisz *et al*., 2023). Jadhav *et al*. (2023) reported that many edible flowers are a good source of vitamin C. Nicknezhad *et al*. (2022) introduced the marigold, gladiolus, yucca, and chrysanthemums as a new source of minerals, vitamin C, and antioxidants. Bayanifar *et al*. (2024) investigated different chrysanthemum cultivars in terms of their vitamin C, minerals, and antioxidant compounds. They revealed that the studied cultivars could partially meet the body's mineral requirements. Pourzarnegar *et al*. (2023) found that rose cultivars, especially the cultivars in red, were acceptable edible sources of vitamin C and antioxidant compounds.

The *Bougainvillea* spp. from the family of Nyctaginaceae is native to hot and semi-hot areas of South America, Peru, Argentina, and Brazil. As an ornamental spiral, the bougainvillea has many fans. Furthermore, it has medicinal and edible applications. Over 105 biologically active compounds have been detected in different parts of the bougainvillea, used for disease treatment in traditional medicine (Saleem *et al*., 2021). There is no significant information about the nutritional value of bougainvillea. Given the need to find new edible flowers and detect their nutritional compounds in each region, this research investigated and compared four bougainvillea cultivars, including *B. glabra* with purple flowers (Kumar *et al*., 2017), *B. glabra* 'Snow White' with white bracts (Gupta *et al*., 2009), *B. glabra* 'Scarlett O'Hara' with magenta

flowers (El-Sayed *et al*., 2020), and *B. glabra* 'Louis Wathen' with orange bracts (Thompson, 2011) as an edible source.

MATERIALS AND METHODS

An experiment based on a completely randomized design was conducted in the summer of 2023 to study four bougainvillea cultivars (*B. glabra*, *B. glabra* 'Snow White', *B. glabra* 'Scarlett O'Hara', and *B. glabra* 'Louis Wathen') (Fig. 1). The flowers were purchased at the fully blooming stage from a commercial producer in Talesh County, Guilan province, and were transferred to the study site (Islamic Azad University, Rasht), taking care of all handling precautions. Then, the healthy flowers that had no symptoms of diseases or mechanical damage were packed in lidded containers in 10-branch groups and were kept at 4°C during the experiment and trait assessment.

Fig. 1. The bougainvilleas flowers used in the research.

Assessment of traits

Minerals (N, P, Ca, K, Fe, and Zn)

To measure N content, 0.5 g of the fresh petal was mixed with acids (100 ml of sulfuric acid, 6 g of salicylic acid, and 18 ml of H_2O_2). After 24 hours, the samples were subjected to digestion using an electric heater. The resulting samples were used to measure the N content using the Kjeldahl method. Finally, the following equation was used to find the N percent:

$$N(\%) = 0.56 \times t \times (a - b) \times \frac{V}{W} \times \frac{100}{DM}$$

In which *t* is the concentration of the acid used for titration in mol/l, *a* is the amount of the acid used in the sample in ml, *b* is the amount of acid used for control in ml, *V* is the volume of the extract derived from the digestion in ml, *W* is the petal sample weight for digestion in g, and *DM* is the dry matter percent of the petal.

To measure the P, K, Ca, Fe, and Zn contents, some petals were converted into ash at 550°C. Then, 1 g of the petal ash was mixed with 1 ml of nitric acid in a 250-mL Erlenmeyer, and it was added with soda 10% and the MOROXAED mixture. Then, the Ca content was determined by titration using ethylenediaminetetraacetic acid (EDTA), the K content was determined by flame-photometry, and the P content was determined by adding the nitro-vanadate-molybdate reagent and using spectrophotometry. The Fe and Zn content of the samples was also evaluated using an atomic absorption device and drawing the standard graph (Rezaee *et al*., 2004).

Vitamin C content

To measure the vitamin C content, 2 g of the fresh petal was extracted using 5 ml of liquid nitrogen. Then, 15 ml of meta-phosphoric acid 3% was added to the extract and mixed with it. The filtered sample was adjusted to 10 ml by adding meta-phosphoric acid 3% and titrated with 2,6-dichlorophenolindophenol until a light pink color emerged. Finally, the vitamin C content of each sample was calculated using the following equation (Mazumdar and Majumder, 2003):

$$Vitamin\ C = \frac{e \times d \times b}{c \times a} \times 100$$

In which *a* is the sample weight, *b* is the amount of meta-phosphoric acid used for extraction, *c* is the solution taken for titration, *e* is the amount of color solution consumed for the sample, and *d* is the color factor. The following equation gives d:

$$d = \frac{0.5}{The\ amount\ of\ color\ solution\ used\ for\ titration\ of\ the\ standard\ sample}$$

Petal anthocyanin

To measure the anthocyanin content, the petal extract was derived using acidic methanol (pure methanol + hydrochloric acid). The resulting extract was filtered through Whatman filter paper and adjusted to 50 ml by adding distilled water. Then, the absorbance was read at 535 nm with a spectrophotometer (APEL, PD-103UV), and the petal's anthocyanin content was estimated by the following equation (Mazumdar and Majumder, 2003):

$$Anthocyanin\ content = \frac{e \times b \times c}{d \times a} \times 100$$

in which *e* is the sample weight, *b* is the sample volume for measurement, *c* is the total solution generated, *d* is the volume of the sample taken, and *a* is the reading by the spectrophotometer.

Antioxidant capacity

The antioxidant capacity of the petal samples was determined by Brand-Williams *et al*.'s (1995) method for which 1 g of fresh petal was extracted using 10 ml of pure methanol. The extracts were kept at room temperature for 2 hours and then filtered through Whatman filter paper. Then, 50 µl of the extract was mixed with 950 µl of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and was kept in darkness at room temperature for some minutes. Then, the absorbance of the samples was read with a spectrophotometer (APEL PD-303UV) at 515 nm, and the following equation was used to determine the antioxidant capacity:

$$\%DPPHsc = (A_{cont} - A_{samp}) \times 100/A_{cont}$$

in which $DPPH_{sc}$% represents the percent inhibition of free radicals, A_{cont} represents the absorbance of the sample and DPPH, and A_{samp} represents the absorbance of DPPH.

Statistical analysis

The collected data were analyzed in the SPSS19 software suite, in which they were subjected to analysis of variance, as well as the comparison of means by the LSD test at the P <0.01 level.

RESULTS

Minerals

Based on the results of ANOVA, the cultivars significantly (P < 0.01) differed in minerals, including N, P, Ca, K, Fe, and Zn (Table 1). The comparison of means showed that *B. glabra* 'Scarlett O'Hara' had the highest levels of N, P, Ca, and Fe. The highest Zn content was recorded by *B. glabra*, whereas the two cultivars *B. glabra* and *B. glabra* 'Scarlett O'Hara' had the lowest one. The lowest N, P, Fe, and Zn contents belonged to *B. glabra* 'Snow White'. On the other hand, *B. glabra* 'Louis Wathen' recorded the lowest Ca and K contents (Table 2).

Table 1. Analysis of variance of the effect of different cultivar on petals minerals in *Bougainvillea* spp.

S.o.V	df	N	P	Ca	K	Fe	Zn
Treatment	3	0.00066**	15.79**	1796.1**	455.6**	0.315**	0.0154**
Error	16	0.0000125	0.202	0.684	2.29	0.0179	0.000947
CV (%)		1.29	1.476	1.024	0.86	3.83	11.17

**: Significant at P < 0.01 based on the LSD test.

Table 2. Mean comparison of the effect of different cultivar on petals minerals in *Bougainvillea* spp.

	N	P	Ca	K	Fe	Zn
Cultivars	**%**			**(mg/100 g F.W.)**		
B. glabra	0.278^{b}	30.74^{b}	96.39^{b}	181.81^{a}	3.53^{b}	0.35^{a}
B. buttiana "Louis Wathen"	0.267^{c}	31.06^{b}	63.80^{d}	161.54^{c}	3.46^{b}	0.24^{bc}
B. specabilis "Scarlett OHara"	0.286^{a}	32.10^{a}	98.00^{a}	181.52^{a}	3.79^{a}	0.28^{b}
B. glabra "Snow White"	0.260^{d}	27.94^{c}	64.99^{c}	172.88^{b}	3.18^{c}	0.22^{c}

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the LSD test.

Minerals are necessary for the health and natural functioning of the human body, so a proper diet must be rich in minerals (Huang *et al*., 2020). Flowers are new sources of minerals in the human food basket (Benvenuti *et al*., 2016). As was already mentioned, bougainvilleas with red and purple petals are richer in minerals than those with white and orange petals. Some researchers have stated that although the daily consumption of edible flowers is trivial, their continuous consumption can supply a part of the human need for minerals and biologically active compounds (Araújo *et al*., 2019).

Rop *et al*. (2012) argue that the mineral contents of edible flowers have a curative effect and are one of the most necessary aspects of edible flower consumption for human nutrition. Benvenuti *et al*. (2016) reported that flowers, as natural sources of minerals, can partially meet the micro-element and macro-element requirements of the human body. We recorded the Ca content of different bougainvillea cultivars at 63.80-98 mg/100 g FW, which was greater than that of white gladiolus (9.11 mg/100 g FW) and purple chrysanthemum (47.25 mg/100 g FW) (Nicknezhad *et al*., 2022). In addition, all four bougainvillea cultivars recorded higher Fe content than marigold, gladiolus, yucca, chrysanthemum, and hollyhock (0.36-2.54 mg/100

g FW) (Nicknezhad *et al*., 2022). Regarding the Zn level, *B. glabra* 'Louis Wathen' (0.24 mg/100 g FW) and *B. glabra* 'Snow White' (0.22 mg/100 g FW) performed weaker than yucca (0.27 mg/100 g FW) and hollyhock (0.25 mg/100 g FW), but *B. glabra* and *B. glabra* 'Scarlett O'Hara' outperformed yucca, chrysanthemum, gladiolus, marigold, and hollyhock as reported by Nicknezhad *et al*. (2022). The comparison of the bougainvilleas with 20 chrysanthemum genotypes studied by Bayanifar *et al*. (2024) in terms of minerals revealed that all four studied paper flower cultivars were superior to the 20 chrysanthemum genotypes in terms of Fe, Zn, and Ca. Also, the studied bougainvilleas had higher P, Ca, and Fe contents but lower Zn and K contents than begonia, roses, daylilies, and pot marigold (Mlcek *et al*., 2021). Based on the results, it can be said that bougainvilleas, especially the cultivars with red and purple flowers, which had the highest mineral contents in this research, can be included in the food regime as a new source of minerals.

Anthocyanins

The four bougainvillea cultivars differed in anthocyanin content significantly ($P < 0.01$; Table 3). The anthocyanin content ranged from 12.64 to 31.14 mg/100 g FW among them. The lowest was for *B. glabra* 'Snow White', which had white petals, and the highest was for *B. glabra* 'Scarlett O'Hara', which had red petals (Fig. 2).

Table 3. Analysis of variance of the effect of cultivar on anthocyanin, vitamin C and antioxidant capacity in *Bougainvillea* spp.

S.o.V	df	Anthocyanin	Vitamin C	Antioxidant capacity
Treatment	3	320**	1.49**	357**
Error	16	0.145	0.257	0.249
CV (%)		1.62	3.89	0.98

**: Significant at $P < 0.01$ based on the LSD test.

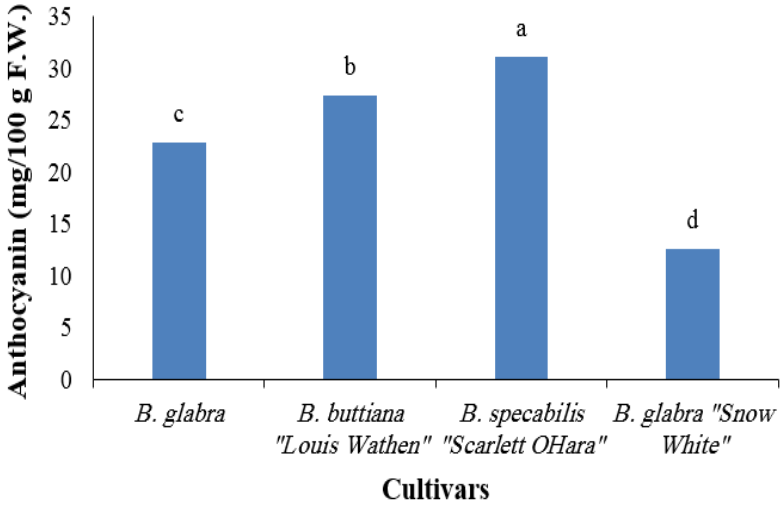

Fig. 2. Effect of cultivar on petals anthocyanin.

Anthocyanins are strong antioxidants that are mainly responsible for a wide range of red, purple, and blue colors in flowers, fruits, and vegetables. These naturally occurring pigments are of high significance in the food industry due to their attractive colors and in the medical industry due to their beneficial curative and antioxidant activities. Humans receive considerable

amounts of anthocyanins through the food regime and the consumption of natural resources (Sadighara *et al*., 2012). The mean anthocyanin intake from natural resources has been reported at 180-215 mg/d among US citizens (Lee *et al*., 2011).

We found that the anthocyanin content was higher in the cultivars with red and orange petals than in those with purple and white petals. A study showed that edible violet flowers with dark (red and purple) colors contained more anthocyanin than those with light-colored petals (Ikeura *et al*., 2023). Islam (2016) reported that the anthocyanin content was higher in red gladioluses than in purple, pink, yellow, and white ones. In Park *et al.*'s (2015) study, the chrysanthemum cultivars with red and purple petals had a higher anthocyanin content than those with orange, green, and white flowers. Similar results were recorded by Bayanifar *et al.* (2024). They revealed that the anthocyanin content was lower in chrysanthemum genotypes with white petals than in those with dark petals, which agrees with our finding about the lower anthocyanin content of the bougainvilleas with white flowers. Since anthocyanins are responsible for generating red, purple, and blue color in plant parts, so there may be a correlation between flower color and anthocyanin content. However, environmental factors, genotype, and growth stage influence anthocyanin content, too (Espejel *et al*., 2019).

Vitamin C

The vitamin C content of the studied cultivars differed significantly at the $P < 0.01$ level (Table 3). It was in the range of 12.24-13.45 mg/100 g FW. *B. glabra* 'Louis Wathen' had the highest vitamin C content, but it did not differ from *B. glabra* 'Snow White' significantly. The lowest was recorded by *B. glabra* 'Scarlett O'Hara' (Fig. 3).

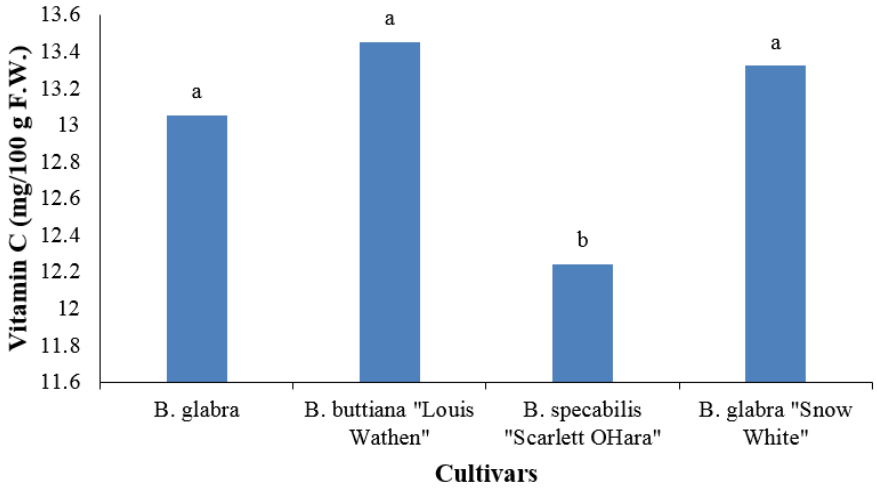

Fig. 3. Effect of cultivar on vitamin C.

Vitamins, including vitamin C, are necessary for body growth and health. The human body does not synthesize vitamin C, so our vitamin C requirement, which amounts to 95-100 mg/day (Bayanifer *et al*., 2024), must be supplied by the food regime. Oranges and kiwifruits, the most famous sources of vitamin C, contain 35 and 93 mg of vitamin C/100 g FW (Demasi *et al*., 2021; Cruz-Rus *et al*., 2012). Although, the bougainvillea cultivars had lower vitamin C content than oranges and kiwifruits, they are a moderate source of vitamin C compared to many

edible flowers, as their vitamin C content has been recorded from 2.6 to 44.9 mg/kg FW (Demasi *et al*., 2021). In a study by Bayanifar *et al*. (2024), the vitamin C content of 20 chrysanthemum cultivars was recorded from 11.71 to 13.58 mg/kg FW. In contrast, Nicknezhad *et al*. (2022) found that the vitamin C content varied from 8.16 mg/100 g for chrysanthemum to 30.6 mg/100 g FW for orange marigolds. The comparison of their results with ours shows that the studied bougainvillea cultivars had higher vitamin C content than chrysanthemum flowers and higher vitamin C content than marigold flowers. In the present work, the orange bougainvilleas had the highest, and the white ones had the second-highest vitamin C content, which agrees with the results reported by Nicknezhad *et al*. (2022), according to which orange marigold flowers had the highest and white yucca flowers had the second-highest vitamin C content.

Antioxidant capacity

As is evident in Table 3, the bougainvillea cultivars differed in antioxidant capacity significantly ($P<0.01$). The antioxidant capacity of the studied cultivars varied from 43.7 to 61.06%. The highest was recorded by *B. glabra* 'Snow White' and *B. glabra*, whereas *B. glabra* 'Louis Wathen' and *B. glabra* 'Scarlett O'Hara' were similarly weaker than the other two (Fig. 4).

Many researchers have stated that there is a relationship between antioxidant capacity and flower color. Flowers in dark colors (red and blue) have higher antioxidant capacity than flowers in light colors (Sadighara *et al*., 2012; Benvenuti *et al*., 2016; Ikeura *et al*., 2023). But, *B. glabra* 'Snow White', which had white petals, recorded the highest antioxidant capacity. Similarly, Chen and Wei (2017) argue that the antioxidant capacity of flowers may be influenced by their chemical compounds. Carotenoids and flavonoids may sometimes be more influential on antioxidant capacity than anthocyanins.

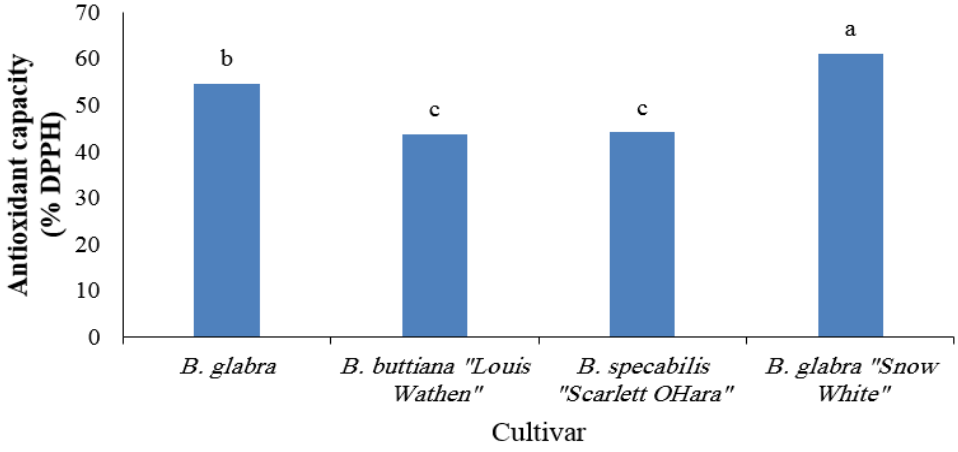

Fig. 4. Effect of cultivar on antioxidant capacity.

Nicknezhad *et al*. (2022) recorded the antioxidant capacity of yucca, marigold, and gladiolus at 47.76, 87.89, and 61.91% HPPH inhibition. So, *B. glabra* 'Snow White' had as much antioxidant capacity as white gladiolus but a higher antioxidant capacity than yucca. However, all four bougainvillea cultivars had lower antioxidant capacities than the marigold.

Anthocyanins and vitamin C are essential antioxidants. According to the correlation test

(Table 4), the anthocyanin content had a negative and significant relationship with the vitamin C content and antioxidant capacity. In the present work, *B. glabra* 'Snow White' had the best, and *B. glabra* had the second-best antioxidant capacity. These two cultivars performed weakly in terms of anthocyanin, but they had acceptable vitamin C content. The correlation between vitamin C and antioxidant capacity was insignificant (Table 4).

Table 4. Correlation between traits.

	An	Vit C	AC	N	P	K	Fe	Zn	Ca
An	1								
Vit C	-0.411*	1							
AC	-0.944**	0.272	1						
N	0.736**	-0.644**	-0.511**	1					
P	0.948**	-0.578**	-0.849**	0.774**	1				
K	0.065	-0.483*	0.229	0.639**	0.173	1			
Fe	0.793**	-0.503**	-0.653**	0.805**	0.822**	0.378	1		
Zn	0.305	-0.012	-0.051	0.602**	0.344	0.567**	0.492**	1	
Ca	0.498**	-0.579**	-0.199	0.886**	0.593**	0.877**	0.673**	0.731**	1

*and**: Significant at $P < 0.05$ and $P < 0.01$ based on the LSD test, respectively. An: Anthocyanin; Vit C: Vitamin C; AC: Antioxidant capacity.

CONCLUSIONS

Although, different bougainvillea cultivars differ in edibility features significantly, they were all good sources of minerals, vitamin C, and antioxidant capacity. The conclusion is that *B. glabra* 'Scarlett O'Hara' can be recommended as a source of minerals, and *B. glabra* can be recommended as a source of vitamin C and antioxidant capacity.

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Biochemical Responses of *Calendula officinalis* to Drought Stress Under Abiotic Elicitor Treatments

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Abstract

The effect of the foliar application of abiotic elicitors was studied on biochemical compounds of marigold under drought stress in a two-factor factorial experiment based on a randomized complete block design with three replications conducted. The first factor was the foliar application of elicitors at 8 levels (control, arginine, glutamine, estradiol, arginine + glutamine, arginine + estradiol, glutamine + estradiol, glutamine + arginine + estradiol) and the second factor was irrigation interval at three levels (1 day, 3 days, and 6 days). Biochemical metabolites, including carotenoids, flavonoids, phenolics, flower and leaf °Brix were measured. Based on the results, the elicitors significantly influenced biochemical traits including carotenoids, flavonoids, phenolics, and leaf and flower ° Brix index. The irrigation interval influenced proline content significantly ($P < 0.05$), but its effect was not significant on other traits. The analysis of variance for the interaction of the foliar application and irrigation interval showed that it was significant ($P < 0.01$) on phenolics content. The highest leaf total soluble solids (°Brix) were obtained from glutamine × 6-day irrigation interval, the highest flower °Brix from glutamine+estradiol×1-day irrigation interval. Finally, it was revealed that glutamine outperformed arginine and estradiol in improving the studied traits. In water stress conditions, some traits like total soluble solids increased. In general, glutamine is superior to arginine and estradiol in improving the studied traits.

Keywords: Arginine, Glutamine, Estradiol, Phenolics, Water deficit stress.

INTRODUCTION

Marigold (*Calendula officinalis* L.) is an herbaceous plant belonging to the Asteraceae family, known for its medicinal and ornamental properties (Sedaghathoor and Raof Haghparvar, 2021; Olennikov and Kashchenko, 2022). Lutein, the primary carotenoid found in marigold, has been linked to a decrease in the risk of various chronic diseases, including cancer and cardiovascular conditions. Studies have highlighted the potential health benefits (the reduction of suffering from many chronic diseases, including cancers and cardiovascular diseases) of incorporating lutein into one's diet (Wang *et al.,* 2006; Yari *et al.,* 2024).

Bio-elicitors, as defined by Thomas *et al.* (2009) and Gawronska (2008), are compounds that stimulate plant growth and enhance both the quantitative and qualitative yields of plants. These elicitors can act as compounds that optimize plants' response to environmental conditions, as well as growth-promoting compounds. Amino acids, for example, are a type of bio-elicitor that can enhance the growth of plants (Starck, 2005). Categorized into eight groups, bio-elicitors include humic matter, organic compounds, inorganic salts (such as phosphite), seaweed extracts, chitin and chitosan derivatives, antitranspirants, and amino acids or nitrogen compounds (Yari *et al.,* 2024). Amino acids, crucial for plant growth, may be lacking in adverse environmental conditions, and their application as fertilizers can fulfill the plant's need for nitrogen. In times of environmental stress or reduced nutrient uptake, foliar application of amino acid-based fertilizers has proven effective for over three decades, offering various benefits for crops (Faten *et al.,* 2010). These organic compounds directly and indirectly contribute to plant growth and development, with specific amino acids like asparagine and glutamine influencing important metabolic cycles in plants (Abaspour Esfaden *et al.,* 2019). Furthermore, amino acids such as glutamic acid and glycine can aid in the uptake of micronutrients by chelating them (Torab Ahmadi *et al.,* 2019).

Drought stress is a significant constraint on crop productivity. The availability of water plays a crucial role in determining the distribution of plants and can lead to various changes at morphological, physiological, biochemical, and molecular levels in plants. It is evident that plants undergo significant biochemical and physiological alterations, negatively impacting their photosynthetic capacity (Seleiman *et al.,* 2021). As drought stress increases to around 25% of field capacity (FC), the dry weights of stems, roots, leaves, and flowers tend to decrease. However, optimal nutrition has the potential to enhance crop growth and yield while mitigating the effects of drought stress (Khalid Hussein and Qader Khursheed, 2014). Amino acids play a crucial role in enhancing crop growth and production by influencing various physiological processes in plants (Radkowski, 2018). Glutamine, arginine, and asparagine are key amino acids involved in metabolic and biochemical reactions that contribute to detoxification, stress tolerance, and chlorophyll formation. Research has shown that exogenous application of amino acids can increase leaf chlorophyll capacity and improve plant resilience to salinity stress (Amin *et al.,* 2011). Additionally, studies on mammalian hormones like estrogen and androgen demonstrate their potential to stimulate cell division and antioxidant enzyme activity. Water stress can negatively impact plant growth by reducing nitrogen uptake, but the application of amino acids has been shown to mitigate these effects and improve crop yield in various plant species (Janeczko and Skocczowski, 2005; Erdal and Dumlupinar, 2011; Sedaghathoor and Zakibakhsh-Mohammadi, 2019). The objective of this study was to investigate the potential effects of elicitors such as glutamine and arginine, along with estradiol, biochemical responses of marigold under conditions of drought.

MATERIALS AND METHODS

The research carried out as a two-factor factorial experiment based on a randomized complete block design with three replications (Table 1). It was done in a greenhouse at the Ornamental Plants Research Center of Lahijan, Iran. The marigold seedlings were transferred to the main pots on December 1, 2020. They were maintained at 21°C, 70% humidity, and 16/8 hours of day/night photoperiod. Each pot received 120 mL of water, with the only variation being the irrigation interval. The first foliar spraying was carried out on January 16, 2021, followed by a second application on February 14. The experimental treatments included different levels of abiotic elicitors (arginine, glutamine, and estradiol) and three irrigation intervals of 1 day, 3 days, and 6 days. Each experimental plot contained three tested plants. On March 30, 2021, marigold samples were collected a week after the anthesis phase and transferred to a laboratory to measure traits.

Table 1. The treatments used in the foliar application of the marigolds with estradiol and the amino acids glutamine and arginine at different rates and different irrigation intervals.

Treatment	Symbol
Control	a_1
100 mg/L arginine	a_2
100 mg/L glutamine	a_3
100 mg/L estradiol	a_4
100 mg/L arginine + 100 mg/L glutamine	a_5
100 mg/L arginine + 100 mg/L estradiol	a_6
100 mg/L glutamine + 100 mg/L estradiol	a_7
100 mg/L glutamine + 100 mg/L arginine + 100 mg/L estradiol	a_8
Irrigation interval of 1 day	b_1
Irrigation interval of 3 days	b_2
Irrigation interval of 6 days	b_3

In the study conducted to assess the carotenoid and anthocyanin content of leaf samples, a meticulous procedure was followed. For carotenoid analysis, 0.5 g of each sample was carefully weighed and subsequently crushed in a china mortar with 50 mL of 80% acetone solution. The resulting extract was filtered, adjusted to a volume of 50 mL, and transferred to cuvettes for spectrophotometric analysis at wavelengths 645 nm, 663 nm, and 660 nm. Carotenoid content was determined using the formula: Carotenoid content = 4.69 (A660) – 0.268 (A645) + 8.02 (A663), as outlined by Mazumdar and Majumder (2003). On the other hand, for anthocyanin analysis, 0.5 g of each sample was weighed and crushed in a china mortar with 50 mL of ethanol-hydrochloric acid solution. Following filtration and adjustment to 50 mL, the samples were refrigerated at 4°C for 24 hours, and then kept in darkness for 2 hours before being read at 535 nm using a spectrophotometer. The anthocyanin content was calculated using the formula:

$$\text{Total absorbance} = (e \times b \times c) / (d \times a) \times 100$$

Where b is the volume measured (5 mL), c is the total volume (50 mL), d is the sample fraction (-0.1), e is the reading at 535 nm, and a is the sample weight (0.5 g), as Mazumdar and Majumder's methodology (2003).

To analyze the proline and flavonoid content in plant samples, specific reagents and procedures were employed. For proline measurement, a reagent was prepared by dissolving acid ninhydrin in acetic acid, followed by the addition of phosphoric acid. After preparing a tissue extract in sulfosalicylic acid, the supernatant was mixed with ninhydrin reagent and acetic acid, heated, and then cooled for measurement at 520 nm (Bates *et al.,* 1973). For flavonoid analysis, frozen leaf samples were processed in acidic ethanol, and the resulting supernatant was analyzed at different wavelengths (270, 300, and 330 nm) using a spectrophotometer. The concentration of flavonoids was calculated using the coefficient of extinction at 33000 mol/cm (Sun *et al.,* 1998; Rabeie and Jozghasemi, 2013).

The total phenolics content was determined using the Folin-Ciocalteu reagent method. Fresh leaves (1 g) were ground in methanol (10 mL) for 2 minutes and filtered. Subsequently, 5 mL of diluted Folin-Ciocalteu reagent (1:10 with distilled water) and 4 mL of sodium carbonate solution (7.5 v/v) were added to 0.5 mL of the diluted extract (1:10 g/mL). After 15 minutes at room temperature, the absorbance was measured at 765 nm using a spectrophotometer (Slinkard and Singleton, 1977; McDonald *et al.,* 2001). The °Brix measurement (total soluble solids/ TSS) was conducted using a manual refractometer (1-PAL-A model, Atago, Japan). A drop of filtered extract from flowers and leaves was placed on the pyramid glass of the device, and the device was then directed towards the light to measure the light refraction, indicating the °Brix content (Mahmood *et al.,* 2012). The data was analyzed using the MSTATC software package, with means compared using the LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

The results of the ANOVA in table 2 indicate that the foliar application of elicitors, such as amino acids and estradiol, had a significant effect ($P < 0.01$) on biochemical metabolites, including carotenoids, flavonoids, phenolics and leaf °Brix. Additionally, the irrigation interval had a significant impact on proline content ($P < 0.05$), but did not significantly affect other chemicals. Furthermore, the interaction between the foliar application of elicitors and irrigation interval was found to be significant ($P < 0.01$ or 0.05) on phenolics, leaf and flower °Brix, while not significantly influencing other pigments and metabolic indices.

According to the comparison of means presented in table 3, the highest carotenoid content was achieved through the application of estradiol. Brassinosteroids have been shown to enhance carotenoid levels, thereby inhibiting chlorophyll degradation and increasing chlorophyll a and b in various plant species such as marigolds, wild pears, mustard, and peas (Ahmadi Lashaki *et al.,* 2018; Zahedi *et al.,* 2017; Fariduddin *et al.,* 2009; Ali *et al.,* 2007). Conversely, the lowest carotenoid synthesis rate was observed in plants treated with glutamine and estradiol. Drought stress has been found to elevate carotenoids, dry matter, proline, and gas exchange through stomata in wild pear plants (Zarafshar *et al.,* 2014). Furthermore, research has demonstrated that the use of bio-elicitors on thyme can boost essential oil yield, chlorophyll a content, and carotenoid levels (Miri *et al.,* 2015). Carotenoids, as crucial light-absorbing pigments in thylakoids, serve as light receptors and protectors of photosystems, combating reactive oxygen species (ROS). By dissipating excess energy from photosystems I and II as heat or chemical reactions, carotenoids safeguard chloroplast membranes, thereby enhancing plant resilience to stress factors (Juan *et al.,* 2005). These pigments indirectly diminish ROS production and regulate free oxygen activity. Studies have indicated that the highest carotenoid content in marigold petals was associated with estradiol treatment, while the lowest was linked to the control group (Sedaghathoor and Raof Haghparvar, 2022). Leaf carotenoid content

has been shown to increase with the foliar application of glutamine and amino acids, with reported benefits on plant growth and biochemical properties. Amino acids play a vital role in synthesizing various plant compounds, including proteins, enzymes, phenolics, and flavonoids, thereby influencing plant processes positively (Aghaye Noroozlo *et al.,* 2019; Haghighi *et al.,* 2022).

Table 2. The analysis of variance for the effect of the foliar application of elicitors (amino acids and estradiol) and irrigation interval on the marigold plants.

S.o.V	df	Carotenoid	Anthocyanin	Proline	Flavonoid	Antioxidant capacity	Phenolics	Leaf TSS	Flower TSS
Replication	2	0.32^{ns}	0.05^{ns}	0.02^{ns}	19.13^{ns}	1.71^{ns}	3.32^{ns}	0.25^{ns}	0.94^{ns}
Elecitors (A)	7	2.30^{**}	0.15^{ns}	0.02^{ns}	62.72^{**}	1.28^{ns}	12.45^{**}	5.32^{**}	1.17^{ns}
Irrigation interval (B)	2	0.38^{ns}	0.76^{ns}	0.07^{*}	37.91^{ns}	2.67^{ns}	4.96^{ns}	0.54^{ns}	1.13^{ns}
Interaction (AB)	14	0.78^{ns}	0.69^{ns}	0.02^{ns}	25.32^{ns}	0.72^{ns}	9.34^{**}	3.46^{**}	2.10^{**}
Error	46	0.504	0.657	0.013	15.717	0.868	2.514	1.16	0.67
CV (%)	-	12.17	12.31	8.63	7.29	1.09	20.35	11.92	11.17

*, **, and ^{ns} represent significance at the $P < 0.01$ and $P < 0.05$ levels and insignificance, respectively.

Based on results all treatments and interaction did not affect significantly anthocyanin content of marigold. The results indicated that the proline content was not significantly affected by the interaction of irrigation interval and foliar application, nor by the simple effect of foliar application. However, the simple effect of the irrigation interval was found to be significant ($P < 0.05$) on this trait. Upon comparing the means, it was observed that the highest proline content was achieved with an irrigation interval of three days, while the lowest was recorded with daily irrigation (Fig. 1). The study examined the impact of irrigation intervals and foliar application on the proline content of pomegranates cv. 'Naderi'. The application of a fertilizer containing amino acids through foliar spray was shown to effectively mitigate the detrimental impacts of drought stress on the pomegranate plants (Hasanzade *et al.,* 2017). Plants respond to various stresses by synthesizing osmolytes such as proline. The rate of proline synthesis is influenced by the species of the plant and the type of stress it experiences. Proline, a compound found in many higher plants, is known to accumulate in high concentrations in response to environmental stresses (Sawahel Wagdy and Hassan, 2002). It plays a crucial role in safeguarding cell structure and macromolecules, as well as in inhibiting free radicals and reducing cell oxidation potential during osmotic stress (Porcel and Ruiz-Lozano, 2004). Overall, the findings underscore the importance of irrigation management and foliar application of amino acid-based fertilizers in enhancing the stress tolerance of plants, particularly in terms of proline accumulation and protection against the adverse effects of drought stress (Roosens *et al.,* 2002).

Table 3. The mean comparison for the simple effect of elicitors on biochemical compounds of marigold.

Organic compounds	Carotenoid (mg/L)	Flavonoid (mg/g)	Phenolics (mg/100 g)	Leaf TSS (°Brix)
Control	5.99 bc	50.22 c	5.74 d	8.09c
Arginine	5.81 bc	55.90 ab	7.04 bcd	8.89bc
Glutamine	5.41 bc	55.62 ab	9.37 a	10.22a
Estradiol	6.8 a	54.04 abc	8.11 abc	8.39c
Arginine + glutamine	5.74 bc	51.53 bc	8.77 ab	10.11ab
Arginine + estradiol	5.56 bc	55.39 ab	7.81 abc	8.78c
Glutamine + estradiol	5.18 c	58.61 a	8.58 abc	9.22abc
Glutamine + arginine + estradiol	6.09 ab	53.67 bc	6.91 cd	8.67c

Means with different letters on the same column are significantly different (P <0.05) based on LSD test.

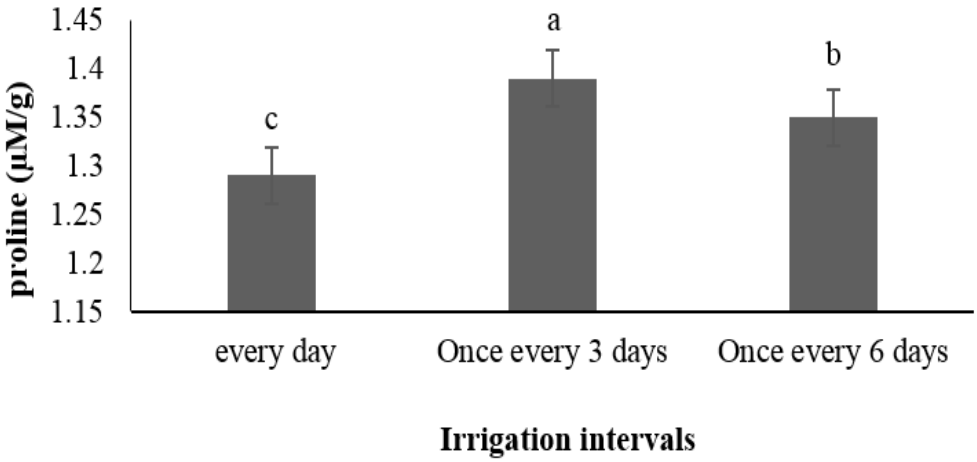

Fig. 1. The simple effect of irrigation on proline content.

The highest flavonoid content was associated with the treatment involving glutamine and estradiol, while the lowest was observed in the control group without any elicitor (Table 3). The application of amino acids has been shown to enhance plant resistance to stressful conditions. Sedaghathoor and Raof Haghparvar (2022) found that the highest flavonoid content in marigolds resulted from the application of estradiol, indicating the positive impact of estradiol on yield improvement and flavonoid enhancement, consistent with our findings. Flavonoids synthesized in the cytoplasm and endoplasmic reticulum play a crucial role as antioxidants, safeguarding plants against various stresses (Pourcel *et al.,* 2006). In the present experiment, the application of elicitors during stressful conditions led to substantial changes compared to the control, with increased flavonoid synthesis providing significant stress protection. The rise in Kala's flavonoid levels can be attributed to their antioxidant activity, activated during drought stress to counter oxidative damage (Seyoum *et al.,* 2006). Studies have also demonstrated that flavonoids enhance membrane resistance to oxidative factors by reducing fluidity and preventing the release of free radicals (Harborne and Williams, 2000).

Antioxidant capacity was one of trial traits that did not affected significantly under experimental factors and their interaction. Based on the results, the maximum phenolics synthesis

was associated with the treatment involving glutamine and estradiol with a six-day irrigation interval, while the lowest was observed in the control group (Table 4). As indicated in table 3, the application of elicitors resulted in a notable increase in phenolics content, with the highest level recorded at 9.37 mg/g for the glutamine treatment and the lowest at 5.74 mg/g for the control. This enhancement in phenolics and flavonoids in marigolds due to elicitor application aligns with previous findings demonstrating increased phenolic compounds in various plants (Schwambach *et al.,* 2008) under drought conditions. Notably, research by Khajehhosseini *et al.* (2020) emphasized the significant impact of irrigation and foliar application of amino acids on total phenolics. Additionally, studies by Rezaie Alulu *et al.* (2020) highlighted that heightened water deficit stress led to increased phenolics, total flavonoids, and antioxidant activity in carla plants, corroborating our observations. The rise in phenolics serves as a defensive antioxidant mechanism in plants facing water deficit stress, aiding in the stabilization of cell membranes and the prevention of lipid peroxidation by scavenging reactive oxygen species. Phenolic compounds possess potent antioxidant properties, capable of scavenging free radicals and inhibiting the breakdown of hydroperoxides into free radicals (Razali *et al.,* 2008).

Table 4. The comparison of means for the interactive effects of the foliar application of organic compounds (amino acids + estradiol) and irrigation.

Treatments	**Phenolics (mg/100 g)**	**Leaf TSS (°Brix)**	**Flower TSS (°Brix)**
Control × 1-day irrigation interval	5.11gh	7.77d	6.00f
Control × 3-day irrigation interval	7.09c-h	8.50cd	8.80ab
Control × 6-day irrigation interval	5.04h	8.00d	6.73def
Arginine × 1-day irrigation interval	8.10c-h	8.67cd	7.00c-f
Arginine × 3-day irrigation interval	5.91e-h	8.67cd	8.50abc
Arginine × 6-day irrigation interval	7.12c-h	9.33bcd	6.83def
Glutamine × 1-day irrigation interval	9.12bcd	8.17d	6.83def
Glutamine × 3-day irrigation interval	9.06b-e	10.67abc	7.50b-f
Glutamine × 6-day irrigation interval	9.91abc	11.83a	7.67a-e
Estradiol × 1-day irrigation interval	7.23c-h	7.83d	7.50b-f
Estradiol × 3-day irrigation interval	9.14bcd	9.17bcd	6.83def
Estradiol × 6-day irrigation interval	7.97c-h	8.17d	7.00c-f
Arginine + glutamine × 1-day irrigation interval	11.42ab	10.50abc	6.67def
Arginine + glutamine × 3-day irrigation interval	6.73c-h	10.67abc	6.83def
Arginine + glutamine × 6-day irrigation interval	8.15c-h	9.17bcd	6.83def
Arginine + estradiol × 1-day irrigation interval	8.31b-f	9.00bcd	8.10a-e
Arginine + estradiol × 3-day irrigation interval	7.35c-h	9.17bcd	7.17b-f
Arginine + estradiol × 6-day irrigation interval	7.76c-h	8.17d	7.50b-f
Glutamine + estradiol × 1-day irrigation interval	6.98c-h	11.00ab	9.167a
Glutamine + estradiol × 3-day irrigation interval	6.18d-h	7.67d	7.17b-f
Glutamine + estradiol × 6-day irrigation interval	12.58a	9.00bcd	7.67a-e
Glutamine + arginine + estradiol × 1-day irrigation interval	8.26b-g	8.17d	8.17a-d
Glutamine + arginine + estradiol × 3-day irrigation interval	6.6 d-h	9.00bcd	7.17b-f
Glutamine + arginine + estradiol × 6-day irrigation interval	5.79fgh	8.83bcd	6.50ef

Means with different letters on the same column are significantly different (P <0.05) based on LSD test.

Based on results (Table 2) the combined effects of foliar elicitor application and irrigation intervals, along with the individual effect of elicitors, significantly influenced °Brix (TSS) ($P < 0.01$). However, irrigation intervals did not have a significant impact on this trait. Analysis of means for the interactive effect on leaf TSS (Table 4) showed that the highest leaf TSS (11.83 °Brix) was associated with the treatment involving glutamine and a 6-day irrigation interval. The second-highest °Brix was observed with the foliar application of "glu + est × 3-day irrigation interval" (7.67°Brix). °Brix reflects the ratio of soluble solids to total solution weight, indicating higher soluble solids and lower water content in a liquid. Research by Noktehsanj *et al.* (2018) highlighted the importance of TSS in plant quality, with Miri Nargesi *et al.* (2022) noting the impact of TSS on fruit quality in olive cultivars. The study suggests that higher TSS in leaves and flowers signifies greater value in pharmaceutical industries. For marigolds, applying glutamine with a 6-day irrigation interval can enhance leaf TSS, thereby improving product quality. ANOVA results showed a significant ($P < 0.01$) impact of elicitors and irrigation intervals on flower TSS interaction, while individual factors did not significantly affect flower TSS. Comparison of means for flower TSS (Table 4) revealed that plants treated with "glu + est × daily irrigation" had the highest flower TSS (9.16 °Brix), while the control group (without elicitor + 1-day interval) had the lowest flower TSS at 6 °Brix. Glutamine and estradiol performed better than arginine and the control, significantly increasing Brix levels.

CONCLUSION

The results of the study indicate that the elicitors had a significant impact on various biochemical traits such as carotenoids, flavonoids, phenolics, and leaf and flower ° Brix index. The irrigation interval was found to have a significant effect on proline content, but not on other traits. The interaction between foliar application and irrigation interval was significant on phenolics content. The highest leaf total soluble solids (°Brix) were observed with glutamine and 6-day irrigation interval, while the highest flower °Brix was seen with glutamine and estradiol with 1-day irrigation interval. Finally, glutamine was found to be more effective than arginine and estradiol in improving the studied traits. Additionally, under water stress conditions, some traits such as total soluble solids showed an increase. In conclusion, glutamine proved to be superior to arginine and estradiol in enhancing the studied traits.

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Evaluation of Stenting for the Propagation of *Syringa vulgaris* on *Ligustrum vulgare* Rootstock

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Abstract

Lilac (*Syringa vulgaris*) is a valuable shrub used in the landscape due to its beautiful leaves and flowers and pleasant fragrance. Grafting of lilac on wild privet (*Ligustrum vulgare*) was evaluated in this study to reduce the time required for propagation and the use of rootstock. Stenting or simultaneous rooting and grafting is a novel method for propagating of some woody plants. In this research, the stenting technique was applied to propagate lilac for the first time. Two stenting methods (splice and omega) and three concentrations of IBA (0, 1000 and 2000 mg L^{-1}) were evaluated. According to the results, stenting via the splice method significantly increased the percentage of rooted stentings and leafed scions and reduced the rate of dried stentings in comparison to the omega method. The percentage of rootstock callus formation, percentage of leafed scions, and fresh weight of produced roots and shoots were significantly enhanced by application of IBA. The best results were obtained with 2000 mg L^{-1}. In addition, the lowest percentage of dried stenting was observed in this treatment. Also, final success of stenting showed that the use of the splice method and IBA had the highest achievement. In conclusion, the application of 2000 mg L^{-1} IBA in comparison with splice stenting method is recommended for propagation of *Syringa vulgaris*.

Keywords: Cutting-grafting, Grafting, *Ligustrum vulgare*, Lilac, Privet, Propagation.

INTRODUCTION

Common lilac, scientifically known as *Syringa vulgaris,* is native to mountainous regions of Eastern Europe and is domesticated as an ornamental deciduous shrub or small tree (Bean, 1921). It is thus prevalent in park and landscape architecture due to its elegant flowers and leaves (Royal Horticultural Society, 2008). Moreover, the common lilac is widely produced as a cut flower that can be forced effortlessly (Jędrzejuk *et al.*, 2013). Because lilac cuttings are hard to root, the most common method of lilac propagation is to graft shoots onto wild privet (*Ligustrum vulgare*) rootstocks (Waldenmaier and Bünemann, 1991). Wild privet, also called common or European privet, is an indigenous deciduous or almost evergreen shrub in Europe, Western Asia, and Morocco (Bean, 1921). Stenting or cutting-rooting is advantageous for reducing the time required for achieving commercially grafted seedlings due to the synchronicity of rooting and grafting applied in numbers of ornamental plants and fruit trees (Nazari *et al.*, 2009; Babaie *et al.*, 2014; Izadi and Zarei, 2014; Karimi, 2011; Solgi *et al.*, 2022; Brar and Khehra, 2017). The stenting method lacks several grafting adversities; in addition, it mostly improves the degree of rooting and scion growth (Ohkawa, 1980). Plants from stenting generally have heavier root weights than cutting plants; furthermore, this technique is appropriate for studying and screening the interactions between rootstocks and scions (van de Pol and Breukelaar, 1982). Thus, it can be a valuable technique for rapid mass multiplication and year-around production of different plants to meet the increasing demand (Rawat and Kumar Das, 2020).

Auxins are phytohormones that play essential roles in nearly every facet of plant growth and development and have complex biosynthesis (Zhao, 2010). These hormones are pivotal to the growth mechanisms of leaves, buds, fruits, roots, and flowers, as well as to seed functions (Osborne and McManus, 2005). Indole-3-butyric acid (IBA) is a synthetic auxin. At the same time, it has been extracted from the leaves and seeds of some plant species (Ludwig-Müller, 2000), with which many of the plant species with hard-rooting cuttings are currently commercially rooted (Hartmann *et al.,* 2010). The grafted ornamental shrubs like the cut rose cultivars, on productive rootstocks had superior flower yield and performance relative to plants growing on their roots (Cabrera, 2002). The utilization of IBA in stenting has been studied in some commercial ornamental roses, which due to the complicated decreasing interaction between scion and rootstock, experienced weaker rooting and shooting compared to those of cutting (Pourghorban *et al.*, 2019). Although, this compound is recommended for rose growers due to the generation of heavier roots and shoots as well as higher leaves, shoots, and roots numbers (Yeshiwas *et al*., 2018). Further, indole-3-butyric acid can result in more numerous and heavier roots in *Ficus benjamina* propagated via the stenting method (Babaie *et al.*, 2014). It has a substantial positive direct impact on callus formation at the grafting union and length of the roots and shoots; moreover, the number of leaves due to the use of IBA in mulberry stenting is determined (Solgi *et al.*, 2022). As far as our knowledge, there is no study on the application of this technique in combination with IBA to propagate *Syringa vulgaris* on wild privet rootstock. The purpose of this study was to determine the performance and yield of stenting as an alternative method of cutting and to evaluate wild privet potential as a rootstock. Recently, Kiran *et al.* (2022) recommended to stenting in the month of January for the production of quality plants of rose cv. "Top Secrete". The research question is how much the stenting propagation method can be successful in overcoming the problems associated with grafting and cutting methods problems like hard rooting and being time-consuming.

MATERIALS AND METHOD

In this study, both types of plant materials (lilac scions and privet rootstocks) were collected from the Arak University landscape. Scions were prepared from mature shoots of *Syringa vulgaris* (Fig. 1).

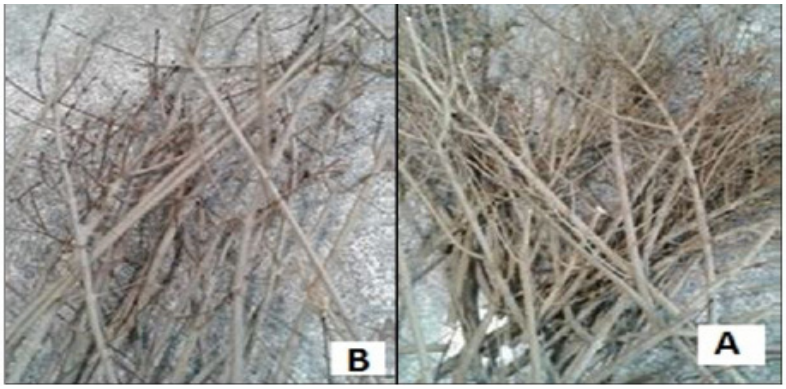

Fig. 1. Wild privet shoots as rootstocks (A) and common lilac shoots as the scions (B).

Treatments and stenting methods

Two factors including two stenting methods (splice and omega) and IBA as a rooting hormone at three levels (0, 1000, and 2000 mg L^{-1}) were used (Fig. 2). Shoots selected from the middle were sliced into pieces with at least two buds and prepared for splice grafting and omega grafting method (Fig. 2). The cuts were made 3 cm below the lowest bud of the scions and 2 cm above the uppermost rootstock buds using a clipper for the splice method. Also, with the cuts were made for the omega technique using a grafting tool (Fig. 3). Immediately after cutting the scions, necessary rootstock procedures were performed. The rootstocks and scions, which had almost the same diameter, overlapped to conjoin the vascular cambium layers in the best way (Fig. 4). The grafted cuttings were then immersed into the IBA treatment for 5 s. Light-color parafilm tapes were applied to furl the grafting union (Fig. 5). Afterward, the plants were placed in a growth medium containing 70% sand and 30% perlite. Moreover, Benomyl (0.3 % V/V) was used to disinfect the propagation medium.

Fig. 2. Applied omega grafting tool for this study.

Fig. 3. Cutting types for splice (left) and omega (right) stenting.

Fig. 4. Overlapping of the scions and rootstocks using a splice (left) and an omega grafting tool (right).

Fig. 5. Covering the grafting union with parafilm tapes.

Plant maintenance conditions

The greenhouse equipment for environmental control includes convective heating, evaporative cooling pads, and exhaust fans. The mean mid-day temperature and relative humidity during the study period were maintained at 25 °C ± 2 °C and 70 % ± 5 %, respectively. Also, due to the plants' requirement of a relative humidity of 95-100%, particularly during the first week after cutting the grafting, a polyethylene coating was thrown over the box of the grafted plants.

Evaluated characteristics

Three months after grafting, the cutting grafts were removed from the medium box and washed with water. Some morphological characteristics, such as the percentage of rooted rootstocks, percentage of leafed scions, percentage of dried grafted plants (did not show any symptoms like as callus formation in junction and/or rootstock and rooting formation after three months), percentage of callus formation from rootstocks, fresh weight of produced roots and leaves, and length of the longest produced root and shoot, were recorded. Following the evaluation of the characteristics, the grafted plants were planted in pots with (10 cm diameter) having the beds made of sand and agricultural soil (1:1). Two months next to the cultivation of cutting grafts (five months after stenting), the final grafting success rate was evaluated.

Experimental design and statistical analysis

This experiment was conducted in a factorial arrangement based on a completely randomized design with three replications, each consisting of 10 samples. Statistical significance between mean values was assessed using analysis of variance (ANOVA) and the conventional Duncan's multiple range test (DMRT) at $P < 0.05$ using SAS (9.1).

RESULTS

According to ANOVA results, the effect of stenting method on the percentages of rooted rootstocks ($P < 0.05$), dried grafted plants characteristics ($P < 0.05$), and percentage of leafed scions ($P < 0.01$) were significant. However, other traits did not show significant differences. The various IBA concentrations significantly influenced the percentage of leafed scions and the length of the tallest shoot at a 5% probability, and the percentages of callus formation from rootstocks, dried grafted plants, and fresh weight of produced roots and leaves were significant at a 1% probability. In contrast, it did not have any significant effect on the percentage of rooted rootstocks and the length of tallest roots. The interaction effect of these factors only affected the percentage of leafed scions at a 1% probability (Table 1).

Table 1. Analysis of variance of three levels of IBA and two methods of stenting's impact on various traits after 3 months.

S.o.V	df	MS							
		Longest root length	Longest shoot length	Shoot fresh weight	Root fresh weight	Dried stenting	Produced leaves	Produced roots	Callus formation
Type of Stenting (A)	1	0.320^{ns}	2.420^{ns}	0.031^{ns}	0.00001^{ns}	16.155*	21.505**	1.008*	0.016^{ns}
IBA Concentration (B)	2	1.416^{ns}	4.402*	0.231**	0.08**	18.722**	12.518*	0.211^{ns}	2.57**
A × B	2	0.0003^{ns}	0.715^{ns}	0.083^{ns}	0.006^{ns}	7.055^{ns}	8.845*	0.141^{ns}	0.191^{ns}
Error	12	0.616	0.678	0.03	0.01	2.0	2.121	0.123	0.212
CV (%)		30.62	24.46	16.17	11.67	24.71	25.23	29.95	24.46

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the DMRT test, respectively.

The comparison between the stenting methods indicated that the percentage of rooted stenting was affected by the stenting method, and the splice method had a better rooting percentage (17%) than the omega method (5%) after three months (Fig. 6a).

Based on the mean comparisons, the influence of the stenting method was significant for a significant percentage of dried grafted plants, and the splice method produced lower dried grafts (48%) compared with the omega method (67%) (Fig. 6b). These outputs are similar to those of previous root stenting.

The splice method significantly influenced the leaf production of stenting. Also, the interaction effect of stenting methods and IBA concentrations significantly impacts on the leaf production. The splice stenting method produced more leaves (48%) than the omega method (27%) (Fig. 6c).

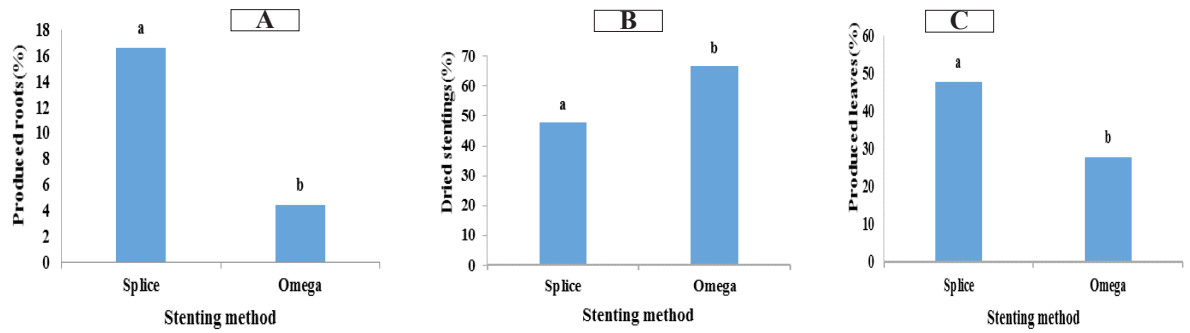

Fig. 6. The influence of the two methods of stenting on the produced roots percentage (a), on the dried stenting percentage (b), and produced leaves percentage (c).

Moreover, the application of IBA especially 2000 mg L^{-1} increased the leaf formation. The comparison effect revealed that IBA levels had less influence on leaf formation grafted via the splice method than the omega method (Fig. 7a). Therefore, the best result was achieved using the omega method with 2000 mg L^{-1} of IBA (53.3%).

The impact of various concentrations of IBA on the percentage of rootstock callus formation was significant. The highest percentage of callus formation was in 2000 mg L^{-1} (63.3%), which is comparable with the lowest percentage observed for control treatment (15%) (Fig. 7b).

The mean IBA level significantly influenced the percentage of dried stenting percentage (Fig. 7c). Increasing the IBA concentration reduced the proportion of dried grafted plants. The highest and lowest dried cutting graft yields were observed for the control treatment (73%) and 2000 mg L^{-1} IBA (38%) treatments, respectively. These results were similar to previous characteristics, including the percentage of rootstock callus formation and leaf production, which were enhanced at higher IBA concentrations (Fig. 7c).

According to the mean comparison of root fresh weight, this trait increased with increasing application of higher IBA concentrations (Fig. 7d). Thus, the most considerable root fresh weight was recorded in 2000 mg L^{-1} (0.55 g), and the least was for the control treatment (0.14 g). The result of this parameter was similar to the above mentioned characters like percentage of rootstock callus formation and leaf production.

Like the roots fresh weight, the shoot fresh weight and length of the longest produced shoot were significantly enhanced by using IBA. Thus, the largest fresh weight and longest shoot were produced because of 2000 mg L^{-1} IBA and the least amount were obtained in the control treatment (Fig. 7e and 7f).

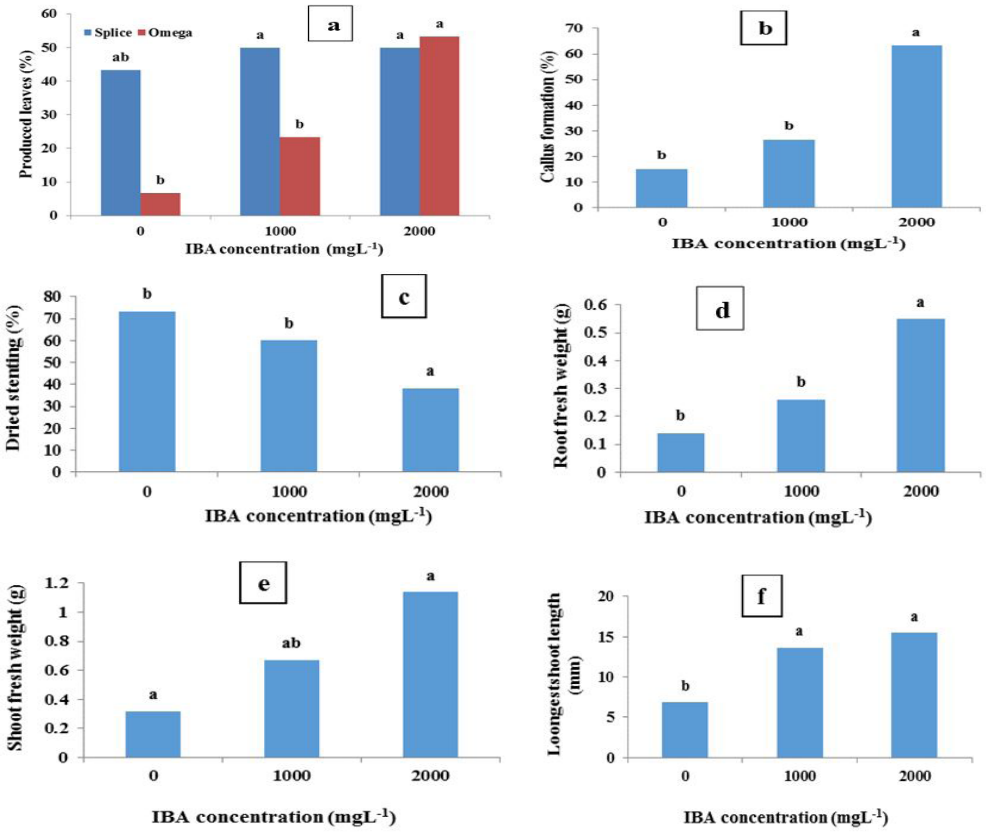

Fig. 7. The effects of two stenting methods and different IBA concentrations on produced leaves percentage (a), on callus formation percentage (b); on the proportion of dried stenting percentage (c), root fresh weight (d), fresh weight (e), and length of the longest shoot (f).

Two months since the remained cutting grafts were established from the previous step and planted in the pots, the final stenting success was evaluated. Analysis of variance revealed that there is a significant effect of the stenting method at 1% and various IBA levels at 5% (Table 2).

Table 2. Analysis of variance of three IBA levels and two stenting methods on the final percentage of stenting success after 5 months.

S.o.V	df	MS
		The final percentage of stenting success
Type of Stenting (A)	1	24.07**
IBA Concentration (B)	2	15.35*
A × B	2	1.37ns
Error	12	1.93
CV (%)		30.12

*, ** and ns: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the DMRT test, respectively.

The comparison of the success of the two stenting methods on cutting grafts revealed that the splice method significantly has a superior success rate (32%) compared with the omega method (13%) after 5 months (Fig. 8a). These outcomes correspond to other characteristics during the prime step (after three months). Also, the final success of stenting was increased with superior amounts of IBA. 2000 mgL^{-1} treatment caused the highest success rate (32%), compared with the control treatment with only 8% success (Fig. 8b).

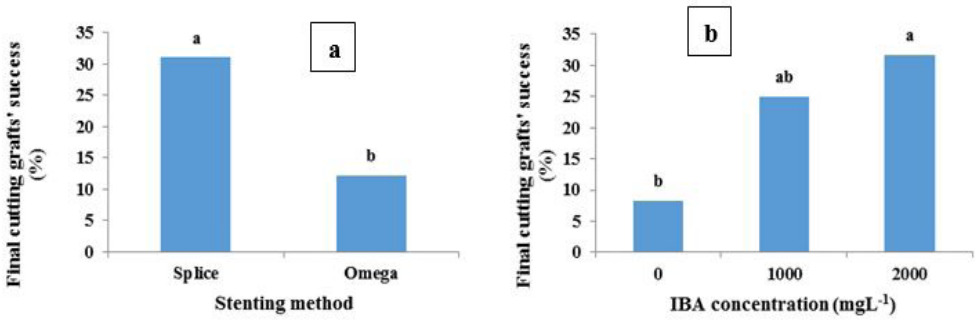

Fig. 8. The influence of the two stenting methods on the final cutting-grafting success after 5 months (a) and the influence of different IBA concentrations on the final cutting-grafting success after 5 months (b).

DISCUSSION

There is no report to investigate the stenting of common lilac. Our study used IBA levels for rooting wild privet as rootstock and two stenting methods on grafting of lilac, simultaneously. According to the present findings, the stenting splice method produced more rooted grafted plants and leaves. Further, the splice technique generated fewer dried stenting. Besides, after five months, the final cut-graft achievement was higher for the splice method. The current study indicated that the splice stenting method has higher final success, survival, and growth factor percentages than the omega stenting method. Generally, a connection between the scion chamber and rootstock is prerequisite for successful grafting. So, failure to create a stable connection between the scion and rootstock vessels and consequently the insertion of the cambium in an abnormal position causes unsuccessful grafting and finally lack of grafting survival and subsequent growth (Ramos, 1998; Solgi *et al.*, 2012; Solgi *et al.*, 2022). Some grafting methods have a minor connection between scion and rootstock cambiums, lowering the grafting success rate (Nowrozi *et al.*, 2016).

In this study, the stenting splice method had a higher success rate owing to earlier root creation and early establishment on rootstock, causing superior vegetative growth of the grafting combination. There is no report related to any stenting method for this shrub to date, and the reported researches are mostly on fruit trees and rose production. For instance, Khalili *et al.* (2011) suggested the application of the omega stenting method due to its desired impact on rose commercial production. In another study, greenhouse rose varieties were grafted onto *R. manetti* rootstock. Results indicated that the omega stenting method was more successful than the tongue method. The "Peach Avalanch" rose cultivar on *R. manetti* rootstock has the highest number of roots, stems, and leaves and produces the longest roots (Izadi *et al.*, 2013). Contrary to our previously mentioned results, the omega stenting method was found suitable for rose propagation.

Based on our results, the application of higher concentrations of IBA, there were significant impacts on rootstocks callus formation, scion leaf production, and fresh weight of roots and shoots. The maximum effect was observed at a concentration of 2000 mg L^{-1}. In addition, the lowest dried cutting graft proportion was observed at this concentration of IBA. Solgi *et al*. (2022) investigated the propagation of black mulberry into white mulberry by stenting. They demonstrated that 62% of white mulberry rootstocks produced rooting at 1000 mg L^{-1} IBA. Whereas, no significant effects were observed using a combination of IBA and stenting as a new technique for propagating lilac in this study. Babaie *et al.* (2014) reported that *Ficus benjamina* produced 50% rooting by the omega stenting method without IBA. In contrast, in our study, just 4.5% of the cutting grafts formed roots via the omega grafting method without IBA consumption. Also, according to a stenting survey of black mulberry onto white mulberry by Solgi *et al.* (2022), 39% rooting percentage was observed, compared to 4.5% in this study. Furthermore, 6.66% of lilac leaves were produced in this research in relation to 0% leaf formation in black mulberry (Solgi *et al.*, 2022). Pourghorban *et al.* (2020) indicated that IBA concentration significantly affected root and shoot characteristics in stenting and that the effect is cultivar dependent in rose. The highest rooting and healing percentages, root length, fresh and dry weights of root, leaf number, shoot percentage, and shoot length were observed in "Samurai" cultivars treated with 4500 mg L^{-1} IBA.

In general, successful rooting during vegetative propagation via cutting grafting depends on diverse physiological conditions from which the cuttings originated, plant genotype, and environmental conditions. Some of the most severe operatives in cutting-grafts rooting are node condition, leaf number, cutting time, light intensity, temperature, humidity, cultivation bed type, and phytohormones (Izadi *et al.*, 2013; Park and Jeong, 2012; Solgi *et al.*, 2022; Hartman *et al.*, 2010).

On the other hand, prominent factors on grafting success can be divided into internal and external. External factors like grafting time, method, and environmental conditions such as temperature and relative humidity influence grafting success. Besides, internal compounds like phytohormones, phenols, and vegetative activity of scion and rootstock are the most influential factors in terms of internal factors influencing the final grafting achievement and success (Nowrozi *et al.*, 2016; Solgi *et al.*, 2012; Hartmann *et al.*, 1990). The suggested and applied stenting method is typically linked to plant species and genetic differences. Genetic differences among plant cultivars influence on internal grafting factors like tissues water content, soluble carbohydrates, starch, C/N ratio, phenolic compounds, and hormone content in scion tissues. These factors can cause differences in cultivar grafting success (Pinghai and Rongting, 1993; Stanisavljevic and Mitrovic, 1997). Also, choosing proper rootstocks is vital to improving future plant scion growth. Nazari *et al.* (2009) reported that propagation of *R. canina* via stenting produces smaller flower shoots than that achieved via cutting.

Furthermore, the stenting success rate ascended in the next five months because IBA was applied at higher levels. Rootstock rooting can be an influential factor in the success of cutting-grafting. It has been observed in some of the cutting grafts that there was grafting success; at the same time, roots were not developed in rootstocks, causing some grafted plants not to attain the compatibility circumstances at the next step and waste. It is suggested to use the optimum conditions like rooting hormones, appropriate beds, and appropriate environmental conditions to avoid this challenge (Izadi *et al.*, 2013).

The splice grafting method increased the proportion of rooted cutting grafts, grafting success, and growth ability of plants analogous to the omega technique. Next to the primary evaluation (after three months), the percentage of rooted cutting grafts was lower than that in the final assessment (after five months). The reason is the lack of adequate roots and weak rooting at earlier grafting times. Sometimes when the roots are insufficient and weak, grafting success and scion growth reduction occur due to the lack of water and nutrient absorption and transfer (Hartmann *et al.*, 2010; Nowrozi *et al.*, 2016).These findings prove that the grafting method may be a prerequisite to successful grafting (Izadi *et al*., 2013; Hartmann *et al.,* 1990). The lilac cuttings on wild privet rootstocks experienced low grafting success percentages (8% for omega without IBA application and 32% for 2000 mg L^{-1} IBA) compared to those of *Ficus benjamina* (75.83% for omega without IBA application and 87.61% for 2000 mg L^{-1} IBA) according to (Babaie *et al.,* 2014).

CONCLUSION

This study was conducted to find an alternative method of lilac propagation that would be faster, easier, and more justifiable economically. However, the evaluation of the elements indicated that stenting for the propagation of common lilac on wild privet is not commercially justifiable due to its low success rate and significantly weak growth factors. Based on these results, further studies on the effects of changing IBA concentrations, grafting time, and quality of scions and rootstocks are suggested for the improvement and success of stenting in *Syringa vulgaris*.

AUTHOR CONTRIBUTIONS

Mousa Solgi has received research grants from Arak University and rolled in planning, analyzing, calculating, comparing, and writing the article in collaboration with Mohammad Sajad Asheghi. Hossein Bagheri was in charge of plant cultivation and measurement.

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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In Vitro Propagation is an Optimal Method for the Production of Orchid *Phalaenopsis schilleriana* 'Karen Rockwell'

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Abstract

Phalaenopsis is an orchid genus of high economic value in world floriculture used as a pot plant and cut flowers. High genetic variation and the lack of uniformity in vegetative and reproductive propagation make the production of this orchid economically uninteresting. *In vitro* proliferation is the only large-scale feasible method for *Phalaenopsis* propagation. The purpose of the present study was to evaluate the effect of types and concentrations of α-naphthaleneacetic acid (NAA) and 6-benzyladenine (BA) (both at the concentrations of 0.0, 0.5, 1.0, 1.5 and 3.0 mg l^{-1}, individually or in combination), as a completely randomized design, on the *in vitro* propagation of *Phalaenopsis schilleriana* 'Karen Rockwell'. Activated charcoal (AC; 0.0, 0.5 and 1.0 mg l^{-1}) was added to the media for prevention of the browning of the media and tissues. Murashige and Skoog (MS), and protocorm were used as culture medium and explant, respectively. The results showed that the highest leaf number was obtained in medium enriched with 1.0 mg l^{-1} NAA together with 1.5 mg l^{-1} BA along with 1.0 mg l^{-1} AC. The treatment containing 1.5 mg l^{-1} NAA together with 0.5 mg l^{-1} BA along with 1.0 mg l^{-1} AC induced the highest number of roots. Fully *in vitro*-produced plantlets were transferred to pots containing a mixture of LECA (Light Expanded Clay Aggregate), peat moss, coco peat, charcoal soil, coco chips and perlite, and acclimatized in greenhouse conditions with 100% survival rate.

Keywords: Activated charcoal, Orchidaceae, Plant growth regulators, Protocorm-like bodies, Tissue culture.

INTRODUCTION

Orchids from family Orchidaceae are one of the most diverse flowering plant families with 800 genera, 25000 species, and thousands of hybrids from different regions of the world (Chugh *et al.,* 2009; Christenhusz and Byng, 2016). Orchids, including *Phalaenopsis*, cultivated as cut flowers and pot plants, are commercially important plants in world floriculture because of their medicinal and exotic values, such as variety in colors, sizes, shapes, and fragrances, as well as high durability of their flowers (Khoddamzadeh *et al.,* 2011; Park *et al.,* 2018; Cardoso *et al.,* 2020). *Phalaenopsis schilleriana* is a hybrid orchid. The genus *Phalaenopsis* (as epiphytic plants) comprises approximately 60 species native to tropical rainforests of South and South-East Asia, Australia and New Guinea (Winkelmann *et al.,* 2006).

The characteristics of seedlings propagated by vegetative means are not uniform, also propagation by seeds results in high genetic variability and the production of heterozygous plants; therefore, lots of tissue culture protocols have been developed in this genus (Murthy *et al.,* 2018; Asa and Kaviani, 2020). Natural clonal propagation of orchids is a slow process, which results in traits segregation and is, therefore, not possible for *Phalaenopsis*. Although, the micropropagation of genus *Phalaenopsis* has been demonstrated good development, the wide spread application of micropropagation is still limited due to some problems such as contamination, the exudation of phenolic compounds and somaclonal variation (Zahara, 2017). *In vitro* culture of *Phalaenopsis* could be considered reliable for guaranteeing the uniformity of flowers (Lee *et al.,* 2013; Zanello *et al.,* 2022). *In vitro* propagation is an extremely important and useful technique for clonal propagation of many species, particularly ornamental plants like orchids (Guo *et al.,* 2024).

Different procedures have been established for *in vitro* proliferation of orchids species, including *Phalaenopsis*, by various explants such as seeds, node, shoot tips, floral stalks, protocorm, protocorm-like bodies (PLBs), leaf, root, inflorescence, tuber, and rhizome, as well as somatic embryos, callus, thin cell layer, and plantlets obtained from seed (Roy *et al.,* 2011; Panwar *et al.,* 2012; Baker *et al.,* 2014; Mahendran, 2014; Chen *et al.,* 2015; Bhattacharyya *et al.,* 2016; Kaviani *et al.,* 2017; Yam and Arditti, 2018; Zakizadeh *et al.,* 2019; Mohammadi *et al.,* 2019; Asa and Kaviani, 2020). Many studies have shown that the optimization of medium composition was an important approach to improve the micropropagation process of orchids by culturing PLBs that is species-specific (Shimura and Koda, 2004; Luo *et al.,* 2009; Guo *et al.,* 2024). Protocorms and PLBs are tuberous embryonic masses of cells that are developed from seeds and vegetative tissues, respectively; they can grow into new plantlets and be applied in commercial micropropagation (Cui *et al.,* 2014; Lo *et al.,* 2022). PLBs derived from different types of somatic tissues represent a clonal method applied to produce large numbers of plantlets from few mother plants and explants (Zanello *et al.,* 2022). PLBs are similar to protocorms in morphology and biological characteristics (Lee *et al.,* 2013; Cardoso *et al.,* 2020). The main difference between protocorms and PLBs is basically the origin of the tissue. Induction of PLBs facilitate the micropropagation of orchids (Chen *et al.,* 2019). In orchids, the formation of protocorms and PLBs is regulated by various factors, and plant growth regulators (PGRs) are among the most important ones (Cardoso *et al.,* 2020).

Cytokinins are the most important factors to improve the plant regeneration from PLBs (Luo *et al.,* 2009). α-naphthaleneacetic acid (NAA) and 6-benzyladenine (BA) are widely applied for the regeneration of shoots from protocorms or PLBs in many *Phalaenopsis* species or hybrids (Park *et al.,* 2002; Paek *et al.,* 2011; Bali Lashaki *et al.,* 2014; Zanello *et al.,* 2022). Another PGRs such as indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), N-phenyl-N′-1,2,3-thiadiazol-5-yl-urea (TDZ), and 6-furfurylaminopurine or kinetin (Kin)

have been used for tissue culture of some orchids like *Cymbidium*, *Phalaenopsis*, *Dendrobium*, and *Paphiopedilum* and others (Roy *et al.,* 2011; Panwar *et al.,* 2012; Zeng *et al.,* 2012; Baker *et al.,* 2014; Bhattacharyya *et al.,* 2016; Kaviani *et al.,* 2017; Zakizadeh *et al.,* 2019; Mohammadi *et al.,* 2019; Asa and Kaviani, 2020; Cardoso *et al.,* 2020; Guo *et al.,* 2024).

Internal factors (such as genotype) as well as external factors (such as culture media, PGRs, and growing conditions) play a crucial role in enhancing the multiplication rate to improve the efficiency of micropropagation and plantlet production in *Phalaenopsis* (Khatun *et al.,* 2020; Zanello *et al.,* 2022). Therefore, the purpose of the present study was to evaluate the effect of different concentrations of NAA and BA, individually and in combination, on *in vitro* propagation of *Phalaenopsis schilleriana* 'Karen Rockwell' *via* organogenesis using protocorm explant.

MATERIALS AND METHODS

Plant material

Experiments were carried out on orchid *Phalaenopsis schilleriana* 'Karen Rockwell' (Fig. 1A) in June 2020 in tissue culture laboratory and greenhouse of the Hyrcan Agricultural Sciences and Biotechnology Research Institute, Amol, Mazandaran, Iran. The geographical coordinates of Amol are as follows: Latitude: 36°28′10″ N, longitude: 52°21′02″ E, and elevation above sea level: 96 m (314 ft). Healthy and sterilized PLBs prepared from a plant tissue culture in Austria was used as explant. *P. schilleriana* as Moth Orchid and the most popular species in this genus has relatively oblong leaves and the pink flowers.

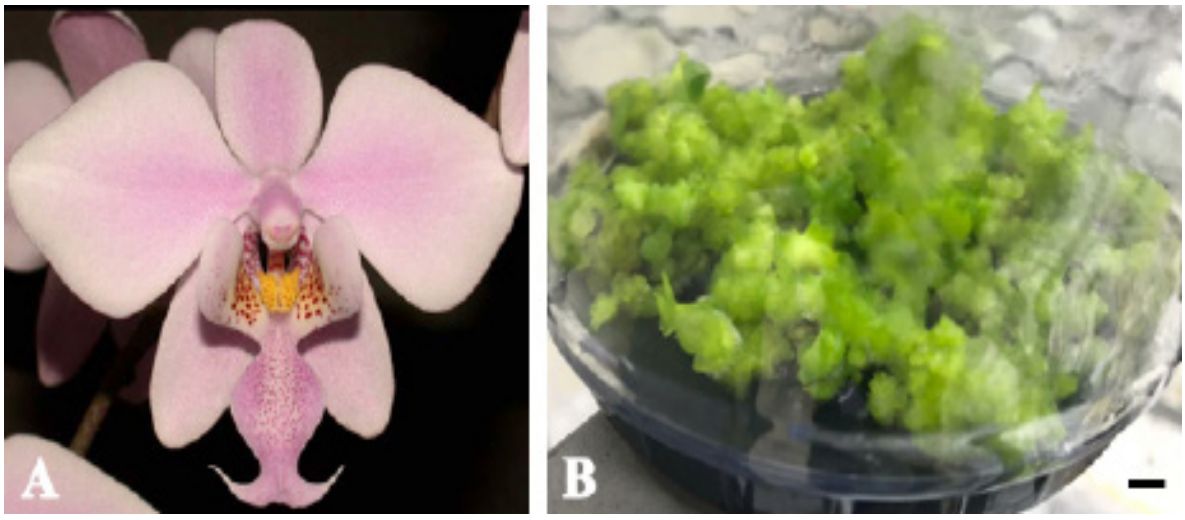

Fig. 1. Effect of NAA and BA on PLBs mass proliferation of *Phalaenopsis schilleriana*. A) In flowering stage; B) Protocorms produced from *in vitro* culture of seeds (scale bar = 10 mm).

Culture media and treatments

The explants (seed-originated protocorms) (Fig. 1B) were cultured on MS (Murashige and Skoog, 1962) medium containing 3% sucrose and 0.8% agar. The pH of the media was adjusted to 5.6–5.8 with 0.1 N NaOH or HCl prior to autoclaving. All media contained in culture bottles were autoclaved at 105 kPa and 121°C for 20 min. The media were enriched with different concentrations of BA and NAA both at the concentrations of 0.0, 0.5, 1.0, 1.5 and 3.0 mg l^{-1}, individually or in combination. Explants secrete phenolic compounds into the media; therefore, activated charcoal (AC; 0.0, 0.5 and 1.0 mg l^{-1}) was added to the media for prevention of the browning of the media. AC absorbs phenolic compound. All the cultures were

incubated at 24 ± 2 °C, 70−80% RH, and 16-h photoperiod of 50–60 µmol m^{-2} s^{-1} irradiance provided by cool−white fluorescent tubes.

Measured traits

Observations on leaf length, leaf width, leaf number, root length, root number, callus number and viability percentage were recorded 60 days after the culture initiation. Leaf length and width, as well as root length were measured with a ruler. The number of organs was counted with the naked eye.

Plantlets acclimatization

For *ex vitro* establishment, *in vitro*-rooted plantlets were taken out from culture vessels and washed with sterile distilled water to remove adherent medium from the plantlet body and transferred to plastic pots (18 cm height × 12 cm diameter) filled with a potting mixture of LECA (Light Expanded Clay Aggregate), peat moss, coco peat, charcoal soil, coco chips and perlite in the proportion of 15:10:20:5:30:20%. All the pots were then transferred to a greenhouse with temperature of 24 ± 2 ºC to 20 ± 2 ºC day/night (light intensity of 3,500 lux, RH of 80−90% and a 14h/10h day/night photoperiod) for acclimatization. The pots were covered with another plastic pots (18 cm height × 12 cm diameter) to retain moisture. Plantlets were exposed gradually to external environment. Thus, these pots were removed after two weeks. The plantlets were watered every five days. The number of surviving plants was recorded after two months of transfer.

Experimental design and data analysis

The experiments were established in a completely randomized design. For each treatment, three replicates and for each replicate, three specimens (explants) were taken (in total 75 treatments, 225 replicates, and 675 explants). PGR-free MS medium was used as control in the experiments. Data were subjected to analysis of variance (ANOVA) and means were compared by the LSD test at $P < 0.05$ using the SPSS ver. 17 (SPSS Inc., USA).

RESULTS

The results of Kolmogorov-Smirnov's test showed that the significance level was greater than 0.05 in all the traits measured in relation to shoot proliferation and there was no significant difference between the data and the data had a normal distribution (data not shown). Also, the results of Levene's homogeneity test showed that in all measured traits related to shoot proliferation, the significance level was greater than 0.05 and there was no significant difference between the data. Therefore, the data had a homogeneous distribution (data not shown). Analysis of variance (ANOVA) showed significant differences among different concentrations of NAA and BA for leaf length, root length, root number and viability percentage (all at $P<0.05$), as well as leaf number, leaf width and callus number (all at $P<0.01$) (data not shown).

Multiplication or shoot proliferation

Based on tables 1-3, simulatanious presence of NAA and BA is more suitable than the presence of each one of them for shoot proliferation. The highest leaf length (4.3 cm per explant) was achieved on medium enriched with 1.5 mg l^{-1} NAA together with 1.5 mg l^{-1} BA along with 1.0 mg l^{-1} AC. The content of 3.0 mg l^{-1} NAA together with 3.0 mg l^{-1} BA along with 1.0 mg l^{-1} AC in medium was suitable for inducing the leaf length. The lowest leaf length (1.13 cm per explant) was obtained on medium without PGRs (control). The highest mean values

of leaf width was 2.66 cm per explant, followed by 2.53 cm per explant, observed on media supplemented with 1.0 mg l^{-1} NAA together with 1.5 mg l^{-1} BA without AC, and 1.5 mg l^{-1} NAA together with 1.0 mg l^{-1} BA along with 1.0 mg l^{-1} AC, respectively. The lowest leaf length (0.4 cm per explant) was observed on medium without PGRs. Our results demonstrated that leaf number on medium enriched with 1.0 mg l^{-1} NAA in combination with 1.5 mg l^{-1} BA was significantly different compared to other treatments, as they produced the maximum number of leaf per explant (5.76 with 1.0 mg l^{-1} AC and 5.4 with 0.5 mg l^{-1} AC, respectively) (Tables 1-3, Fig. 2A, B). The medium without BA and AC, but containing 0.5 mg l^{-1} NAA induced the minimum number of leaf per explant (1.66). Present research showed that the 10 treatments containing different concentrations of NAA and BA induced the production of leaf more than 4 per explant.

Table 1. Mean comparison of the effect of different concentrations of NAA and BA without activated chaircol on the measured characteristics of *Phalaenopsis schilleriana* 'Karen Rockwell'.

NAA × BA (mg l^{-1})	Leaf length (cm)	Leaf width (cm)	Leaf number	Root length (cm)	Root number	Callus number	Viability (%)
0.0 × 0.0	1.33^{h}	0.76^{fg}	2.26^{d-g}	2.50^{i}	3.63^{bc}	4.36^{cde}	70.0^{d}
0.0 × 0.5	1.63^{e-h}	0.53^{g}	3.36^{bcd}	2.70^{i}	3.93^{abc}	4.63^{cde}	83.0^{bcd}
0.0 × 1.0	1.73^{d-h}	0.80^{efg}	2.53^{c-g}	3.13^{f-i}	3.60^{bc}	4.70^{cde}	73.0^{cd}
0.0 × 1.5	2.46^{b-e}	1.16^{d-g}	1.8^{fg}	4.33^{b-f}	4.46^{abc}	4.13^{cde}	83.0^{bcd}
0.0 × 3.0	2.00^{c-h}	1.60^{cde}	2.43^{d-g}	2.90^{hi}	3.36^{c}	7.56^{ab}	96.0^{ab}
0.5 × 0.0	1.43^{f-h}	1.26^{c-g}	1.66^{g}	3.80^{b-i}	4.40^{abc}	3.23^{cde}	83.0^{bcd}
0.5 × 0.5	1.90^{c-h}	1.16^{d-g}	2.8^{c-g}	3.56^{c-i}	4.16^{abc}	2.76^{e}	90.0^{ab}
0.5 × 1.0	2.03^{c-h}	2.03^{abc}	2.7^{c-g}	4.73^{bcd}	4.76^{abc}	5.20^{cd}	100.0^{a}
0.5 × 1.5	1.86^{c-h}	2.46^{ab}	3.7^{abc}	4.03^{b-h}	4.13^{abc}	3.36^{cde}	93.0^{ab}
0.5 × 3.0	1.40^{gh}	0.86^{d-g}	2.6^{c-g}	3.80^{b-i}	3.66^{bc}	4.20^{cde}	100.0^{a}
1.0 × 0.0	2.00^{c-h}	0.76^{fg}	2.16^{efg}	4.90^{b}	4.56^{abc}	3.16^{cde}	100.0^{a}
1.0 × 0.5	2.03^{c-h}	1.40^{c-f}	3.36^{bcd}	3.80^{b-i}	5.00^{ab}	8.70^{a}	90.0^{ab}
1.0 × 1.0	2.70^{a-d}	1.63^{cd}	3.33^{b-e}	6.20^{a}	5.00^{ab}	4.36^{cde}	90.0^{b}
1.0 × 1.5	2.36^{b-g}	2.66^{a}	4.7^{a}	4.70^{bcd}	5.23^{a}	3.00^{de}	96.0^{ab}
1.0 × 3.0	3.26^{ab}	1.56^{c-f}	3.33^{b-e}	4.56^{bcde}	4.50^{abc}	4.20^{cde}	86.0^{abc}
1.5 × 0.0	1.76^{d-h}	0.93^{d-g}	2.43^{d-g}	4.83^{bc}	4.50^{abc}	4.33^{cde}	86.0^{abc}
1.5 × 0.5	1.93^{c-h}	1.30^{c-g}	3.06^{b-e}	4.00^{b-h}	4.80^{abc}	3.53^{cde}	96.0^{ab}
1.5 × 1.0	2.50^{a-e}	1.66^{bcd}	3.26^{b-e}	4.80^{bc}	4.10^{abc}	4.46^{cde}	96.0^{ab}
1.5 × 1.5	3.23^{ab}	0.93^{d-g}	4.2^{ab}	4.26^{b-g}	4.16^{abc}	3.00^{de}	96.0^{ab}
1.5 × 3.0	1.80^{c-h}	0.90^{d-g}	2.8^{c-g}	3.00^{ghi}	4.66^{abc}	4.33^{cde}	86.0^{abc}
3.0 × 0.0	1.86^{c-h}	1.16^{d-g}	2.46^{d-g}	3.33^{e-i}	4.16^{abc}	4.03^{cde}	96.0^{ab}
3.0 × 0.5	1.73^{d-h}	1.26^{c-g}	2.73^{c-g}	3.80^{b-i}	4.23^{abc}	5.33^{bc}	86.0^{abc}
3.0 × 1.0	2.43^{b-f}	1.56^{c-f}	2.63^{c-g}	3.46^{d-i}	3.93^{abc}	4.53^{cde}	90.0^{ab}
3.0 × 1.5	2.80^{abc}	1.23^{c-g}	4.16^{ab}	3.46^{d-i}	4.30^{abc}	3.63^{cde}	93.0^{ab}
3.0 × 3.0	3.50^{a}	1.00^{d-g}	2.86^{c-e}	3.66^{b-i}	4.16^{abc}	3.80^{cde}	86.0^{abc}

Means with different letters on the same column are significantly different ($P<0.05$) based on LSD test.

Table 2. Mean comparison of the effect of different concentrations of NAA and BA along with 0.5 mg l^{-1} activated chaircol on the measured characteristics of *Phalaenopsis schilleriana* 'Karen Rockwell'.

NAA × BA (mg l^{-1})	Leaf length (cm)	Leaf width (cm)	Leaf number	Root length (cm)	Root number	Callus number	Viability (%)
0.0 × 0.0	1.13^{g}	0.40^{f}	3.00^{cd}	2.8^{g}	3.46^{gh}	2.60^{g}	86.0^{abc}
0.0 × 0.5	1.36^{efg}	1.33^{b-e}	2.26^{d}	2.93^{efg}	2.83^{h}	5.20^{bcd}	83.0^{bc}
0.0 × 1.0	2.20^{b-e}	0.90^{c-f}	3.23^{bcd}	2.76^{g}	3.70^{fgh}	3.03^{fg}	90.0^{abc}
0.0 × 1.5	2.36^{a-d}	1.20^{b-e}	2.50^{d}	2.83^{fg}	4.73^{b-g}	2.83^{g}	100.0^{a}
0.0 × 3.0	1.93^{b-g}	0.93^{c-f}	2.50^{d}	2.93^{efg}	4.40^{b-g}	3.33^{d-g}	86.0^{abc}
0.5 × 0.0	1.93^{b-g}	0.73^{ef}	2.80^{d}	4.66^{a-d}	5.40^{a-e}	2.86^{fg}	96.0^{ab}
0.5 × 0.5	1.70^{d-g}	1.20^{b-e}	2.26^{d}	3.36^{d-g}	5.76^{abc}	5.10^{bcd}	93.0^{abc}
0.5 × 1.0	2.03^{b-g}	1.66^{abc}	2.46^{d}	3.83^{c-g}	5.43^{a-e}	6.16^{bc}	93.0^{abc}
0.5 × 1.5	1.26^{fg}	2.26^{a}	4.73^{a}	3.46^{c-g}	5.56^{a-d}	3.00^{fg}	96.0^{ab}
0.5 × 3.0	2.13^{b-f}	1.16^{b-f}	2.93^{d}	4.30^{a-e}	4.50^{b-g}	3.13^{efg}	96.0^{ab}
1.0 × 0.0	2.43^{a-d}	0.80^{def}	2.56^{d}	5.56^{ab}	3.33^{gh}	3.50^{d-g}	80.0^{c}
1.0 × 0.5	2.23^{b-e}	1.53^{a-d}	2.86^{d}	4.23^{b-f}	5.70^{abc}	4.96^{cde}	96.0^{ab}
1.0 × 1.0	1.83^{c-g}	1.86^{ab}	3.40^{bcd}	5.66^{a}	6.66^{a}	5.03^{b-e}	90.0^{abc}
1.0 × 1.5	1.76^{c-g}	1.43^{b-e}	5.40^{a}	4.86^{abc}	5.16^{a-f}	8.16^{a}	96.0^{ab}
1.0 × 3.0	2.83^{ab}	1.33^{b-e}	3.43^{bcd}	3.6^{c-g}	4.10^{d-h}	2.76^{g}	90.0^{abc}
1.5 × 0.0	1.70^{d-g}	0.96^{c-f}	2.70^{d}	4.00^{c-g}	5.00^{b-f}	6.90^{ab}	90.0^{abc}
1.5 × 0.5	2.30^{a-d}	1.23^{b-e}	4.23^{abc}	3.40^{d-g}	5.83^{ab}	3.93^{d-g}	96.0^{ab}
1.5 × 1.0	2.33^{a-d}	1.50^{a-e}	2.46^{d}	4.13^{c-g}	4.53^{b-g}	4.76^{c-f}	93.0^{abc}
1.5 × 1.5	3.16^{a}	1.06^{c-f}	4.36^{ab}	3.60^{c-g}	4.26^{c-h}	4.36^{c-g}	83.0^{bc}
1.5 × 3.0	2.53^{a-d}	1.30^{b-e}	3.13^{bcd}	4.16^{b-g}	3.73^{fgh}	4.50^{c-g}	100.0^{a}
3.0 × 0.0	2.63^{abc}	1.16^{b-f}	2.46^{d}	3.26^{d-g}	4.33^{b-h}	2.80^{g}	90.0^{abc}
3.0 × 0.5	1.96^{b-g}	1.26^{b-e}	2.96^{cd}	4.16^{b-g}	4.13^{d-h}	5.20^{bcd}	80.0^{c}
3.0 × 1.0	2.53^{a-d}	1.53^{a-d}	3.00^{cd}	4.13^{c-g}	4.03^{d-h}	4.23^{d-g}	90.0^{abc}
3.0 × 1.5	2.50^{a-d}	1.06^{c-f}	4.33^{ab}	3.86^{c-g}	3.73^{fgh}	3.53^{d-g}	83.0^{bc}
3.0 × 3.0	2.33^{a-d}	1.23^{b-e}	3.36^{bcd}	3.93^{c-g}	3.80^{fgh}	3.53^{d-g}	96.0^{ab}

Means with different letters on the same column are significantly different (P<0.05) based on LSD test.

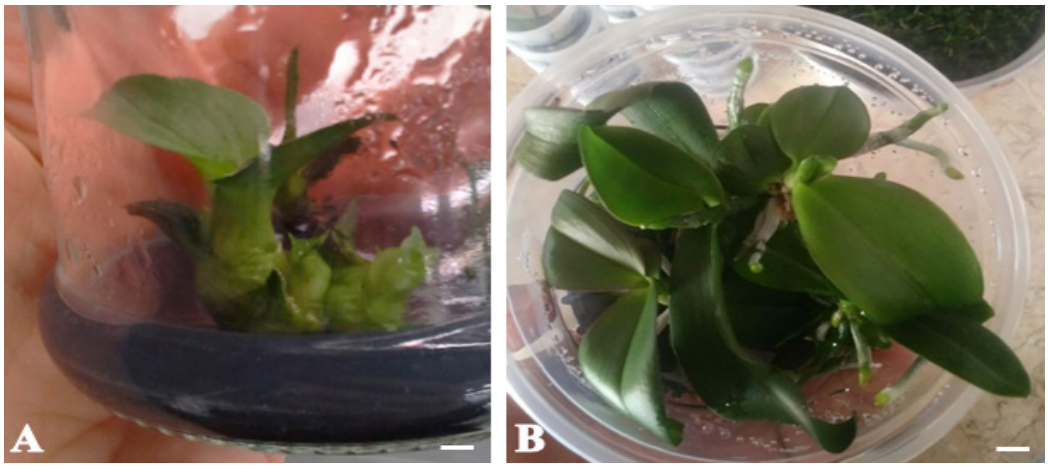

Fig. 2. Effect of NAA and BA along with activated chaicol on PLBs growth of *Phalaenopsis schilleriana* 'Karen Rockwell'. A) On medium enriched with 1.0 mg l^{-1} NAA together with 1.5 mg l^{-1} BA along with 0.5 mg l^{-1} AC; B) On medium enriched with 1.0 mg l^{-1} NAA together with 1.5 mg l^{-1} BA along with 1.0 mg l^{-1} AC (scale bar = 5 mm).

Table 3. Mean comparison of the effect of different concentrations of NAA and BA along with 1.0 mg l^{-1} activated chaircol on the measured characteristics of *Phalaenopsis schilleriana* 'Karen Rockwell'.

NAA × BA (mg l^{-1})	Leaf length (cm)	Leaf width (cm)	Leaf number	Root length (cm)	Root number	Callus number	Viability (%)
0.0 × 0.0	1.9^{de}	1.01^{d}	2.90^{e-h}	3.06^{ef}	4.10^{c-f}	5.40^{abc}	90.0^{a-d}
0.0 × 0.5	2.16^{cde}	1.20^{d}	2.70^{e-h}	2.80^{f}	3.50^{f}	4.16^{bc}	73.0^{e}
0.0 × 1.0	2.33^{cde}	1.80^{a-d}	3.00^{d-h}	3.76^{c-f}	4.43^{b-f}	5.26^{abc}	76.0^{de}
0.0 × 1.5	1.93^{de}	1.50^{bcd}	3.03^{d-h}	3.33^{def}	4.50^{b-f}	4.03^{bc}	76.0^{de}
0.0 × 3.0	1.80^{de}	1.90^{a-d}	2.66^{e-h}	4.13^{b-f}	3.93^{def}	3.46^{c}	90.0^{a-d}
0.5 × 0.0	1.90^{de}	1.10^{d}	2.60^{e-h}	3.70^{c-f}	4.96^{b-e}	3.43^{c}	96.0^{ab}
0.5 × 0.5	2.76^{cd}	1.60^{a-d}	2.43^{gh}	4.13^{b-f}	4.93^{b-f}	6.50^{ab}	90.0^{a-d}
0.5 × 1.0	1.83^{de}	1.70^{a-d}	2.86^{e-h}	5.73^{ab}	5.46^{abc}	5.40^{abc}	83.0^{b-e}
0.5 × 1.5	3.06^{bc}	2.23^{ab}	4.63^{ab}	4.73^{a-d}	5.36^{a-d}	4.80^{abc}	93.0^{abc}
0.5 × 3.0	2.66^{cd}	1.66^{a-d}	2.50^{fgh}	4.10^{b-f}	4.33^{b-f}	3.86^{bc}	96.0^{ab}
1.0 × 0.0	2.50^{cde}	1.03^{d}	1.86^{h}	4.30^{b-f}	5.70^{ab}	3.40^{c}	80.0^{cde}
1.0 × 0.5	2.80^{cd}	1.50^{bcd}	3.40^{b-g}	3.66^{c-f}	4.00^{def}	6.50^{ab}	100.0^{a}
1.0 × 1.0	2.20^{cde}	2.16^{abc}	2.83^{e-h}	6.26^{a}	5.70^{ab}	4.63^{bc}	86.0^{a-e}
1.0 × 1.5	2.33^{cde}	2.46^{a}	5.76^{a}	5.00^{abc}	4.03^{cdef}	3.96^{bc}	83.0^{b-e}
1.0 × 3.0	3.03^{bc}	1.60^{a-d}	3.80^{b-f}	4.80^{a-d}	4.46^{b-f}	3.40^{c}	90.0^{a-d}
1.5 × 0.0	1.80^{de}	1.40^{bcd}	2.60^{e-h}	4.26^{b-f}	4.96^{b-e}	5.76^{abc}	83.0^{b-e}
1.5 × 0.5	2.30^{cde}	1.16^{d}	4.23^{bcd}	4.00^{c-f}	6.73^{a}	4.86^{abc}	93.0^{abc}
1.5 × 1.0	2.40^{cde}	2.53^{a}	3.33^{b-g}	3.46^{c-f}	3.70^{ef}	4.06^{bc}	96.0^{ab}
1.5 × 1.5	4.30^{a}	1.03^{d}	4.53^{abc}	3.96^{c-f}	4.36^{b-f}	3.63^{c}	96.0^{ab}
1.5 × 3.0	2.50^{cde}	1.36^{bcd}	3.26^{c-g}	4.00^{c-f}	4.10^{c-f}	4.70^{abc}	96.0^{ab}
3.0 × 0.0	2.6^{cde}	1.66^{a-d}	2.66^{e-h}	3.56^{c-f}	4.6^{b-f}	3.73^{c}	80.0^{cde}
3.0 × 0.5	2.13^{cde}	1.26^{cd}	2.93^{d-h}	4.66^{a-e}	4.46^{b-f}	7.33^{a}	93.0^{abc}
3.0 × 1.0	1.56^{e}	1.66^{a-d}	3.46^{b-g}	4.46^{b-e}	4.56^{b-f}	4.50^{bc}	90.0^{a-d}
3.0 × 1.5	2.46^{cde}	1.46^{bcd}	3.90^{b-e}	3.90^{c-f}	4.36^{b-f}	4.16^{bc}	83.0^{b-e}
3.0 × 3.0	4.06^{ab}	1.30^{bcd}	3.40^{b-g}	3.40^{c-f}	4.43^{b-f}	3.40^{c}	80.0^{cde}

Means with different letters on the same column are significantly different (P<0.05) based on LSD test.

Root induction and growth

Based on tables 1-3, simulatanious presence of NAA and BA is more suitable than the presence of each one of them for root induction and growth. We found that a combination of 1.00 mg l^{-1} of both NAA BA, with and without AC, provoked the highest length of root (6.26, 6.2 and 5.66 cm per explant, respectively). The lowed root length (2.5 cm per explant) was achieved on medium without NAA, BA and AC. The media containing 0.5, 1.0 and 1.5 mg l^{-1}

NAA combined with 0.5 and 1.0 mg l^{-1} BA were effective for root production. The medium fortified with 1.5 mg l^{-1} NAA together with 0.5 mg l^{-1} BA along with 1.0 mg l^{-1} AC resulted in the highest increase in root number (6.73) (Fig. 3A). High root number was also observed using 1.0 mg l^{-1} NAA together with 1.0 mg l^{-1} BA along with 0.5 mg l^{-1} AC. The difference between these two media was not significant. The highest reduction in root production was found in medium containing 0.5 mg l^{-1} BA without NAA.

Fig. 3. Rooting and transplanting of *Phalaenopsis schilleriana* 'Karen Rockwell'. A) Rooting plantlet on medium enriched with 1.5 mg l^{-1} NAA together with 0.5 mg l^{-1} BA along with 1.0 mg l^{-1} AC; B) Plantlets transplanted to trays filled out with a mixture of LECA (Light Expanded Clay Aggregate), peat moss, coco peat, charcoal soil, coco chips and perlite (A: scale bar = 5 mm; B: scale bar = 20 mm).

Callus induction and plantlets viability

The treatments containing 1.0 mg l^{-1} NAA together with 0.5 and 1.5 mg l^{-1} caused an increase in the number of callus per explant (more than 8), compared with the control (2.6). Explants cultured on medium supplemented with 3.0 mg l^{-1} NAA together with 0.5 mg l^{-1} BA along with 1.0 mg l^{-1} AC showed high callus number. Plantlets obtained from six treatments resulted in the 100% viability. Plantlets produced in medium without NAA, BA and AC showed least viability (70%) (Tables 1-3).

***Ex vitro* establishment of plantlets**

Well-developed plantlets were transferred to plastic pots for *ex vitro* establishment and acclimatization (Fig. 3B). A 100% establishment rate was obtained and plantlets were morphologically identical to the mother plants.

DISCUSSION

Our findings demonstrated that the spontaneous use of an auxin and a cytokinin is important in the shoot and root production in *Phalaenopsis schilleriana* 'Karen Rockwell'. The PGR-free culture medium resulted in low survival of plantlets and low shoot multiplication and root induction rates. Reports of many researchers showed better results when they were used an auxin and a cytokinin, in combination (Panwar *et al.,* 2012; Zakizadeh *et al.,* 2019; Mohammadi *et al.,* 2019; Asa and Kaviani, 2020). Bhattacharyya *et al.* (2016) revealed that when the explants were grown in medium containing cytokinin and auxin, a higher rate of response frequency of shoot buds and PLBs was observed in all PGRs combinations. A combination of 1.0 mg l^{-1} KIN and 1.0 mg l^{-1} IBA was found to be suitable for regeneration of most measured characteristics especially leaf and root number in *Phalaenopsis amabilis* (L.) Blume var. Jawa. Also, the

maximum number of plantlet was obtained on medium supplemented with 1.0 mg l^{-1} KIN and 0.5 mg l^{-1} IBA (Asa and Kaviani, 2020). In our study, the percentage of the explant response to leaf, root and callus formation was generally enhanced through the use of 0.5-1.5 mg l^{-1} of both NAA and BA. BA treatment was significantly better for shoot induction compared with NAA. On the other hand, NAA treatment was significantly better for root induction compared with BA. Although, the differences between NAA and BA were insignificant. Contrary to our finding, BA individually was better than in combination with NAA for shoot production of orchid *Oncidium* (Kalimuthu *et al.,* 2007). BA is known to promote seedling leaf formation in some *Paphiopedilum* species (Chen *et al.,* 2015). BA is the cytokinin most commonly used in plant tissue culture, and it is also efficient in promoting shoot development (Zanello *et al.,* 2022). The ratio of auxin and cytokinin for PLB, shoot and root formation depends upon the species studied. Similar findings were reported on *Phalaenopsis* and other orchid's species (Baker *et al.,* 2014; Zakizadeh *et al.,* 2019; Lo *et al.,* 2022; Zargar *et al.,* 2023; Kiaheirati *et al.,* 2024). Study on orchid *Dendrobium nobile* demonstrated that when explants were cultured in medium enriched with BAP solely, PLBs was formed but direct shoot formation was not observed (Bhattacharyya *et al.,* 2016). The presence of cytokinins alone promoted optimal shoot proliferation from protocorm explants in some orchids like *Dendrobium nobile* and *C. aloifolium* (Nayak *et al.,* 1997b), *C. ensifolium* (Chang and Chang, 1998), *Rhynchostylis gigantea* (van Le *et al.,* 1999), *D. nobile* and *C. aloifolium* (Nayak *et al.,* 2002), and *Dendrobium* (Ferreira *et al.,* 2006). The effect of NAA and BA at different concentrations on the induction of PLBs, leaf, root and callus was assessed on *Phalaenopsis* (Bali Lashaki *et al.,* 2014).

The most commonly used auxins in orchid culture media are indole-3-acetic acid (IAA), NAA, IBA, and 2,4-D. On the other hand, Kin, BA, BAP, TDZ, and zeatin (Zt) are the most commonly used cytokinins in orchid culture media (Yam and Arditi, 2018). Some orchid tissue culture studies found that auxins alone or in combination with cytokinins increased overall shoot growth (Parvathy, 2022). Some researchers have recognized that the effect of a single PGR alone on shoot multiplication is better than the effect of that in combination with another PGRs in orchids (Martin and Madassery, 2006; Zhao *et al.,* 2007; Mahendran and Narmatha Bai, 2009; Luo *et al.,* 2009; Panwar *et al.,* 2012; Parthibhan *et al.,* 2015). These findings are in contrast with our findings, because maximum shoot number was produced in media fortified with NAA in combination with BA. Study of Hossain *et al.* (2010) on *Cymbidium giganteum* and Bali Lashaki *et al.* (2014) on *Phalaenopsis amabilis* var. 'Manila' revealed that multiple shoot formation were induced on medium supplemented with different concentrations and combinations of BAP together with NAA, and BA together with NAA, respectively.

The present study showed that BA in combination with NAA induced better rooting. Some reports showed that cytokinins would be associated with a subsequent inhibition of *in vitro* rooting (Podwyszynska, 2003). However, many researches has shown that BA-derived shoots resulted in better rooting compared with shoots derived from BA-free culture medium (Iiyama and Cardoso, 2021; Zanello *et al.,* 2022). Our study also showed that NAA was suitable for root length and number. Similar to our finding, in *Vanda coerulea* Griff ex. Lindl. (Blue Vanda), NAA was found to be the most effective for production of maximum numbers of PLBs, shoots and roots which simultaneously differentiated in the same medium (Roy *et al.,* 2011). NAA was found more effective than IBA for micropropagation of *Orchis catasetum* (Baker *et al.,* 2014). Kiaheirati *et al.* (2024) demonstrated that the longest roots in *Phalaenopsis circus* were induced using both NAA and Kin in combination. The best root induction in *Phalaenopsis amabilis* cv. Cool 'Breeze' was achieved with 1.0 mg l^{-1} IAA (Bali Lashaki *et al.,* 2014). Baker *et al.* (2014) showed that a combination of 0.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA induced the

largest number of root and the highest length of root in orchid *Catasetum*. These researchers also showed that a combination of 1.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA was a suitable treatment for induction of root number and root length.

Callus was produced at the base of shoots of *Eulophia nuda* cultured on medium supplemented with higher concentration of BA, while lesser number of shoots were produced on medium with lower BA concentration (Panwar *et al.,* 2012). In the present study, induction of callus was occured in some treatments. This is in contradict with the findings for *Paphiopedilum* spp. (Guo *et al.,* 2024; Kiaheirati *et al.,* 2024). Similarity, calluses have been successfully induced from the seeds or protocorms of some species of *Paphiopedilum* (Zeng *et al.,* 2013; Guo *et al.,* 2024).

The better development and proliferation of shoots and induction of roots in culture media containing AC may be related to the effect of AC as an anti-browning agent. Browning is one of the major problems affecting *in vitro* cultivation of *Phalaenopsis* and some other orchids. This problem is frequently associated with high content of phenolics and increases in polyphenol oxidase activity (Xu and Li, 2006; Zanello *et al.,* 2022). The high rate of explants browning, and its association with phenolic oxidation, has been previously reported in the genus *Phalaenopsis* and is caused by physical damage to tissues, with phenolic oxidation being toxic to plant tissues and in some cases leading to plant death (Minamiguchi and Machado Neto, 2007; Zanello *et al.,* 2022).

The *in vitro* rooted plantlets were successfully acclimatized in the greenhouse through their cultivation in pots containing a mixture of LECA (Light Expanded Clay Aggregate), peat moss, coco peat, charcoal soil, coco chips and perlite in the proportion of 15:10:20:5:30:20%. Similar results were reported on *Phalaenopsis circus* (Kiaheirati *et al.,* 2024). Coconut powder, sphagnum, and vermiculite, also cocochips and sphagnum moss were also applied for acclimatization of *Phalaenopsis* plantlets (Venturieri and Arbieto, 2011; Asa and Kaviani, 2020; Zanello *et al.,* 2022). The successful use of clay, sand, vermicompost (1:1:1), moss and charcoal (1:1), charcoal and brick pieces (1:1), sand, vermiculite and chopped dry leaves (1:1:1), moss, peat and perlite (3:1:1); vermiculite, bark, soil (1:2:2), sphagnum and coconut fibres (1:1), and peat and perlite (1:1) was reported in some other orchid species (Panwar *et al.,* 2012; Teixeira da Silva *et al.,* 2017; Lo *et al.,* 2022; Zargar *et al.,* 2023). The highest water-holding capacity was pointed out as the main difference between various substrates (Venturieri and Arbieto, 2011).

CONCLUSION

In conclusion, our investigation into the tissue culture of *Phalaenopsis schilleriana* 'Karen Rockwell' under varying exogenous NAA and BA treatments has presented an efficient and reliable procedure. The treatments containing 1.0 mg l^{-1} NAA together with 1.5 mg l^{-1} BA, and 1.5 mg l^{-1} NAA together with 0.5 mg l^{-1} BA, both along with 1.0 mg l^{-1} AC induced the highest number of leaves and roots, respectively. These findings have significant implications for optimizing *P. schilleriana* 'Karen Rockwell' micropropagation protocols.

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Evaluation of the Foliar Spraying Effects of Chitosan Nanoparticles and Salicylic Acid on the Petal Senescence and Postharvest Quality of Cut Roses cv. "Samuraie"

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Abstract

Extending the flower vase life is very important in the floriculture industry. In present study, the effectiveness of chitosan and salicylic acid nanoparticles in delaying of the senescence of cut roses was evaluated. Experiments were performed in a completely randomized design with 9 treatments in six repetitions, and the treatments were applied by petal spraying every three days. Chitosan nanoparticles with two concentrations (0.1 and 0.5 mg L^{-1}) and SA with two concentrations (0.1 and 0.3 mM) on the postharvest quality of cut roses were tested. Biochemical and enzymatic traits were measured during a period of 15 days (1, 4, 7, 11, 15 days after harvest), whereas, the vase life, RWU and electrolyte leakage were recorded till the end of flowers vase life. The maximum vase life (17.8 days) observed in flowers subjected to foliar spraying of 0.5 mg L^{-1} of chitosan nanoparticles with 0.3 mM salicylic acid, while shortest flower longevity (13.5 days) obtained in untreated flowers. Also, the amount of relative solution uptake of flowers and flavonoid content with significant increase were observed in 0.5 mg L^{-1} chitosan nanoparticles with 0.3 mM salicylic acid treatment. Treatments containing chitosan nanoparticles, especially the combined treatment of 0.5 mg L^{-1} of chitosan nanoparticles with 0.3 mM SA, reduced the amount of electrolyte leakage of the petals and increasing the vase life. Moreover, the lowest activity of antioxidant enzymes including GPX, PPO, POD and hydrogen peroxide were observed in flowers sprayed with 0.5 mg L^{-1} chitosan nanoparticles + 0.3 mM SA. Among chitosan nanoparticle treatments, the longest vase life was related to 0.5 mg L^{-1} (15 days), which increased flowers vase life by 11% compared to the control. Between SA treatments, the concentration of 0.3 mM was the most effective with a 6% increase in flowers vase life comparing untreated flowers. Finally, the combined treatments of chitosan nanoparticles and SA beside improving postharvest quality of cut roses, caused a 31% increase in flower longevity comparing untreated flowers.

Keywords: Chitosan, Longevity, Rose, SA, Spray treatment.

INTRODUCTION

Rose from Rosaceae family is considered as the most important cut flower in the world. The export value of this cut flower in the floriculture industry of the world is about 11 billion dollars (Chalabi and Nasreen Khalil, 2013). Cut roses are the most important cut flowers from the economic point of view. Rose cut flowers are sensitive to water stress under postharvest adverse conditions and often lose rapidly their marketability as well as their beauty value under such conditions (In *et al.,* 2016). Postharvest technology is able to increase the lifespan of cut flowers (Scariot *et al*., 2014). The vase life of cut roses is determined by many factors, including water relations (Doi *et al*., 2000; Hassan *et al*., 2020; In *et al.,* 2017). Therefore, increasing the vase life of cut flowers through proper management and care after harvesting is important from a commercial and economic point of view. In this regard, the lack of nutrients, bacterial and fungal contamination, wilting caused by water stress and blockage of vessels are considered to be the main factors that shorten the life of cut flowers. By using techniques to delay the senescence of cut flowers, the marketability of cut flowers can be significantly increased because the postharvest life is a vital and important factor for cut flowers. In this regard, to increase the longevity of cut flowers, a suitable preservative solution should be used (Parween and Gupta, 2022). Various postharvest treatments using growth regulators, sugars, signaling molecules and substances such as salicylic acid can inhibit post-harvest senescence and increase the postharvest life of the flower (Zulfiqar *et al*., 2020).

The pre-harvest life of cut flowers depends on various factors such as environment, genetics, handling systems and harvesting time, however, the postharvest life is determined by the water relations, microorganism's infections, storage conditions and packaging methods. Microbes have the ability to grow rapidly and settle at the ends of the cut stems after harvesting the flowers, which results in the blockage of the xylem vessels. Due to microbial blockage, the water absorption via stems is disturbed and ultimately causes water imbalance and unwanted wilting of cut flowers (He *et al*., 2018). Therefore, to prevent or slowing senescence process in cut flowers, preventing microbial blockage is considered a useful approach to increase postharvest quality of cut flowers. The use of different chemicals, including silver nitrate, silver thiosulfate, silver nanoparticles, calcium and hydrogen gas, was used in the past to increase the longevity of cut flowers (Ahmad *et al*., 2016; Alimoradi *et al.,* 2013; Bai *et al.,* 2009). However, the high cost of these chemicals and their hazards on the environment and human health have caused the attention of researchers to change to the use of environmentally friendly agents.

The use of nanoparticles plays an important role in postharvest management of horticultural products and plant protection due to their biocidal properties. For instance, in gerbera cut flowers, the floral preservative solution containing silver nanoparticles with antimicrobial activity increased their postharvest life (Solgi *et al*., 2009). Previous studies have shown that the use of different antimicrobial compounds can increase the life of cut flowers. The process of petal senescence is very complex and includes physiological and biochemical changes such as changes in the permeability of cell membranes, which lead to loss of color, wilting and finally senescence of petals (Arora and Singh, 2004). During the last decade, nanotechnology has made significant progress and various types of nanoparticles have been synthesized and introduced in different laboratories, and with the increase in the use of nanoparticles in various industrial and research sectors, studies on the applications of nanoparticles in the field of agriculture. Also, the interaction of plants and nanoparticles, the impact of nanoparticles on the environment, food chain and human health have been the focus of researchers all over the world (Ioannou *et al*., 2020).

Chitosan is a valuable biopolymer with many remarkable properties. This composition is

compatible with plants and has antioxidant, antiperspirant and antimicrobial properties. Also, it is a biodegradable and economic compound that is obtained from the skin of animals such as crabs and shrimps. In ornamental plants, chitosan has been used as an antitrannspirant compound to increase the longevity of cut flowers (Bañuelos-Hernández *et al*., 2017). Based on research, compounds containing chitosan called chito-oligosaccharide (COS) by improving the water absorption capacity of roses increased the vase life up to 6.4 days comparing control.

These compounds, while increasing the activity of antioxidant enzymes such as glutathione reductase, have improved the amount of glutathione in cut rose petals, so they are recommended as a commercial protective solution to increase the lifespan of cut roses (Hong-Juan and Huan- Qing, 2015). The use of chitosan or its derivatives increase the quality and postharvest life of various horticultural crops due to its germicidal properties and also by stimulating the defense mechanism of plant tissues (Terry and Joyce, 2004).

Salicylic acid (SA) as a plant growth regulator causes many physiological and biochemical effects in plants. Meanwhile, salicylic acid delays senescence process in cut flowers by increasing the activity of antioxidant enzymes and strengthening the cellular antioxidant system (Armitage and Laushman, 2003). They concluded that the salicylic acid treatment significantly prevents the formation of lignin. The reduction of lignin formation is directly related to the inhibitory effect of SA on the activity of enzymes related to lignin production and indirectly to the reduction of oxidative damage, which is caused by the reduction of O_2 and H_2O_2 accumulation (Wang *et al*., 2016). It has been reported that the pre- and postharvest treatments of SA influenced the physico-chemical properties of cut roses , improved the longevity of roses via increase of enzymatic antioxidant capacity, the improvement of water relations and the increase of cumulative water absorption (Alaey *et al*., 2011).

In some ethylene sensitive cut flowers, ethylene is the main responsible for petal senescence. As internally produced ethylene causes senescence and regulates gene expression coordination in flower petals (Mohammadi Kabari and Jadid Soleimandarabi, 2019; Hassan *et al*., 2020), but rose is not more sensitive to ethylene, and petal senescence may occur due to internal factors other than ethylene, such as the increase of reactive oxygen species (ROS) (Jones and McConchie, 1995). Antioxidant enzymes such as peroxidase and guaiacol peroxidase (GPX) play an important role in the defense against oxygen free radicals (Okigbo and Ogbonnaya, 2006; Bayat and Aminifard, 2017).

The aim of this study was to investigate the role of chitosan nanoparticles and salicylic acid as a preservative treatment to slowing the senescence process of rose cut flowers. Therefore, in this research, the optimal concentrations of preservative treatments (chitosan nanoparticles and salicylic acid in the form of foliar spraying) in order to increase the vase life, maintain moisture and improve water absorption (preventing the xylem blockage) and especially preventing the ROS accumulation has been tested.

MATERIALS AND METHODS

Plant materials and treatments

Cut roses were obtained from a commercial greenhouse located in Ajabshir, Iran. The flowers were harvested early morning and homogenized based on their visual appearance and openness. The flowers were cut to a height of 35 cm and then placed in containers containing distilled water. Cut roses were sprayed with different solutions prepared using various concentrations of salicylic acid and/or chitosan nanoparticles. The treatments included salicylic acid in two concentrations (0.1 and 0.3 mM), chitosan nanoparticles in two levels (0.1 and 0.5 mg L^{-1}) and four levels of combined aforementioned substances (CSNPs 0.1 mg L^{-1} + SA 0.1 mM), (CSNPs 0.5 mg L^{-1} + SA 0.1 mM), (CSNPs 0.1 mg L^{-1}+

SA 0.3 mM), (CSNPs 0.5 mg L^{-1} + SA 0.3 mM) in addition with distilled water as control (Figs. 1 and 2). The treated cut flowers were kept at a temperature of 10 ± 2 °C, relative humidity of 60-70% and a photoperiod of 12 hours at an intensity of 15 µmol m^2 S^{-1}.

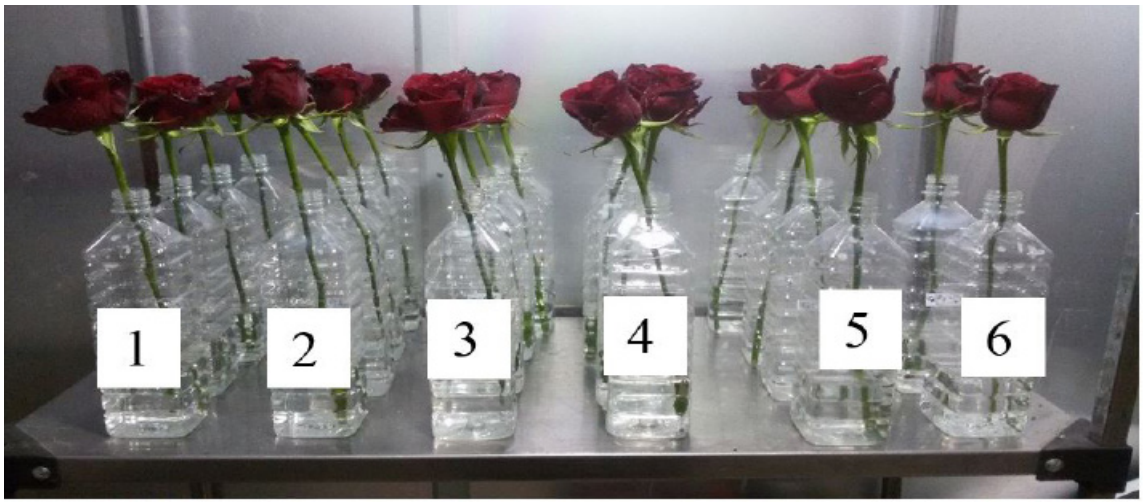

Fig. 1. Effect of chitosan nanoparticles (CSNPs) and salicylic acid on cut rose "Sammuraie" 13th day after harvest. 1: SA 0.1 mM; 2: SA 0.1 mM+CSNPs 0.5 mg/L; 3: Control; 4: CSNPs 0.5 mg/L; 5: SA 0.3 mM+CSNPs 0.1 mg/L; 6: SA0.3 mM+CSNPs 0.1 mg/L.

Fig. 2. Effect of chitosan nanoparticles (CSNPs) and salicylic acid on cut rose "Sammuraie" on the 11th day of vase life.

The flowers were evaluated for morphological traits (such as relative water uptake, and vase life) and membrane electrolyte leakage, while the petals of each treated groups were collected, frozen in liquid nitrogen and stored at - 40 ˚C for further biochemical and enzymatic assays.

Preparation of chitosan nanoparticles solution

Pure chitosan with molecular characteristics of Mw = 100 kD, DD = 85%, and purity = 97% obtained from Sabz Gostaresh Aazin Turkan Company (Maragheh, Iran), tripolyphosphate (TPP) from Merck (Germany) and salicylic acid with a purity 99 % were obtained from Sigma Aldrich (USA). To prepare 5 L of chitosan solution with a concentration of 1% by weight, first, 50 g of chitosan powder was mixed with 4950 ml of distilled water for one hour on a heater until a uniform solution was obtained. Then 50 ml of acetic acid was added to the chitosan

dispersion and completely dissolved using stirrer. Using this solution, we prepared the desired concentrations of chitosan nanoparticles (Ahmadi *et al*., 2018). Chitosan nanoparticle solution in two concentrations of 0.1 and 0.5% by weight was obtained using chitosan stock solution and TPP ionic binder. Solutions of salicylic acid with concentrations of 0.1 and 0.3 mM were prepared by dissolving 0.373 and 1.12 g of SA in two liters of distilled water, respectively. To prepare chitosan nanoparticles with concentrations of 0.1 and 0.5 mg L^{-1}, 200 and 1000 mL of chitosan solution were diluted with 1800 and 1000 mL of distilled water, respectively. SA was dissolved in distilled water and added to the chitosan solution with a certain concentration and the solution was reached to a volume of two liters. The desired amount of TPP as an ionic interface based on the amount of chitosan was dissolved in 25 ml of distilled water and the TPP solution was slowly added into the chitosan solution, and the chitosan nanoparticle solution was produced.

Traits measurment

Vase life

During the postharvest life, the visual quality of cut roses were inspected daily. In present study, vase life was defined as the period (days) from the time of spray treatments until 50% of flower petals were wilted or abscised or flower necks were bent as symptoms of flower senescence process (Jiang *et al*., 2015).

Relative water uptake (RWU)

Relative water uptake of the flowers was traced by the formula:

Formula (1): Relative water uptake (RWU) = V_t-V_{t-1}/ stem weight at the day zero

Where V_t refers to the vase water volume during the measurements, and V_{t-1} is the water volume at the day before.

This trait was measured at one- day intervals till the end of vase life (van Meeteren and van Gelder, 1999; Pompodakis *et al*., 2004).

Membrane electrolyte leakage (EL)

To measure membrane turgidity using the method (Sairam *et al*., 2002), 0.1 g of petal samples from each treatment were transferred separately (in duplicate) to test tubes containing 20 ml of deionized water. Then, a series of samples were placed at a temperature of 40°C for 30 minutes and another series at a temperature of 100 °C for 15 minutes in a Ben-Marie. The conductance of the samples was measured by EC meter (AL 10Con, AquaLytic, Germany). Then the percentage of ion leakage was calculated in the following way:

Formula (2) $$EL = \frac{EC(2)-EC(1)}{EC(2)} * 100$$

EC (1): Conductance amount in temperature 1, EC (2): Conductance amount in temperature 2.

Measurement of guaiacol peroxidase (GPX)

The activity of GPX enzyme was measured according to method described by Mencarelli *et al*. (1995). 0.5 g petal tissue was homogenized by potassium phosphate buffer (pH=7, 100 mM). The homogenate was centrifuged at 4°C for 15 min. at 15000 g and supernatant ware used for recording enzyme activity. The GPX activity was calculated at 470 nm based on the extinction coefficient of tetraguaiacol (26.16 mmol cm^{-1}) and expressed as micromoles of oxidized guaiacol per minute per gram of fresh weight.

Measurement of peroxidase (POD) and polyphenol oxidase (PPO) activities

Peroxidase enzyme activity was traced based on the conversion of guaiacol to tetraguaiacol by the method (Maehly and Chance, 1995). For this purpose, 200 mg of frozen petal tissue was grinded in sodium phosphate buffer containing 2% PVP and 1.3 mM EDTA. To measure each sample, 450 µL of hydrogen peroxide buffer and 450 µL of guaiacol buffer are mixed together at a low temperature (container containing ice) and 10 µL of enzyme extract is added to it, and finally oxidation of guaiacol at a temperature of 25 °C with a spectrophotometer two visible-ultraviolet rays, model UV-1800 of Shimadzu, Japan, were measured at a wavelength of 470 nm.

Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) activity was assayed by measuring the oxidation of catechol as substrate according to Nguyen *et al*. (2003) and was expressed as IU mg^{-1} protein min^{-1}.

Measurement of H_2O_2 amount

The H_2O_2 content was measured according to Alexieva *et al*. (2001) and expressed in µmol g^{-1} FW.

Measurement of total flavonoids

The amount of total flavonoids was measured as described by Chang *et al*. (2002) which is based on aluminum chloride colorimetric. The absorbance of the mixture was read at 415 nm with a UV-1800 model UV-1800 spectrophotometer from Shimadzu, Japan. Total flavonoids content was expressed as mg Quercetin g^{-1} FW.

Statistical analysis of data

Two-way ANOVA was used for all the traits except vase life which is measured only once at the end of the experiment. The experiment was designed as factorial based on completely randomized design (CRD) with six replications. First factor was 8 levels of treatment solutions in addition with distilled water as control and the second factor was time of sampling for measurements which were first, fourth, seventh, eleventh and fifteenth day of the experiment. For vase life which was measured once at the end of the experiment, one-way ANOVA was used. Mean comparisons were done by Tukey's honestly significant difference (HSD) test ($P<0.05$). All statistical analyses was performed using R statistical software (R foundation for statistical computing, version 4.3.2).

RESULTS AND DISCUSSION

Vase life

ANOVA (Table 1) showed the significant ($P < 0.01$) difference in the vase life of cut roses under tested treatments. The mean comparison indicates that foliar spraying of the combined treatment of SA 0.3 Mm + CSNP 0.5 mg L^{-1} has shown the longest vase life (17.83 days) compared to the control (13.5 days), showed 4.33 days more longevity comparing untreated flowers (Fig. 3).

Table 1. Summary of two-way ANOVA for physiological traits of cut rose cv. "Sammuraie".

S.o.V	df	Vase life	RWU	EL
Treatment	8	108.00**	2.454**	2390.5**
Time	4	58.83	15.872**	196.8**
Treatment * Time	32	166.83	0.09**	15.187**
Error	225	12.18	0.013	5.593
Total	269		0.331	80.504
CV (%)			12.18	6.94

**: Significant at P < 0.01 based on the HSD test. RWU: Relative water uptake, EL: Electrolyte leakage.

The flowers treated with 0.1 Mm SA, alone and without the application of chitosan had the lowest flower longevity (13.6 days) after the control treatment (13.5 days), which did not show a significant difference. Our results are consistent with Jing and Li's study which demonstrated chitosan positive impacts on the longevity of cut roses, which recorded about 6.4 days more than the control (Jing and Li, 2015).

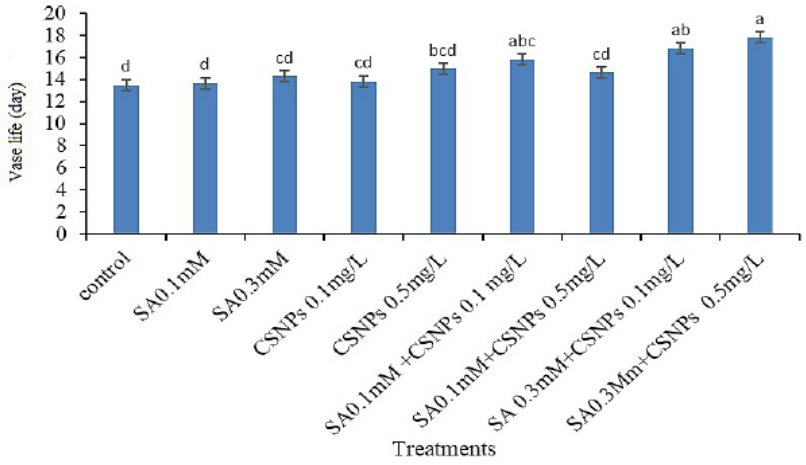

Fig. 3. The effect of different levels of chitosan and salicylic acid nanoparticle treatments on the vase life of cut roses CV. "Samuraie". *In each column, means with similar letter(s) are not significantly different (P < 0.05) using the HSD test.

Relative water uptake (RWU)

The results revealed that the amount of water uptake by rose cut flowers influenced significantly is significant (P < 0.01) by interactive effects of chemical treatments and time. The mean comparisons showed that in all the treatments until the fifth day, the uptake of the water was high. With the increase of time after harvest, the amount of uptake of the water showed a significant decrease. After the fifth day, the amount of water uptake in the treatment containing SA 0.1 mM and CSNP 0.1 mg L^{-1} decreased, while in treatments containing SA 0.3 mM + CSNP 0.5 mg L^{-1} and SA 0.3 mM + CSNP 0.1 mg L^{-1} absorption rate decreased just after 9th day. So, in the last days of flower vase life, the flowers treated with SA 0.3 mM + CSNP 0.5 mg L^{-1} had the highest amount of water uptake (1.29 ml g^{-1} FW), which was significantly different from the treated flowers with 0.1 mM of SA. 0.1 mM SA treatment alone had the lowest average water uptake (0.64 ml g^{-1} FW) (Fig. 4). The minimum amount of water absorption (0.47 ml g^{-1} FW) was recorded in control flowers comparing other treatments.

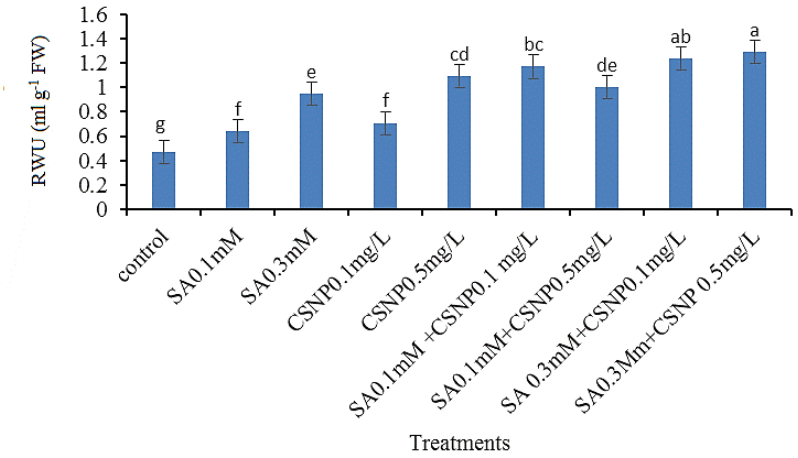

Fig. 4. The effect of different concentrations of chitosan and salicylic acid nanoparticles on the relative solution uptake in the cut flowers of the "Samuraie" rose. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

Since chitosan nanoparticles and salicylic acid act as antibacterial agents, they reduce bacterial infection and prevent vascular occlusion, thus improve solution uptake (van Meeteren, 1978; Mehdikhah *et al*., 2016). The present study in agreement with previous findings showed similar results. In this research, foliar spraying of chitosan nanoparticles along with increasing the solution uptake, significantly improved postharvest quality of cut rose flowers.

The reduction of water solution uptake due to xylem occlusion has the greatest effect on the early wilting of the petals and bending of the stem (Xue *et al*., 2009). The vessels in cut flowers are vital tissues for water absorption, so that the amount of solution absorption decreases with the obstruction of the vessels by bacteria. In this regard, the role and importance of chitosan nanoparticles against bacteria has been investigated, and the current research is in accordance with the research conducted regarding the improvement of water absorption in cut roses due to the antibacterial property of chitosan nanoparticles (Jing and Li, 2015).

Electrolyte leakage (EL)

The analysis of variance of the data showed that the interaction effect of these treatments with the time after harvesting on the amount of electrolyte leakage of petals was significant at the level of 1% ($P < 0.01$) (Table 1). The mean comparisons showed a notable decrease in ion leakage index in the higher concentrations of the preservative treatment, especially in the combined treatment of 0.3 Mm SA + 0.5 mg L^{-1} CSNP by 21.84%, comparing untreated flowers those showed the highest amount of petal electrolyte leakage (49.26%) (Fig. 5).

The mean comparisons among treatments showed that in all the treatments until the third day, the amount of electrolyte leakage was low. With time passage after harvest, the amount of electrolyte leakage increased. In the treatment containing 0.1 mM SA and 0.1 mg L^{-1} CSNP, an increase in the amount of electrolyte leakage after the fifth day, and in the treatments containing 0.3 mM SA + 0.5 mg L^{-1} CSNP and 0.3 mM SA + 0.1 mg L^{-1} CSNP, the amount of petal electrolyte leakage was increased from 10th day after harvest. So, in the last days of the vase life flowers, the petals that were treated with 0.3 mM SA + 0.5 mg L^{-1} CSNP had the lowest amount of electrolyte leakage. Therefore, the membrane integrity of petals sprayed with 0.3 mM SA + 0.5 mg L^{-1} CSNP treatment was higher than other treatments, and as a result of this treatment, the life span of cut flowers was increased compared to other treatments.

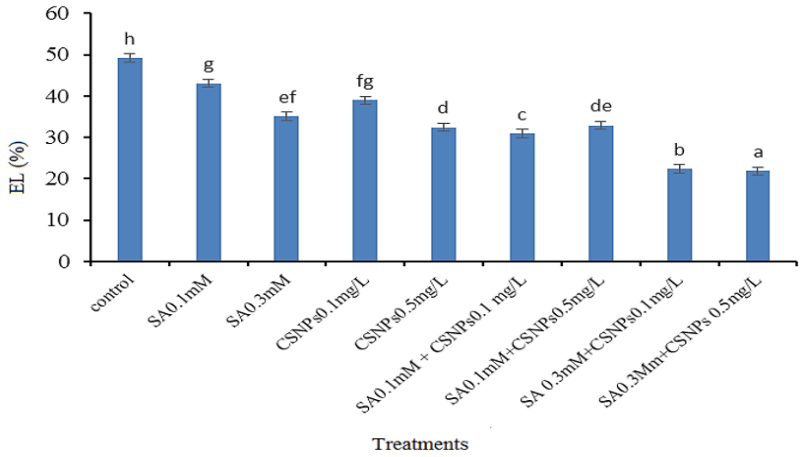

Fig. 5. The effect of different concentrations of chitosan nanoparticles and salicylic acid treatments on the amount of electrolyte leakage on cut roses cv. "Samuraie". *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

Shortly, the combined treatments of chitosan nanoparticles and salicylic acid in this research maintained the strength of the cell wall and increased the vase life of rose cut flowers. Obtained results by Mohammadi and Mortazavi (2014) about the effect of salicylic acid on alstromeria cut flowers are consistent with our research. The findings of Parween and Gupta (2022) regarding the effect of chitosan treatment on membrane stability in gerbera cut flowers are completely consistent with our results.

Flavonoid content

The effect of different concentrations of chitosan nanoparticles with salicylic acid indicate that these treatments increased the flavonoids content compared to the control during post-harvest period. Comparison of the means showed that the highest amount of flavonoid (580.38 µg g^{-1} FW) was in the combined treatment chitosan nanoparticles with a concentration of 0.5 mg L^{-1} along with salicylic acid with a concentration of 0.3 mM. The lowest amount of flavonoids (480 µg g^{-1} FW) was obtained in the control treatment. In foliar spraying with of chitosan nanoparticles with a concentration of 0.5 mg L^{-1} (alone) and salicylic acid with a concentration of 0.3 mM (alone) compared to the combined treatments of SA 0.1 mM + 0.1 mg L^{-1} CSNP and SA 0.1 mM + 0.5 mg L^{-1} CSNP, no significant difference was observed in terms of petal flavonoid content (Fig. 6).

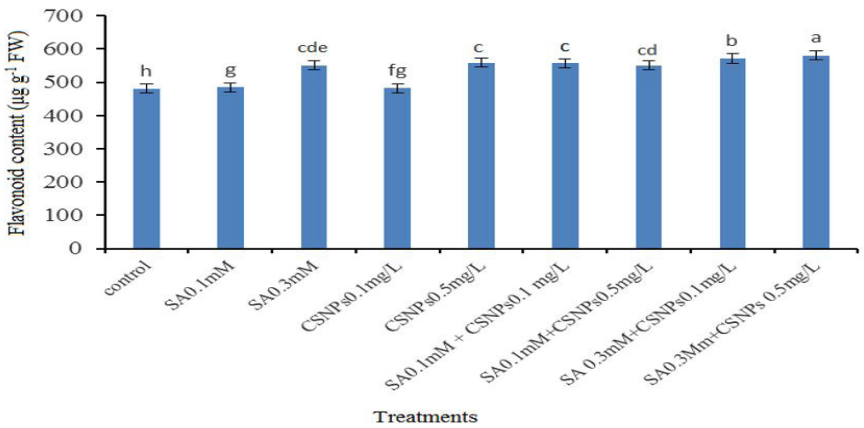

Fig. 6. The effect of different concentrations of chitosan and salicylic acid nanoparticle on the flavonoid content of cut rose flowers cv. "Samuraie". *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

The results showed that the interaction of the nanoparticle treatments with post-harvest times was significant at the 1% probability level. Mean comparison of data showed that with increasing concentration of chitosan nanoparticle complex with salicylic acid, the flavonoid content increased, so that in foliar spraying with 0.3 mM SA + 0.5 mg L^{-1} CSNP, the highest amount of flavonoid in petals (Fig. 4).

Guaiacol peroxidase (GPX) activity

According to the results of analysis of variance (Table 2), the interaction of different concentrations of salicylic acid and chitosan nanoparticles and time caused significantly ($P < 0.05$) the guaiacol peroxidase enzyme activity, where, guaiacol peroxidase activity in flowers treated with different concentrations of salicylic acid and chitosan nanoparticles was lower compared to untreated flowers regardless of their concentration.

Table 2. Summary of two-way ANOVA for biochemical traits of cut rose cv. "Sammuraie".

S.o.V	df	Flavonoid	GPX	PPO	POD	H_2O_2
Treatment	8	49628.12**	13.166**	10.610**	0.0077**	4.909**
Time	4	8532.5**	0.0035**	5.214**	0.00087**	4.83**
Treatment × Time	32	611.19**	0.0003**	0.7080**	0.000054*	0.240**
Error	225	127.4	0.0000	0.170	0.000028	0.0101
Total	269	1782.07	0.3916	0.620	2.741	0.255
CV (%)		2.11	0.05	17.6	7.54	4.01

**: Significant at $P < 0.01$ based on the HSD test.

As shown in Fig. 7, the change of peroxidase enzyme was decreasing with the progress of time, and the highest level of enzyme activity was in the control treatment (3.81 IU mg^{-1} protein min^{-1}) and the lowest level was observed in 0.3 mM salicylic acid chitosan nanoparticles 0.5 mg L^{-1} (1.81 IU mg^{-1} protein min^{-1}).

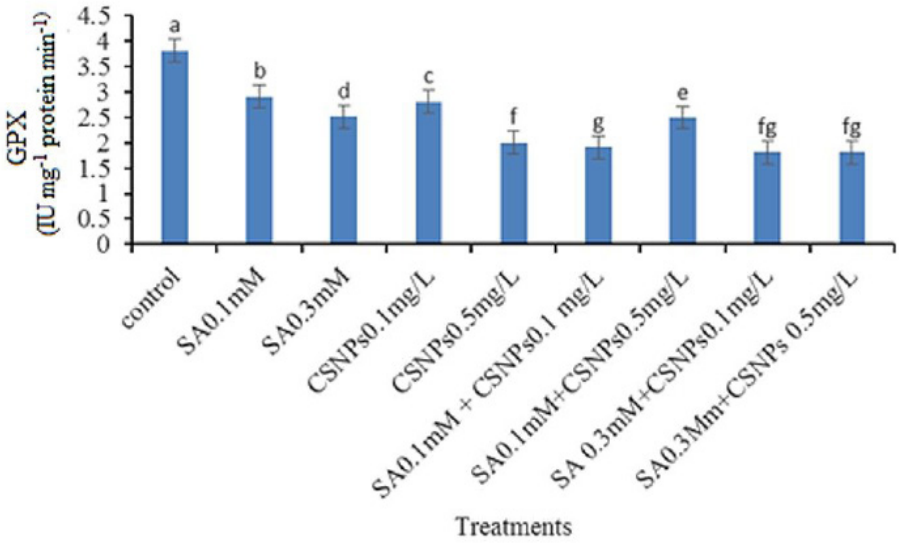

Fig. 7. The effect of different concentrations of chitosan nanoparticles and salicylic acid treatments on the guaiacol peroxidase activity in the cut flowers of the "Samuraie" rose. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

In this research, the activity of guaicol peroxidase enzyme shows an increasing trend in rose cut flowers at postharvest and the senescence stage of petal cells. These findings confirm similar results recently reported for carnation flowers (Moulaei *et al*., 2021). So the results of our research indicate that chitosan nanoparticles of and salicylic acid treatments have a protective effect and increasing the vase life of rose flower petals, which prevent the creation of various stresses (such as osmotic potential) in the petals and as a result increase the activity of antioxidant enzymes, including peroxidase.

Peroxidase (POD) activity

According to the results of analysis of variance (Table 2), the interaction of different concentrations of salicylic acid and chitosan nanoparticles and time caused significantly ($P < 0.05$) the peroxidase enzyme activity, where, POD activity in flowers treated with different concentrations of salicylic acid and chitosan nanoparticles was lower compared to untreated flowers regardless of their concentration. As shown in fig. 8, the change of peroxidase enzyme was decreasing with the progress of time, and the highest level of enzyme activity was in the control treatment (0.091 µmol mg^{-1} FW min^{-1}) and the lowest level was observed in the treatment of 0.3 mM salicylic acid with chitosan nanoparticles 0.5 mg L^{-1} (0.047 µmol mg^{-1} FW min^{-1}).

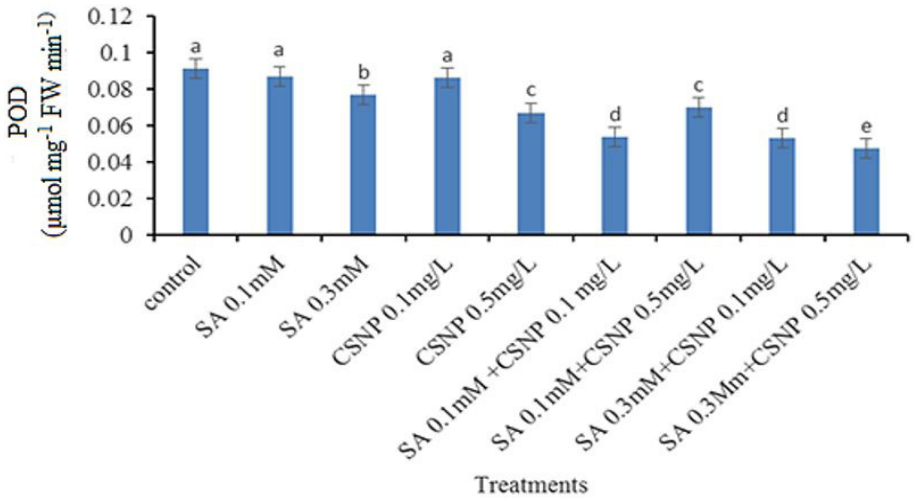

Fig. 8. The effect of different concentrations of chitosan nanoparticles and salicylic acid treatments on the peroxidase activity in the cut flowers of the "Samuraie" rose. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

Peroxidases play an important role in oxidative stress, removal of oxygen free radicals, involvement in auxin metabolism, wound healing in plants, and reaction to environmental pollution. This enzyme breakdown hydrogen peroxide through compounds such as ascorbate or oxidation of substrates such as phenolic compounds (Reddy *et al*., 2008). Peroxidase enzyme is able to remove malondialdehyde and hydrogen peroxide (Hojati *et al*., 2011). In our research, salicylic acid and chitosan nanoparticles caused a significant decrease in peroxidase enzyme activity. One of the causes of senescence in plant tissues are active oxygen species such as O_2^- and H_2O_2, which cause flower senescence by destroying proteins, lipids and nucleic acids (Choudhary *et al*., 2017). Antioxidant enzymes are very effective systems that protect cells against ROS. According to the results of Ezhilmathi *et al*. (2007) 5-sulfo-salicylic acid treatment and the results of Hatamzadeh *et al*. (2012), salicylic acid treatment increases the activities of catalase

and peroxidase antioxidant enzymes, which scavenging ROS resulted to reduce senescence process in cut flowers.

Polyphenol oxidase (PPO) activity

The results of ANOVA revealed that the interaction effects of different concentrations of chitosan-salicylic acid nanoparticle complex and postharvest period was significant ($P<0.01$) on the activity of polyphenol oxidase (PPO) enzyme (Table 2). Mean comparison of data showed that the highest and lowest PPO activity was observed with an average of 3.42 IU mg^{-1} protein min^{-1} in the control treatment and 1.62 IU mg^{-1} protein min^{-1} in 0.3 mM salicylic acid with 0.5 mg chitosan (Fig. 9).

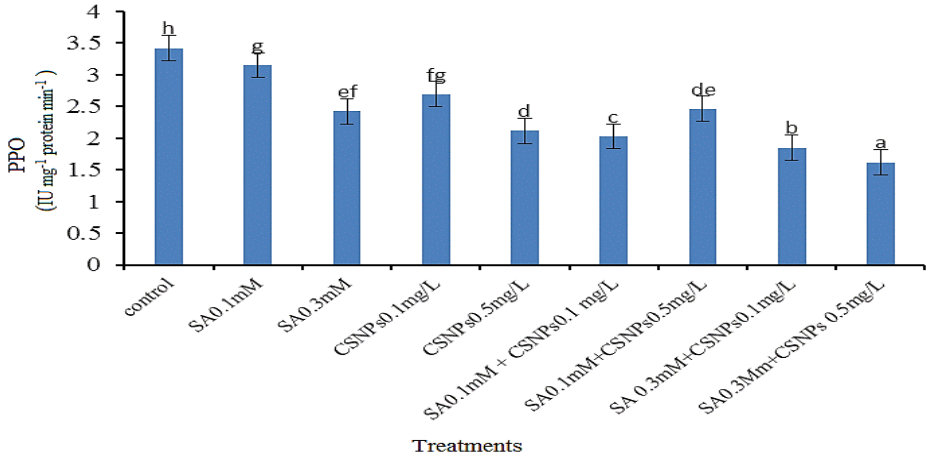

Fig. 9. The effect of different concentrations of chitosan nanoparticles and salicylic acid treatments on the PPO activity in "Samuraie" rose cut flowers. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

During the days after harvest, the activity of PPO increased. So, in the control treatment, the increasing trend of this enzyme started on the fifth day after harvest, and in the treatments containing chitosan nanoparticles and salicylic acid containing treatments, respectively, from the seventh and sixth days, and in the combined treatments of chitosan nanoparticles with salicylic acid, the increasing trend of the activity of this enzyme was started from 9th day. Therefore, considering that foliar spraying with combined treatments of chitosan nanoparticles with salicylic acid slows down PPO activity rate. So, combined treatments of chitosan nanoparticles with salicylic acid, especially the treatment of 0.3 mM SA + 0.5 mg L^{-1} CSNP in increasing the flower longevity showed the greatest effect comparing other treatments. According to previous studies, the reactions related to the polyphenol oxidase enzyme are very important in the postharvest stage (Zeeshan *et al*., 2020), because this enzyme is able to convert the hydrogen peroxide produced in the organs and cytosol to water and oxygen and reduce its harmful effects. Also, the PPO plays an effective role in cleaning these compounds (Michalak, 2006). In addition, increasing the activity of these enzymes may increase the concentration of $NADP^+$ to release electrons from the photosynthetic electron transport chain and thus reduce the production of ROS (Gozukirmizi *et al*., 2015).

Peroxide hydrogen

The results of variance analysis of the data showed that the interaction effects of chitosan and SA nanoparticle treatments with the duration of treatment on hydrogen peroxide (H_2O_2)

were significant ($P < 0.01$) (Table 2). During the days after harvest, the hydrogen peroxide content increased. H_2O_2 increasing was started in untreated flowers from the 4th day after harvest, but in the treatments containing chitosan nanoparticles, from the seventh day, and in the combined treatments of chitosan nanoparticles with salicylic acid, the increasing trend of the hydrogen peroxide content started from the 10th day. Therefore, the combined treatments of chitosan nanoparticles with SA showed notable capacity in slowing down of the H_2O_2 content which resulted to the vase life of treated flowers by compared to other treatments. Mean comparison of the data showed that the highest concentration of hydrogen peroxide with an average (3.31 µmol g^{-1} FW) was recorded in control flowers and the lowest amount of it with an average (1.96 µmol g^{-1} FW) was observed in combined treatment of 0.3 mM salicylic acid with 0.5 mg L^{-1} of chitosan (Fig. 10). Considering that two types of ROS (reactive oxygen species), including hydrogen peroxide and superoxide anion, play an important role in the plant senescence process, especially in cut flower senescence process, where the content of O_2 and H_2O_2 are increasing. But the trend of this increase with the application of chitosan nanoparticles and salicylic acid treatments is less than that of the control, and there is a significant difference between the amount of hydrogen peroxide in the petals of roses treated with chitosan nanoparticles and salicylic acid compared to the control (Fig. 10). According to the obtained results, treatments containing chitosan nanoparticles are able to reduce the activity of reactive oxygen species (ROS) in rose cut flowers.

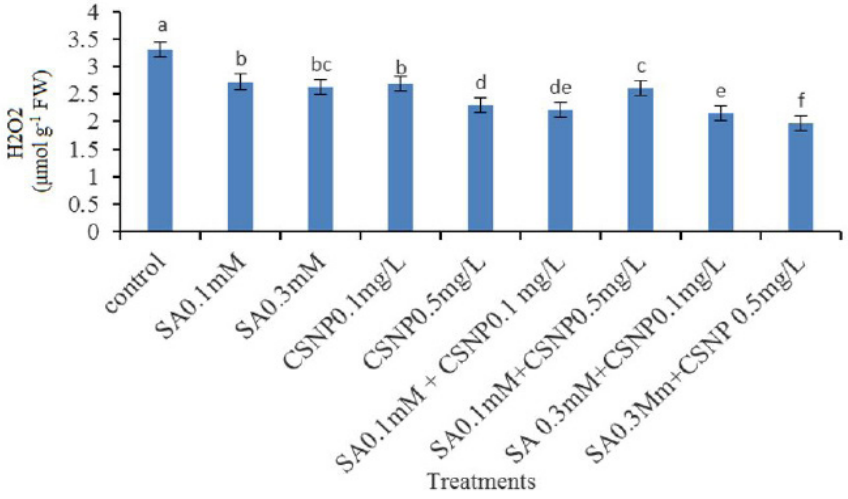

Fig. 10. The effect of different concentrations of chitosan and salicylic acid nanoparticle treatments on hydrogen peroxide content in the cut flowers of the "Samuraie" rose. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

In recent decades, H_2O_2 has received much attention as a reactive oxygen species (ROS). This molecule accumulates more in plants in most environmental stresses, both biotic and non-biotic, and causes more damage to the plant. Thus, hydrogen peroxide plays a role in most physiological processes such as senescence, stomatal opening control, photorespiration and photosynthesis and plant development. On the other hand, the increase and accumulation of H_2O_2 causes oxidative stress, which starts the process of cell death. Therefore, the survival of all aerobic organisms depends on hydrogen peroxide homeostasis. This homeostasis includes the production of H_2O_2 from different pathways and its removal. H_2O_2 removal pathways include enzymatic pathways and non-enzymatic pathways (Shahroodi *et al*., 2020).

Earlier study (Wan *et al*., 2023) has shown that chitosan and its oligosaccharins improve the absorption of the solution in rose cut flowers cv. Gaoyuanhong. Moreover, chitosan activates nutrients and defense mechanisms of cut flowers. In this regard, Ahmed *et al*. (2020), showed the role of chitosan in the growth regulating and improving resistance mechanism to senescence, resulting increase the vase life of cut rose flowers.

CONCLUSION

Rose, as the most important cut flower in the world, has a great economic value and importance in terms of popularity among consumers worldwide. The most basic problem of this cut flower is its relatively short vase life due to the failure in water uptake coming from xylem occlusion beside diverse effects of ROS. Therefore, choosing the proper preservative solution for the post-harvest stage of cut roses is very important considering the various side effects of chemicals. In this research, we use nanoparticles of chitosan and salicylic acid in the form of foliar spray for its bioavailability and environmental friendly nature. In addition, as appropriate biochemical compounds they have the ability to control or inhibit ROS activity and also due to having antibacterial properties, we have used them to increase the vase life of cut flowers. The results showed that the postharvest spraying of chitosan nanoparticles and salicylic acid treatments significantly increased the vase life of rose cut flowers. In short, in cut roses sprayed with solutions containing chitosan nanoparticle +SA, the relative solution uptake and the flavonoid content of the petals increased. In addition, the amount of electrolyte leakage and the activity of POD, PPO and GPX enzymes and the amount of hydrogen peroxide in petal tissues of treated roses sprayed were decreased. Finally, results were demonstrated that the among of different concentrations of solution treatments, the combined treatment of 0.5 mg/L of chitosan nanoparticles with 0.3 mM salicylic acid has the greatest impact on increasing the flower longevity and improving postharvest physiological and biochemical attributes of cut roses.

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Biochemical Evaluation of Ornamental and Native *Muscari* and *Bellvalia* Genotypes with the Attitude of Using them as Valuable Edible Species

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Abstract

Muscari and *Bellvalia* are related ornamental bulbous plants that grow natively in several areas of Iran. These species are highly used as potted and outdoor ornamental plants. Also, these plants have been used as edible species in some regions, but they are not common in Iran. These ornamental plants have the potential to be introduced as valuable edible genotypes and enter the process of domestication and commercial production. Besides ornamental uses, these plants are used primarily as mucilage and filler in cooking. Moreover, various phenolic and saponin compounds affect flavor, too. In the present study, the physicochemical properties of three species of *Muscari*, including *comosum*, *botryoides*, and *neglectum*, and one species of *Bellevalia* from native places were evaluated. These antioxidant capacity, total phenol, and total content of saponin, mucilage, alkaloids, and steroids in leaf and bulb extracts were examined. By evaluating the various metabolites of proposed plants, it can be concluded that *M. comosum* is a suitable genotype for entering the domestication process and as a parent for hybridization. Also, the lesser-known genotype, *Bellevalia paradox*, in addition to being an ornamental plant, has valuable nutritional properties, and further studies on this genotype will have an appropriate approach.

Keywords: Antioxidant capacity, Grape hyacinth, Mucilage, Phenolic compound, Saponin.

INTRODUCTION

Some plants have been used for medicinal purposes for thousands of years. This group of plants is known for being the source of many natural ingredients that heal human health problems. *Muscari* and *Bellevalia* are two native genera that grow naturally across some regions of Iran, and they are usually known and consumed as edible and medicinal plants. Despite the different karyological aspects in these two genera, they have similar morphological and molecular features, making them be considered originating from the same clade (Azizi *et al*., 2016; Johnson, 2003; Pfosser and Speta, 1999).

Muscari, *Pseudomuscari*, and *Leopoldia* are three subgenera in the genus *Muscari,* which is classified in the Asparagaceae family (Speta, 1998). *Muscari* is also known as grape hyacinth due to the shape of the inflorescence. This genus has also been interested in its several ornamental usages, such as its usage as a garden plant, pot plant, and cut flower because of producing colored beautiful and sweet-scented inflorescence in spring (de Hertogh and le Nard, 1993; Qi *et al*., 2013). Some plants of this genus are consumed as edible plants in several countries, such as Iran, Italy, and Turkey. In Turkish traditional medicine, *M. neglectum* species are known as expectorants, appetizers, and diuretics and are used to treat warts, which seems that some compounds in these plants have antiviral properties (Özkan *et al*., 2017).

In Iranian traditional medicine, this species is known as Kalaghak and is used for disorders of the digestive system and uterus. Bulbs of this species are traditionally used in southern Italy as medicine to treat facial blemishes and toothache (Motti *et al*., 2009). The nutritional value and antioxidant capacity of bulbs of this species, which are widely used in Italian food, have been reported in several studies (Casacchia *et al*., 2017; Pieroni *et al*., 2002). The flowers of this species are sweet and are used as flavoring (Lim, 2014; Wright, 2001). There are also edible types among the species of the *Bellvalia* genus. For example, in Turkey, the leaves of *Bellevalia paradoxa* are used in foods (Altundağ, 2009). Phenolic compounds are a leading group of plant antioxidants (Kochan *et al*., 2019). A comparison of some ethnic vegetables in southern Italy showed that the antioxidant activity of *Leopoldia comosa* (L.) Parl. (syn. *Muscari comosum* (L.) Miller) bulbs were higher than that occurred in the other 26 studied species, including *Asparagus acutifolius* L. shoots (Pieroni *et al*., 2002). Another 2018 report indicated that the total phenolic compound in *Muscari armeniacum* Leichtlinex Baker was 88.19 mg GAE/100 g, higher than that found in the other eight tested species. Antioxidant activity in this research was about 11% for *M. armeniacum* (Özcan *et al*., 2018). Saponins are an important group of plant secondary metabolites, which include glycosylated triterpene or steroids. Some properties of saponins are hemolytic activity, cholesterol-binding properties, and bitterness. Most of the properties of saponins are beneficial. Therefore, plants with a specific saponin content are popular as folk medicine (Price *et al*., 1987). Extensive research about *Muscari*'s saponin content, especially *Bellevalia* species, is not satisfying. A few research studies have demonstrated the presence of saponins in the bulb and leaf of some *Muscari* species, such as *Muscari longipes* (Masum and Osw, 2016).

The use of various polysaccharides in the food industry (especially gums and mucilage) has increased due to their edible properties, including thickening and stabilizing the taste and color of food, as well as having medicinal properties in the control and prevention of cardiovascular diseases (Gao *et al*., 2017; Kaur *et al*., 2018) studies on some plants of Asparagaceae, such as *Asparagus racemosus* Wild. Indicate the appropriate ability of this family's plants to produce mucilage (Gheybi *et al*., 2021; Saju and Sivaraman, 2021). Studies have shown the presence of alkaloids and steroids in *Muscari* and *Bellevalia* genera, but their amount has yet to be measured. Some plant alkaloids are medicinal, and some are classified as toxic, so their identification in

edible plants is essential. Steroids are important in maintaining salt balance, preventing thyroid problems, and enhancing sexual power (Nasrudin, 2017).

Given that these two genera include many plant varieties valuable in their health and medicinal properties on one hand and offering many culinary values besides their remarkable worthy ornamental characteristics in the garden and interior spaces on the other hand, it would be highly reasonable to do more investigations for identifying those varieties rich in valuable and healthy compounds which can be later introduced as commercial cultivars in mass field production.

In this study, we aimed to identify and compare several selected genotypes regarding medicinal and edible value by measuring some of their biochemical traits. Hence, three *Muscari* and a *Bellvalia* were analyzed to determine the antioxidant, total phenols, mucilage, alkaloids, and steroids. These parameters are essential to perform the edible and medicinal properties of the selected plants.

MATERIALS AND METHODS

Materials

Some different species of *Muscari* and *Bellevalia* were collected from northwest Iran. These plants were identified by field research and, in some cases, the cooperation of villagers and foresters to find original habitats. Local people consumed all the genotypes collected in the primary habitat. The Center of Genetic Resources of Iran studied and identified the collected samples according to morphological characteristics and keys (Rechinger, 1990). The identified genotypes were classified into three species of *Muscari* and one species of *Bellevalia*, including *M. neglectum* (M1), *L. comosa* (M2), *Muscari botryoides* (M3), and *B. paradoxa* (B5) (Fig. 1). Based on this, the two studied genotypes were *Muscari comosum* species collected from Node village in Ardabil province and Sahand mountain hillsides. Also, *M. botryoides* species were collected from Khalkhal summer areas, *M. neglectum* species were collected from the original areas of Khalatposhan research station in the east of Tabriz, and *Bellevalia paradoxa* species were collected from relatively high areas of Sahand mountain. The identified samples were also matched with the herbarium samples of the Tabriz University Agriculture Faculty, and their codes were recorded in table 1. In the case of sample M3, there was no similar case in the herbarium of the Agriculture Faculty.

Fig. 1. Digital photographs of the collected initial plants used in this study. The width of leaves, shape, and color of flowers and bulbs are the significant characteristics that helped to identify the species. M1: *Muscari neglectum* Guss. ex Ten; M2: *Leopoldia comosa* (L.) Parl. From Ardabil province; M3: *Muscari botryoides*; M4: *Leopoldia comosa* (L.) Parl. From East Azarbaijan province; B5: *Bellevalia paradoxa* (Fisch. & C.A.Mey.) Boiss.

Thirty bulbs were randomly collected from each identified species (Table 1). In all identified regions, almost the same areas were considered first, and then, by throwing a piece of wood, the plants in the place where the wood landed were collected. All bulbs were planted in the same climate and substrate and harvested at the stage of the whole opening of the florets. The harvested plants were washed with distilled water, and after quick freezing in liquid nitrogen, they were kept in a freezer at -80 °C until the experiments.

Table 1. The location of plants of the Asparagaceae family was used to analyze this study.

Species	Another name	Location	Ltitude (M)	Longitude	Latitude	Herbarium code
Muscari neglectum Guss. ex ten (M1)		East Azarbaijan, Tabriz-Basmenj Rd, Khalatposhan	1564	38˚1ʹ54.214''	46˚23ʹ38.927''	13357
Leopoldia comosa (L.) Parl. (M2)	*Leopoldia comosa* (L.) Parl.	Ardabil, nowdeh village, Aq dagh mountain range.	1576	37˚21ʹ23.206''	48˚27ʹ38.892''	6915
Muscari botryoides (M3)		Ardabil, Asalem-Khalkhal Rd, Finaroud.	2024	37˚37'39.049''	48˚37'58.864''	---
Leopoldia comosa (L.) Parl. (M4)	*Leopoldia comosa* (L.) Parl.	East Azarbaijan, Isparaxan, Sahand mountain range.	2582	37˚48'49.716''	46˚24' 24.393''	6915
Bellevalia paradoxa (Fisch. & C.A.Mey.) Boiss. (B5)		East Azarbaijan, Isparaxan hotspring Rd, Sahand mountain range.	3094	37˚45'46.868''	46˚22' 56.392''	1669

Extraction of samples

The bulbs and leaves of samples were lyophilized (DENA®) at -20 °C and ground into a fine powder using mortar and pestle; the 200 mg of fine powder of each sample was added in 1800 µL of methanol and water (70:30). Samples were then being moved slightly for 30 minutes by shaker and centrifuged for 10 minutes at 10000 g. After separating the supernatant, 1800 µL of methanol and water (70:30) mixture was added to the vial, and the previous step was repeated. The supernatant obtained from two steps was transferred to new microtubes and mixed for further studies.

Total phenolic compound content

The total phenolic compound content of the bulbs and leaves extract was determined by spectrophotometric technique with the Folin-Ciocalteu reagent (Singleton and Rossi, 1965) with some modifications. As the standard for the calibration curve, pyrocatechol (Sigma C9510) (1-10 µg mL-) was used, following the mixing of 100 µL of extraction or standard and 2 mL of 2% Na_2CO_3 (Merck 106392). The samples were kept at room temperature for 2 minutes. After this time, 100 µL of Folin-Ciocalteu reagent (1/2 diluted) was added to the mixture, and samples were incubated in the dark for 30 minutes. The absorbance was recorded at 720 nm at the end with a UV-visible spectrophotometer (Specord, Analytik Jena, Germany). All the procedures were followed for the blank sample, though water was added instead of extracted. The results were interpreted as µg pyrocatechol equivalent (PEs) per mg dry weight of plant material.

Evaluation of antioxidant capacity by the DPPH assay

Radical scavenging capacity was determined with 2,2-Diphenyl-1-picrythydrazyl (DPPH) according to the method of Brand-Williams *et al*. (1995). A methanolic solution of DPPH (60 µM) was prepared for use as fresh. After adding of 50 µL of each extract to 1950 µL of DPPH solution, the reaction mixture was shaken vigorously and incubated in darkness at room temperature for 15 minutes. The absorbance of samples and control containing DPPH solution without extract was recorded at 517 nm using a UV-Visible. Methanol is used as a blank. The amount of DPPH radical scavenging activity by the methanolic extract of *Muscari* was calculated with the following formula:

Free radical scavenging activity = [1-(sample absorption- control absorption) / control absorption × 100]

After obtaining the percentage of free radical scavenging capacity, the IC_{50} value of the extract and ascorbic acid (Sigma A5960) were determined. The amount of sample concentration required for inhibition of 50% of the initial concentration of DPPH radicals was defined as the IC_{50} (µg of dry sample per mL), and its value is obtained by plotting different RSA values according to different sample concentrations and calculating the equation of the regression line.

Evaluation of saponin content by spectrophotometric analysis

The saponin content was determined using the method of Nickel *et al*. (2016). At first, 25 mL of 50% ethanol was added to 110 mg of fine powder of dry plant material and allowed to macerate for 72 h at room temperature. The extract was filtrated and marked up to 25 mL with 50% ethanol. To evaluate saponin content, 2 mL of extract or standard was added to 7 mL of Lieberman-Burchard reagent (16.7% of acetic anhydride in sulfuric acid concentrated). The mixture was incubated at room temperature for 30 minutes, and after that time, the absorbance of the mixture was recorded at 528 nm. A standard saponin curve (50-350 µg/mL) was used to measure saponin concentration in the samples, and the results were expressed as % dry weight of plant material.

Evaluation of total mucilage content

Dried samples powdered and defatted using petroleum ether solvent. After removing the solvent, the remaining materials were dried at room temperature. The 200 mg of dried plant material was kept in 1 mL of distilled water for 12 hours and then placed in a bain-marie at boiling temperature for one hour. The mixture was filtered and then mixed with the same volume of 96% ethanol to precipitate mucilage. Finally, the water from the samples was removed in a freeze-dryer, and mucilage powder was obtained. The amount of mucilage was expressed by its weight and its percentage over the weight of plant tissue (Deore and Khadabadi, 2008).

Evaluation of total alkaloid content

The total alkaloid of bulbs and leaf extracts was measured using the UV-visible technique (Sreevidya and Mehrotra, 2003). This method used a Dragendorff reagent (Sigma 44578), and the samples' absorbance was recorded at 435 nm. Different concentrations of bismuth nitrate (Sigma 254150) were used to plot the standard calibration curve, and the alkaloid concentration of all samples was measured using the calibration curve.

Evaluation of steroid content

The content of total steroids of sample extracts was measured by the Ray and Gupta

method (Ray and Gupta, 1994), with some adaption and the use of potassium hexacyanoferrate (Merck 702587). Sulfuric acid 4 N and 0.5% iron chloride (Merck 157740) were added to the methanolic extracts of samples and combined with 0.5% potassium hexacyanoferrate solution. The solution was incubated for 30 minutes at 70 °C; their absorbance was recorded at 780 nm. Cycloartenol (Merck 08172) was used as a standard, and the total steroid content of the samples was calculated in terms of mg of cycloartenol equivalent per gram of the sample's dry weight.

Statistical analysis

The Kolmogorov-Smirnov test analyzed data's normality and homoscedasticity using the Hartley test. The results were statistically evaluated by one-way analysis of variance (ANOVA), the Tukey test determined mean difference at a 5% level of probability, and correlations between variables were determined by the Pearson correlation coefficient ($P < 0.001$). All analyses were performed using IBM SPSS 21.0 software.

RESULTS AND DISCUSSION

Total phenols content

The total phenol content of leaves and bulbs of *Muscari* species and *Bellevalia* are given in table 2. Phenolic compounds are one of the primary metabolites of plants that correspond to the human diet. These compounds have various physiological properties such as anti-allergic, anti-arthrosis, anti-inflammatory, antimicrobial, antioxidant, anti-thrombosis, heart protection, and vasodilation. The beneficial effects of phenolic compounds have been attributed to their antioxidant activity (Balasundram *et al*., 2006). Therefore, total phenol and antioxidant capacity are the primary parameters to consider in plant samples. The content of total phenols in bulb extracts varied from 16.27 to 20.03 µg of PEs/mg of dry weight, and in leaf extracts ranged from 17.51 to 21.27 µg of PEs/mg of dry weight. In bulb extraction, *Bellevalia paradoxa* (B5) had the highest amount of total phenols content, and the lowest amount was determined in M1 and M3 ($P<0.05$). The B5 and M4 had the highest total phenol content in the leaf extracts ($P<0.05$). The leaves of M2 were provided lower phenols compared to the other species. The genotypes perfectly affected total phenol content, and the *Leopoldia* subgenus had more phenol in bulbs than the *Muscari* subgenus. The total phenols content of M2 and M4, which are the same species but coming from different locations, were the same in bulbs and leaves and had no significant difference ($P<0.05$). In the survey literature, the total phenol in *Allium oschaninii* was determined to be 17.18 mg gallic acid/g, which follows the results obtained from our examined cultivars (Lu *et al*., 2011). In the proposed research, the total phenol content of three varieties of edible onion, *Allium cepa* L., was much lower than *Allium toscanini*'s. Hence, these results confirm the high nutritional value of *Muscari*.

Table 2. Total phenolic compound (µg of PEs/mg) of bulb and leaf extracts.

Genotypes	Bulbs	Leaf
M1	16.27^{c*}	17.51^{c}
M2	17.68^{b}	18.56^{b}
M3	16.60^{c}	17.92^{c}
M4	17.37^{b}	19.37^{a}
B5	20.03^{a}	21.27^{a}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

The changes of secondary metabolites in the studied genotypes are affected by the genetic differences of the studied species, although, the climate will also affect the occurrence of these phenotypes. All species had higher total phenol content in their leaves than in their bulbs. Experiments conducted on *Muscari parviflorum* Desf. and *Allium jesdianum* (Ghasemi Pirbalouti, 2019; Mammadov *et al*., 2012), showed that the amount of total phenol in leaf extract was higher than in bulb extract, which is like our findings. It should be noticed that native people generally prefer leaves of M4 and M2 to those of other species.

Antioxidant capacity

Determination of antioxidant capacity by DPPH is an indirect method based on scavenging stable free radicals (DPPH) by hydrogen donors in plant extracts. This method is also the most common and oldest for antioxidant capacity determination (Roginsky and Lissi, 2005). Likewise, evaluating IC_{50} is an efficient way to compare the antioxidant activity of samples. In the current study, ascorbic acid was used as a standard index for measuring and comparing the antioxidant capacity of samples. The IC_{50} value has an inverse relationship with the antioxidant capacity; therefore, the higher the antioxidant capacity of a sample, the lower the IC_{50} value. Consumers are interested in edible flowers as food due to their benefits, such as their anti-allergy and anti-inflammatory properties. Scientific findings indicate that medicinal plants have other potential benefits, such as preventing oxidative damage, reducing blood sugar, and fighting cancer (Chensom *et al*., 2019; Fernandes *et al*., 2017). The antioxidant capacity of the bulb and leaf extracts of samples are listed in table 3. The bulb extract of B5 had significantly the lowest amount of IC_{50} at 195.5 µg/ml ($P<0.05$) comparing the extracts taken from the other bulbs. Therefore, the highest antioxidant capacity was related to the B5. The high antioxidant capacity in the *Bellevalia* bulb can be due to the presence of phenolic compounds in this plant; as seen in table 2, this plant has the highest concentration of phenolic compounds among the other samples. It has been proven in many reports that different levels of total phenol have a direct effect on the antioxidant activity of the plant (Santas *et al*., 2008; Sellappan and Akoh, 2002).

In Mexico, four types of common medicinal plants were examined, and *Myrtillocactus geometrizans* provided the highest antioxidant activity of 675.06 µmol TE/g DW. Also, this plant's phenol was obtained higher than other species (Pinedo-Espinoza *et al*., 2020). Both phenolic acids and flavonoid compounds, which are a subset of phenolic compounds, act as strong and well-known antioxidants by chelating various ions and combining with free radicals, especially superoxide, peroxyl, and hydroxyl radicals, therefore protecting against DNA damage and preventing the peroxidation of phospholipids, which can lead to the damage of biological membranes (Urquiza-Martínez and Navarro, 2016). In recent years, there has been interest in phenolic compounds and their antioxidant activity among consumers and the scientific community. Meanwhile, epidemiological studies have linked diets rich in natural antioxidants with a reduced risk of oxidative stress-related diseases such as cancer and cardiovascular disease (Chen *et al*., 2016). The lowest antioxidant capacity is obtained for M1, with 27.54% and 34.98% in bulb and leaf extracts, respectively. The antioxidant capacity of leaves was significantly higher than that in all species' bulbs ($P<0.05$), as it has been shown in a study that the antioxidant activity of the extract of the herbal parts of *M. neglectum* is higher than its bulb (Özkan *et al*., 2017). Comparing the samples taken from M2 and M4 plants revealed a significant difference in the antioxidant capacity of their extracts, and M2 had the highest antioxidant activity in both leaf and bulb extracts ($P<0.05$). It is probably caused by the climatic effect that ultimately led to changes in the production of metabolites and antioxidant

capacity. However, for the more correct and explicit conclusion, it is necessary to grow both plants in a similar climate at a greenhouse so that the plant's phenotype can be compared. Despite the low antioxidant capacity of genotype M1 compared to other studied genotypes, this species has a higher antioxidant capacity than many plants. For example, in the study about the antioxidant capacity of *Allium sativum* and *Allium ascalonicum* bulbs as two common edible species, the IC_{50} results were 5300 and 1330 µg/ml, respectively (Povichit *et al*., 2010), which are much higher than the genotypes of this study. Accordingly, it can be said that consuming a lower amount of *Muscari* bulbs compared to onions or shallots provides similar antioxidant compounds for the human body.

Table 3. Antioxidant activity of bulb and leaf extracts of different species.

Genotypes	Antioxidant capacity by DPPH			
	DPPH radical scavenging capacity (%)		IC_{50} (µg/ml)	
	Bulb	Leaf	Bulb	Leaf
M1	27.54^{e*}	34.98^{d}	277.3^{a}	322^{a}
M2	40.86^{b}	42.49^{b}	245.2^{c}	258^{c}
M3	30.19^{d}	39.14^{c}	246.4^{b}	273.3^{b}
M4	35.58^{c}	39.25^{c}	240.5^{b}	270.7^{b}
B5	44.55^{a}	66.83^{a}	179.0^{d}	195.5^{d}
Ascorbic acid	94.2	68.3		

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

T he study demonstrated a notable range in phenol content among the different genotypes. *Bellevalia paradoxa* (B5) exhibited the highest phenolic content in bulb and leaf extracts, suggesting a strong potential for antioxidant activity. This test was confirmed by the DPPH assay, where B5, with the highest antioxidant capacity, showed the lowest IC50 value, indicating its efficacy in scavenging free radicals. The higher phenol content and antioxidant capacity in leaves compared to bulbs across all species align with previous findings in similar studies (Ghasemi Pirbalouti, 2019; Mammadov *et al*., 2012; Pinedo-Espinoza *et al*., 2020). This could be due to the greater exposure of leaves to environmental stressors. The significant antioxidant capacity of *Muscari* species, even in the genotype with the lowest performance (M1), underscores their potential as dietary sources of antioxidants. The comparison with *Allium* species, commonly consumed for their health benefits, highlights *Muscari*'s competitive edge in providing similar or superior antioxidant benefits with potentially lower consumption quantities.

Saponins content

Saponin contents and their variation across the species and plant parts are illustrated in Fig. 2. Consuming saponins can reduce cholesterol concentration in the plasma, reducing the risk of heart disease and inducing cancer cell death through different pathways (Lorent *et al*., 2014). This is probably due to the stereo structure of the saponins. However, the health benefits of saponins make it attractive to find natural sources of saponins in the human diet. Among the species, this substance concentration varied from 1.85% to 4.03% in bulbs and from 1.30% to 3.21% in leaf extracts. In fiber-rich powders from asparagus, saponin content ranged from 2.14 to 3.64 mg/g (Fuentes-Alventosa *et al*., 2009). Saponins of some allium species, as common edible plants, were detected between 2 and 3 mg/g dry matter (Smoczkiewicz *et al*., 1982), which are close to the results of genotypes In this study. *Allium nigrum*, also known as black garlic, has a high saponin content in bulbs (19.38 mg/g DW), which is higher than that of leaves

(10.48 mg/g DW) (Mostafa *et al*., 2013). Plants of M2 and M4 showed the highest saponin content in bulb and leaf, respectively. This result illustrates the role of environmental factors in saponin fluctuations over different climatic regions.

Given that the saponin content of the bulbs was higher than in the leaves of all samples, this may be the reason for the higher degree of bitterness of the bulbs when compared to the leaves. The lowest saponin content belonged to the leaf extracts of M3, which were 1.30%. Although, saponin terpenoids increase the medicinal value of plants, due to the bitter and astringent taste, these parts may be less accepted by consumers, especially when eaten fresh.

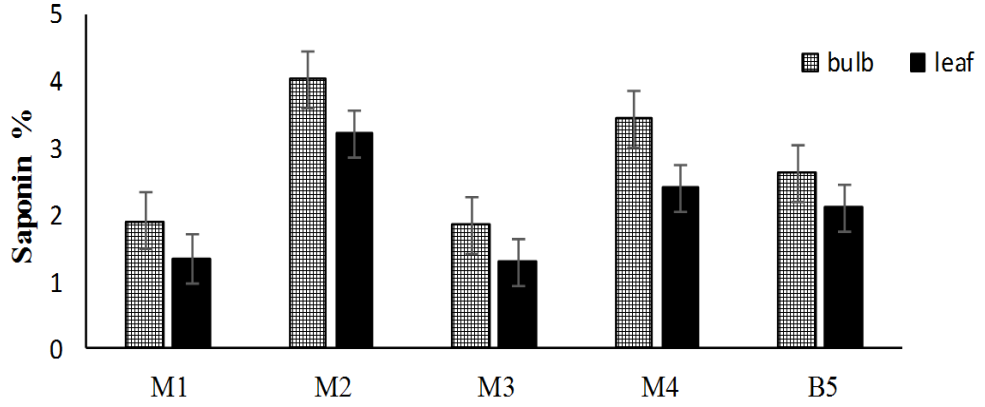

Fig. 2. Saponin content (%) of plant species' bulb and leaf extractions.*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

Saponins, known for their bitter taste and health benefits, varied significantly across the species. M2 and M4 showed the highest saponin content in bulbs and leaves, respectively. These findings suggest that environmental factors significantly influence saponin levels, which are higher in bulbs than in leaves (Ariyanti and Latifa, 2021; Mostafa *et al*., 2013). The bitter taste of high saponin content might affect consumer acceptance, especially for fresh consumption. However, their medicinal properties, such as cholesterol-lowering effects and immune system enhancement, make them valuable.

Total mucilage content

The mean comparison of mucilage percentage of bulb and leaf samples is given in table 4. Mucilage is a sticky, mucus-like substance produced by plants. Its main components are polysaccharides, proteins, minerals, lipids, and uric acid units. Researchers have proven that adding mucilage to food formulations improves nutritional quality (Goksen *et al*., 2023). This substance has also been reported to be beneficial for health; oral Mucilage consumption helps reduce blood cholesterol levels (Dhar, 2005). In general, the mucilage in bulb tissues was higher than in leaves in all plants, but the bulb of M3 had much lower mucilage than other species. Bulb of M2 consumed more than other bulbs and has the highest amount of mucilage, an essential factor in justifying its high use. The mucilage content of the bulb of M2 is higher than the yield of the *O. cuspidatum* bulb's mucilage reported in 2021, a plant of the same family. In this research, the mucilage percentage of the plant's root was obtained as 16.4%, and it was introduced as a suitable plant for use in the food industry with the property of thickening food and increasing its taste (Gheybi *et al*., 2021). Most of the Malvaceae family, such as *Adansonia digitata* L., *Gossypium* spp., *Hibiscus cannabinus*, *Plantago psyllium*, and *Abelmoschus* spp L., are known due to their significant mucilage content (Ahmad *et al*., 2009). The 21 species

of *Okra abelmoschus* spp L., one of the most famous mucilages edible plants, provide 6.52 and 37.67 mg/kg of mucilage in the fruits (Ahiakpa *et al*., 2014). The amount of mucilage in *Plantago ovata* and *P. psyllium* is obtained in the range from 13.52 to 18.60%, and these results showed that two different environments can differentiate genotypes in terms of the amount of mucilage (Shahriari *et al*., 2018).

In addition, mucilage has different properties depending on the type of monosaccharides that constitute it. Various commercial medicines have been formulated from mucilage. In this study, only the total amount of these substances has been evaluated, and accurate studies are needed to fully identify the mucilage components of *Muscari* genotypes.

Table 4. Percentage of mucilage extracted from plant samples.

Genotype	Bulb	Leaf
M1	12.73^{d*}	10.40^{c}
M2	19.29^{a}	15.32^{a}
M3	8.72^{e}	6.48^{e}
M4	16.60^{c}	7.06^{d}
B5	17.88^{b}	12.45^{b}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

The study confirmed that the mucilage content was higher in bulbs than in leaves, with M2 bulbs showing the highest content. This polysaccharide has various applications in the food industry due to its thickening properties and potential health benefits, including improved digestion and bowel health. The high mucilage content in *Muscari* species is advantageous for culinary and health applications, adding to their value as edible plants (Gao *et al*., 2017).

Total alkaloid content

The number of alkaloids in all samples was generally insignificant (Table 5). However, the highest amount was seen in both leaf and bulb extracts of B5 and M4. Alkaloids give the plant a bitter taste; in this sense, the public does not accept their presence and high amount in the edible plant. Some alkaloids, such as caffeine or quinine, cause bitterness in food products (Briand and Salles, 2016). The presence of alkaloids has been detected in *M. neglectum*, but its amount has not been measured (Nasrabadi *et al*., 2013). However, some alkaloids also have medicinal properties. A study reported that the main extracted alkaloids of *M. armeniacum* bulb included hyacinthine A1, A2, A3, and B3. Hyacinthacine alkaloids have glycosidase inhibitory activities. These alkaloids are anti-cancer, anti-viral, anti-diabetic, and anti-obesity compounds (Savaspun *et al*., 2014). In all samples, the amount of alkaloids in bulbs was higher than in leaves, which can justify the bitter taste in bulbs compared to leaves.

Table 5. Total alkaloids content (µg g^{-1} DW).

Genotype	Bulb	Leaf
M1	1.22^{e*}	1.10^{d}
M2	1.47^{d}	1.22^{c}
M3	1.59^{c}	1.45^{b}
M4	2.03^{b}	1.48^{b}
B5	2.21^{a}	2.06^{a}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

Toxicological studies on *Muscari* alkaloids are limited but can be toxic, particularly in high concentrations. However, *Muscari* species, such as hyacinthine A1, A2, A3, and B3, have been shown to exhibit significant bioactivity. These compounds can offer medicinal benefits, such as glycosidase inhibitory activities that may have anti-cancer, anti-viral, anti-diabetic, and anti-obesity effects (Savaspun *et al*., 2014). Still, they can also pose risks if consumed in large amounts.

Total steroids content

In previous studies, the presence of steroids in the leaves and bulbs of some *Muscari* species has been determined, but no study has been done on the total amount of steroids in this genus (Nasrabadi *et al*., 2013). Steroids are known for their medicinal properties, such as antitumor, liver protection, antibacterial, plant growth regulators, and antiparasitic, and they are attractive to herbalists (Petersen and Simmonds, 2003). The number of total steroids in the plant samples of this study is remarkable compared to the amount of the total steroid in the root of *Asparagus racemosus*, which is a well-known medicinal plant of the same family, and it was measured in previous studies as 27.5 µg/mg (Saraswathi *et al*., 2020). In a study on ten medicinal herbals in India, they set the amount of total steroid as the main parameter to determine the medicinal activity of the plant. The *Foeniculum vulgare* plant has shown the highest steroid amount of 68.39 µg/mg (Madhu *et al*., 2016). Comparing these results with our experiment highlights our studied genotypes. In general, the amount of steroid in leaves was higher than in bulbs, and the highest amount in both leaves and bulbs was related to B5, followed by M4 (Table 6).

The difference in the amount of steroids in genotypes was significant and considerable. Steroids do not have a noticeable effect on the taste and color of the product. In many cases, these substances do not have a known role in consumers' bodies, although some can act as compounds like animal steroid hormones. In this study, the total concentration of steroid compounds in the plant has been evaluated, and it is necessary to identify and accurately evaluate the steroids of *Muscari* in the following studies.

Table 6. Total steroids of plant samples (mg g^{-1} DW).

Genotype	Bulb	Leaf
M1	15.25^{d*}	67.45^{e}
M2	54.05^{c}	88.55^{c}
M3	50.80^{c}	77.42^{d}
M4	62.50^{b}	101.65^{b}
B5	70.62^{a}	110.12^{a}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

The study also assessed alkaloid and steroid contents, which, although generally lower than other metabolites, were significant in B5 and M4. Steroids were more concentrated in leaves than bulbs, with B5 again showing the highest content. Alkaloids contribute to the bitter taste but also possess medicinal properties such as anti-cancer and anti-viral activities (Karolkowski *et al*., 2023; Rajput *et al*., 2022). This highlights the dual ornamental and medicinal potential of *Bellevalia paradoxa*, suggesting further exploration and utilization of these compounds.

The present study aimed to evaluate the biochemical properties of different *Muscari* and *Bellevalia* genotypes to assess their potential edible species. The investigation focused on various genotypes' total phenol content, antioxidant capacity, saponin content, mucilage

content, alkaloid content, and steroid content. The results reveal significant variations among the genotypes, highlighting the potential of specific species for both nutritional and medicinal uses.

CONCLUSION

This study demonstrated the considerable potential of the *Muscari* and *Bellvalia* species for cultivation and utilization as a valuable source of nutrition and medicinal plants. Specifically, when comparing different genotypes, the *M. comosum* species, widely recognized as an essential edible genotype in countries like Turkey and Italy, emerged as the most suitable candidate for inclusion in domestication programs among the genotypes examined.

Moreover, the experiment revealed promising results for the less familiar *Bellevalia paradox*, which in certain instances exhibited outcomes comparable to or even superior to those of *Muscari* species. This suggests that *Bellevalia paradox* could be regarded as a valuable genotype with antioxidant-rich compounds, capable of competing with *Muscari* for significant presence within agricultural production settings.

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ارزیابی بیوشیمیایی ژنوتیپ‌های زینتی و بومی موسکاری و بلوالیا با نگرش کاربرد به‌عنوان گونه‌های ارزشمند تغذیه‌ای

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چکیده

موسکاری و بلوالیا دو گونه زینتی پیازی خویشاوند هستند که به‌صورت بومی در بسیاری از مناطق ایران رشد می‌کنند. این گونه‌ها به‌عنوان گیاهان زینتی گلدانی و فضای آزاد کاربرد بالایی دارند. از این گیاهان در نقاطی از دنیا به‌عنوان گونه‌های خوراکی نیز استفاده شده است ولی استفاده از آن‌ها در ایران بسیار محدود است. به‌نظر می‌رسد این گیاهان زینتی پتانسیل معرفی به‌عنوان ژنوتیپ‌های دارای ارزش خوراکی جهت ورود به فرآیند اهلی‌سازی و تولید تجاری را دارا هستند. کاربرد اصلی این گیاهان به‌جز موارد زینتی به‌عنوان مواد موسیلاژی و پر کننده در آشپزی مطرح است، گرچه به‌علت دارا بودن ترکیبات فنولی و ساپونینی، در ایجاد طعم و مزه نیز دخالت دارند. در پژوهش اخیر سه جنس موسکاری شامل *botryoides*, *comosum* و *neglectum* و یک گونه از جنس بلوالیا از رویشگاه‌های طبیعی جمع‌آوری شده و مورد ارزیابی قرار گرفتند. این گیاهان برای سنجش ظرفیت آنتی‌اکسیدانی، فنول کل، ساپونین، موسیلاژ، آلکالوئید و استروئید کل در عصاره برگ و پیاز انتخاب شدند. با بررسی جمیع موارد و ارزیابی متابولیت‌های گوناگون این گیاه، می‌توان چنین جمع‌بندی نمود که گونه *M. comosum* ژنوتیپی مناسب برای ورود به فرآیند اهلی‌سازی و همچنین به‌عنوان والد برای برنامه‌های هیبریداسیون است. از طرفی ژنوتیپ کمتر معرفی‌شده *Bellevalia paradoxa* نیز در مجموع دارای خصوصیات ارزشمندی است و به‌نظر می‌رسد ادامه مطالعات در مورد این ژنوتیپ نیز رویکردی صحیح خواهد بود.

کلید واژه‌ها: ظرفیت آنتی‌اکسیدانی، کلاغک، موسیلاژ، ترکیبات فنولی، ساپونین.

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ارزیابی تاثیر محلول‌پاشی نانوذرات‌کیتوسان و سالیسیلیک اسید بر روی روند کاهش پیری گلبرگ‌ها و افزایش کیفیت پس از برداشت گل‌های شاخه بریده رز رقم "سامورایی"

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چکیده

افزایش عمر پس از برداشت گل‌های شاخه بریده، اهمیت بسیاری در صنعت گل و گیاهان زینتی دارد. در مطالعه حاضر، اثربخشی نانوذرات کیتوسان و سالیسیلیک اسید در تأخیر پیری گلبرگ‌های گل رز شاخه بریده مورد ارزیابی قرار گرفت. این تحقیق در قالب طرح کاملا تصادفی با ۹ تیمار در شش تکرار انجام شد که اعمال تیمارها به روش محلول‌پاشی گلبرگی هر سه روز یک‌بار صورت گرفت. تیمار نانوذره کیتوسان با دو غلظت (۰/۱ و ۰/۵ میلی‌گرم بر لیتر) و سالیسیلیک اسید با دو غلظت (۰/۱ و ۰/۳ میلی‌مولار) بر کیفیت گل‌های شاخه بریده رز بررسی شد. صفات بیوشیمیایی و آنزیمی طی دوره زمانی ۱۵ روزه (۱، ۴، ۷، ۱۱، ۱۵ روز پس از برداشت) و صفات عمر گلجایی و جذب نسبی آب و نشت الکترولیت روزانه و تا پایان عمر گل‌ها اندازه‌گیری شدند. حداکثر عمر گلجائی ۱۷/۸ روز بود و در گل‌هایی که تحت محلول‌پاشی گلبرگی با ترکیب تیماری ۰/۵ میلی‌گرم در لیتر نانوذرات کیتوزان با ۰/۳ میلی‌مولار سالیسیلیک اسید قرار گرفتند، بیشترین عمر پس از برداشت و در تیمار شاهد (آب مقطر) کمترین عمر گلجائی (۱۳/۵ روز) مشاهده شد. همچنین میزان آب جذب شده نسبی گلبرگ‌ها و محتوای فلاونوئید در گلبرگ‌های تیمار شده توسط تیمار ترکیبی ۰/۵ میلی‌گرم در لیتر نانوذرات کیتوسان با ۰/۳ میلی‌مولار سالیسیلیک اسید به‌طور چشمگیری افزایش یافت. تیمارهای حاوی نانوذرات کیتوسان به‌ویژه تیمار ترکیبی ۰/۵ میلی‌گرم در لیتر نانوذرات کیتوسان با ۰/۳ میلی‌مولار سالیسیلیک اسید باعث کاهش میزان نشت الکترولیت با حفظ شاخص غشایی گلبرگ‌ها و افزایش عمر گلجایی شد. ضمنا فعالیت آنزیم‌های آنتی‌اکسیدانی از جمله گایاکول‌پراکسیداز، پلی‌فنول‌اکسیداز، پراکسیداز و هیدروژن‌پراکسید در گل‌های محلول‌پاشی شده با ترکیب تیماری ۰/۵ میلی‌گرم در لیتر نانوذرات کیتوسان با ۰/۳ میلی‌مولار سالیسیلیک اسید، کمترین میزان را نشان دادند. در میان غلظت‌های نانوذرات کیتوسان، طولانی‌ترین عمر گلجایی (۱۵ روز) مربوط غلظت ۰/۵ میلی‌گرم در لیتر کیتوسان بود که افزایش ۱۱ درصدی را در مقایسه با تیمار شاهد نشان داد، در حالی‌که بین تیمارهای اسید سالسیلیک، موثرترین تیمار برای افزایش عمر گلجایی با ۶ درصد افزایش نسبت به شاهد مربوط به تیمار ۰/۳ میلی‌مولار اسید سالسیلیک بود. در نهایت تیمارهای ترکیبی نانوذرات کیتوسان و سالیسیلیک اسید در مقایسه با تیمار شاهد ضمن افزایش کیفیت پس از برداشت رزهای شاخه بریده رقم "سامورایی" باعث افزایش چشمگیر ۳۱ درصدی عمر گلجائی رزها در مقایسه با گل‌های شاهد شدند.

کلید واژه‌ها: کیتوسان، ماندگاری، رز، اسید سالیسیلیک، محلول‌پاشی.

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تکثیر درون‌شیشه‌ای موثر ارکید فالانوپسیس رقم Karen Rockwell

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چکیده

فالانوپسیس یک جنس ارکید با ارزش اقتصادی بالا در گلکاری جهان است که به‌عنوان یک گیاه گلدانی و گل شاخه‌بریده استفاده می‌شود. تنوع ژنتیکی بالا و عدم یکنواختی رویشی و تکثیر تولیدمثلی، تولید این ارکید را از نظر اقتصادی غیرجالب می‌کند. تکثیر درون‌شیشه‌ای تنها روش امکان‌پذیر در مقیاس بزرگ برای تکثیر فالانوپسیس است. هدف از مطالعه حاضر، بررسی اثر انواع و غلظت‌های نفتالن استیک اسید (NAA) و بنزیل‌آدنین (BA) (هر دو در غلظت‌های ۰، ۰/۵، ۱، ۱/۵ و ۳ میلی‌گرم در لیتر، به‌صورت جداگانه یا ترکیبی)، در قالب طرح کاملا تصادفی در تکثیر درون‌شیشه‌ای فالانوپسیس بود. زغال فعال (AC؛ ۰، ۰/۵ و ۱ میلی‌گرم در لیتر) برای جلوگیری از قهوه‌ای‌شدن محیط و بافت‌ها به محیط اضافه شد. موراشیگ و اسکوگ (MS) و پروتوکورم به‌ترتیب به‌عنوان محیط کشت و ریزنمونه استفاده شدند. نتایج نشان داد که بیشترین تعداد برگ در محیط غنی‌شده با ۱ میلی‌گرم در لیتر NAA همراه با ۱/۵ میلی‌گرم در لیتر BA و ۱ میلی‌گرم در لیتر AC به‌دست آمد. تیمار حاوی ۱/۵ میلی‌گرم در لیتر NAA همراه با ۰/۵ میلی‌گرم در لیتر BA و ۱ میلی‌گرم در لیتر AC بیشترین تعداد ریشه را القا کرد. گیاهچه‌های کامل تولیدشده در شرایط درون‌شیشه‌ای به گلدان‌های حاوی مخلوطی از LECA (دانه‌های خاک رس منبسط‌شده سبک)، پیت ماس، کوکوپیت، خاک زغال چوب، کوکو چیپس و پرلیت منتقل شدند و در شرایط گلخانه‌ای با درصد بقای ۱۰۰ درصد سازگار شدند.

کلید واژه‌ها: زغال فعال، ارکیداسه، تنظیم‌کننده‌های رشد گیاهی، اجسام شبه‌پروتوکورم، کشت بافت.

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ارزیابی روش قلمه-پیوند برای تکثیر درختچه زینتی یاس خوشه‌ای پیوند شده بر روی پایه برگ نو

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چکیده

یاس بنفش (*Syringa vulgaris*) درختچه‌ای با ارزش است که به‌دلیل داشتن برگ‌ها و گل‌های زیبا با رایحه‌ای مطبوع در مناظر طبیعی مورد استفاده قرار می‌گیرد. پیوند یاس بنفش روی برگ نو (*Ligustrum vulgare*) به‌منظور کاهش زمان لازم برای تکثیر و استفاده از فواید پایه مورد ارزیابی قرار گرفت. روش تکثیر قلمه-پیوند یا ریشه‌زایی و پیوند همزمان یکی از روش‌های جدید برای تکثیر برخی از گیاهان چوبی است. در این تحقیق برای اولین بار از تکنیک قلمه-پیوند برای تکثیر یاس بنفش استفاده شد. دو روش قلمه-پیوند (نیمانیم و امگا) و سه غلظت (۰، ۱۰۰۰، ۲۰۰۰ و ۳۰۰۰ میلی‌گرم در لیتر) مورد ارزیابی قرار گرفت. با توجه به نتایج، قلمه-پیوند به‌روش نیمانیم به‌طور معنی‌داری درصد قلمه-پیوندهای ریشه‌دار شده و پیوندک‌های برگ‌دار شده را افزایش و میزان قلمه-پیوندهای خشک شده را نسبت به روش امگا کاهش داد. درصد تشکیل کالوس پایه، درصد پیوندک‌های برگ‌دار و وزن تر ریشه و اندام هوایی تولید شده با کاربرد ایندول بوتیریک اسید به‌طور معنی‌داری افزایش یافت. بهترین نتایج با ۲۰۰۰ میلی‌گرم در لیتر به‌دست آمد. کمترین درصد قلمه-پیوند خشک شده در این تیمار مشاهده شد. همچنین موفقیت نهایی قلمه-پیوند نشان داد که استفاده از روش نیمانیم و IBA بیشترین دستاورد را داشته است. به‌طورکلی، استفاده از غلظت ۲۰۰۰ میلی‌گرم در لیتر IBA همراه با روش قلمه-پیوند نیمانیم برای تکثیر درختچه زینتی یاس خوشه‌ای در این تحقیق توصیه می شود.

کلید واژه‌ها: قلمه-پیوند، پیوند، برگ نو، یاس بنفش، تکثیر.

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پاسخ‌های بیوشیمیایی گیاه همیشه‌بهار *Calendula officinalis* به تنش خشکی تحت تیمار الیستورهای غیر زیستی

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چکیده

اثر محلول‌پاشی الیسیتورهای غیرزیستی بر ترکیبات بیوشیمیایی گل همیشه بهار تحت تنش خشکی طی یک آزمایش فاکتوریل دو عاملی در قالب طرح بلوک‌های کامل تصادفی با سه تکرار مورد بررسی قرار گرفت. عامل اول محلول‌پاشی الیسیتورها در ۸ سطح (شاهد، آرژنین، گلوتامین، استرادیول، آرژنین + گلوتامین، آرژنین + استرادیول، گلوتامین + استرادیول، گلوتامین + آرژنین + استرادیول) و عامل دوم دور آبیاری در سه سطح (۱ روز، ۳ روز و ۶ روز) بود. متابولیت‌های بیوشیمیایی شامل کاروتنوئیدها، فلاونوئیدها، فنل‌ها، درجه بریکس گل و برگ اندازه‌گیری شد. براساس نتایج، الیسیتورها به‌طور معنی‌داری بر صفات بیوشیمیایی شامل کاروتنوئیدها، فلاونوئیدها، مواد فنولی و شاخص بریکس برگ و گل تأثیر معنی‌دار داشتند. دور آبیاری بر محتوای پرولین تأثیر معنی‌داری داشت ($P < 0.05$)، اما تأثیر آن بر سایر صفات معنی‌دار نبود. تجزیه واریانس اثرمتقابل محلول‌پاشی و دور آبیاری نشان داد که اثر متقابل بر محتوای مواد فنلی معنی‌دار بود ($P < 0.01$). بیشترین میزان مواد جامد محلول برگ (°Brix) تحت ترکیب تیماری گلوتامین × دور آبیاری ۶ روزه، بالاترین درجه بریکس گل تحت تیمار گلوتامین + استرادیول × دور آبیاری ۱ روز بدست آمد. در نهایت مشخص شد که گلوتامین در بهبود صفات مورد مطالعه بهتر از آرژنین و استرادیول عمل می کند. در شرایط تنش آبی برخی از صفات مانند مواد جامد محلول افزایش یافت. به‌طورکلی گلوتامین نسبت به آرژنین و استرادیول در بهبود صفات مورد مطالعه برتری دارد.

کلید واژه‌ها: آرژنین، گلوتامین، استرادیول، فنل‌ها، استرس کمبود آب.

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بررسی ۴ رقم گل کاغذی از نظر مواد معدنی، ویتامین ث و ظرفیت آنتی‌اکسیدانی

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چکیده

گل کاغذی (*Bougainvillea* spp.) به‌عنوان یک گیاه زینتی، دارویی و خوراکی شناخته شده است اما در پژوهش‌های پیشین به خوراکی بودن ارقام مختلف گل کاغذی کمتر پرداخته شده است. در این پژوهش ۴ رقم گل کاغذی (*B. glabra*, *B. glabra* Snow White, *B. specabilis* Scarlett OHara, *B. buttiana* Louis Wathen) به‌جهت مصارف خوراکی بررسی شدند. گل‌های کاغذی در مرحله گل کاملا باز از یک تولید کننده تجاری در استان گیلان-شهرستان تالش خریداری و از گلبرگ‌ها جهت تعیین درصد مواد معدنی، ویتامین C، آنتوسیانین گلبرگ و ظرفیت آنتی‌اکسیدانی استفاده شد. نتایج نشان داد که *B. specabilis* Scarlett OHara دارای بالاترین سطح فسفر (۳۲/۱۰ میلی‌گرم در ۱۰۰ گرم وزن تر)، کلسیم (۹۸ میلی‌گرم در ۱۰۰ گرم وزن تر)، آهن (۳/۷۹ میلی‌گرم در ۱۰۰ گرم وزن تر) و آنتوسیانین (۳۱/۱۴ میلی‌گرم در ۱۰۰ گرم وزن تر) است. بیشترین مقدار پتاسیم به‌ترتیب با ۱۸۱/۸۱ و ۱۸۱/۲۵ میلی‌گرم در ۱۰۰ گرم وزن تر برای *B. glabra* و *B. specabilis* Scarlett OHara و بیشترین مقدار روی (۰/۳۵ میلی‌گرم در ۱۰۰ گرم وزن تر) برای *B. glabra* ثبت شد. *B. glabra* Snow White ضعیف‌ترین منبع از نظر روی، آهن، فسفر و آنتوسیانین بود. *B. glabra* Snow White و *B. buttiana* Louis Wathen بهترین ارقام از نظر ویتامین C و *B. glabra* Snow White" و *B. glabra* نیز بهترین ارقام گل کاغذی از نظر ظرفیت آنتی‌اکسیدانی بودند. در کل مشخص شد که هر چهار رقم گل کاغذی دارای ارزش غذایی هستند اما با توجه به اینکه *B. specabilis* Scarlett OHara از نظر مواد معدنی و *B. glabra* Snow White از نظر ویتامین C و ظرفیت آنتی‌اکسیدانی نسبت به سایر ارقام برتری داشتند به‌عنوان بهترین ارقام جهت مصرف توصیه می‌شوند.

کلید واژه‌ها: گیاهان زینتی، گل خوراکی، ارزش غذایی، ترکیبات آنتی‌اکسیدانی، آنتوسیانین.

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