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Effect of Nitrate, Ammonium and Mesos on *In Vitro* Multiplication of *Gerbera jamesonii*: New Findings

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Abstract

Gerbera jamesonii (gerbera) is a commercially cultivated cut flower worldwide. Owing to high levels of heterozygosity, micropropagation is the most suitable approach for the rapid propagation of gerbera cultivars. The optimization of a suitable culture medium is critical for the micropropagation protocol and requires the adjustment of medium components, such as macro- and micronutrients. A full factorial experiment (2 × 2 × 3 × 3) was designed using KNO_3 at 0.5X and 1X levels, NH_4NO_3 at 0.5X, 1X, and 1.5X levels, mesos ($MgSO_4.7H_2O$, $CaCl_2.2H_2O$, and KH_2PO_4) at 0.5X, 1X, and 1.5X levels, and two cultivars (Artist and Brilliance) to analyze the effect of their single and multiple interactions on several *in vitro* growth parameters of gerbera. The current study indicated that the triple interaction among nitrate, ammonium, and mesos affects all growth parameters, and interestingly, KNO_3 × NH_4NO_3 did not affect shoot number. As a result, the highest number of shoots, the lowest number of roots, and the lowest length of the roots were obtained using low concentrations of KNO_3 (0.5X) and NH_4NO_3 (0.5X) and increased levels of mesos (1.5X). This research reports new findings on the interaction effects among macroelements and suggests how they can be modified to improve the rate of micropropagation as well as the quality of regenerated gerbera plants.

Keywords: Ammonium, Gerbera, Mesos, Micropropagation, Nitrate.

INTRODUCTION

Gerbera jamesonii Bolus is an ornamental perennial herb of Asteraceae and native to South Africa and Asia (Kumar *et al*., 2020). It is favored for its large, colorful flowers, and long-lasting blooming. Flowers can be produced in protected greenhouses under various climatic conditions. Gerberas can propagate both sexually and clonally. However, for commercial purposes, vegetative methods are too slow, and plant tissue culture is an effective method for rapid mass propagation. An efficient and productive *in vitro* culture system depends on the physiological status of the donor plant, genotype, explant type, medium composition, and their interactions. Moreover, the quality of regenerated plants directly affects not only the in vitro multiplication process, but also the adaptation and post-harvest stages. MS (Murashige and Skoog, 1962a) is commonly used basal culture medium for gerbera micropropagation (Cardoso and Teixeira da Silva, 2013).

The composition of nutrients in the multiplication medium determines the proliferation rate and quality of *in vitro* plantlets. Supplementation of culture medium with adequate amounts of major essential elements (N, P, K, Ca, Mg, and S) contributes to high efficiency of propagation rate, high-quality plants for post-harvest procedures, and low cost of commercial production. In a current study, ¼ or ½ MS was used to improve multiplication rate of gerbera cultivars (da Silva *et al*., 2020). The same results reported by Shabanour *et al*. (2011) and half strength MS supplemented with 2 mg L^{-1} BAP (6-benzylaminopurine) induced low number of shoots. Cioc *et al.* (2022) used MS medium supplemented with 1.1 mg L^{-1} BAP for multiplication of gerbera. However, there is no information regarding the quality of the plants after several subcultures. Additionally, the NH^{+}_{4} / NO_3 ratio is the main factor affecting the quality of the produced plants. Their excessive application causes vitrification during the first or second subculture in some varieties or after successive subcultures in some tolerant varieties. Therefore, it is crucial to determine the optimum concentration of macronutrients in the culture medium.

Overall, in previous studies, optimization was limited only to reducing all macronutrients together by the same ratio (Cardoso and Teixeira da Silva, 2013), but generally, each macronutrient has a different concentration, and even two of them, including KNO_3 and NH_4NO_3 are present in a higher proportion relative to KH_2PO_4, $MgSO_4.7H_2O$, and $CaCl_2.2H_2O$. To date, there have been no reports on the interaction between different concentrations of dominant salts, including KNO_3, NH_4NO_3, and mesos ($MgSO_4.7H_2O$, $CaCl_2.2H_2O$, and KH_2PO_4), in the culture medium of gerbera. This study aimed to examine the relationship between different concentrations of these macronutrients and analyze their single effects and interactions. These results provide important clues for improving the micropropagation protocol of gerbera.

MATERIALS AND METHODS

Explant preparation and culture media

Young and immature capitulum explants (with 10 – 15 mm in diameter) of two G*erbera jamesonii* cultivars (Brilliance and Artist) were collected and used for establishment step. The explants were washed under tap water for 30 min and then immersed in 70% alcohol for one minute followed by thorough washing three times with sterilized distilled water. For the final surface sterilization, explants were immersed in 20% commercial bleach with 0.01% Tween-20 for 20 min, followed by washing three times with sterilized distilled water.

The basal medium used in this research was MS containing 30 g L^{-1} sucrose, and 0.7% agar. The pH of all media was adjusted to 5.7 before autoclaving at 121 °C and 110 kPa for 30 min.

Establishment step

Sterilized explants were cut into three pieces and cultured in the establishment culture

medium containing 0.5 mg L^{-1} TDZ (Thidiazorun). Cultures were kept in a growth chamber at 25 °C with a 16 h photoperiod, cool white fluorescent bulbs (PPFD = 35 µmol m^{-2} s^{-1}), and 8 h darkness. After two months, regenerated shoots were transferred to the multiplication medium and used for subsequent experiments.

Multiplication step

A full factorial experiment (2 × 2 × 3 × 3) was designed to evaluate the effects of the main macrosalts at various concentrations, including KNO_3 at 0.5X and 1X levels; NH_4NO_3 at 0.5X, 1X, and 1.5X levels; mesos (including KH_2PO_4, $MgSO_4$. $7H_2O$, and $CaCl_2$. $2H_2O$) at 0.5X, 1X, and 1.5X levels; and two cultivars (Brilliance and Artist) on *in vitro* propagation parameters. All media contained 0.3 mg L^{-1} BA (6-benzyladenine) and 0.05 mg L^{-1} NAA (naphthalene acetic acid). Finally, all possible combinations of factors at all levels produced 36 treatments (Table 1). The concentration of macronutrients was calculated according to the MS medium. In other words, 1X is the corresponding concentration of a mineral in the MS medium. Three replicates (jars) were prepared for each treatment, and each jar included three gerbera axillary shoots. Treatments were transferred to a growth chamber with a 16 h photoperiod, temperature of 25 °C and irradiance of 35 µmol photon m^{-2} s^{-1}. The characteristics evaluated after 45 d of culture were the number of shoots, shoot length of the highest shoot (cm), length of the roots (cm), and number of roots.

Table 1. The four factors and their components were used to construct a 2 × 2 × 3 × 3 full factorial design. Concentrations are expressed as MS levels.

Factors	**Component**		
Cultivar	**Artist**	**Brilliance**	**-**
KNO_3	0.5X	1X	-
NH_4NO_3	0.5X	1X	1.5x
Mesos	0.5X	1X	1.5x

In vitro rooting

Rooting medium was composed of half-strength MS medium supplemented with 0.5 mg L^{-1} NAA, 15 g L^{-1} sucrose, 1.5 g L^{-1} activated charcoal, and 0.7% agar. Single shoots were cultured on rooting medium and maintained in a growth chamber under the conditions described above for 30 days.

Acclimatization

Rooted plantlets were carefully washed with tap water to remove agar from the roots and transplanted into pots containing peat moss and perlite at a ratio of 1:1. The pots were then transferred to the greenhouse at 24 / 22 ± 2°C (day/night), 80% humidity, and natural light conditions. The pots were kept under plastic sheets, which were then gradually removed.

Statistical analysis

This study used a 2 × 2 × 3 × 3 factorial experiment arranged in a completely randomized design with three replicates. The effects of the treatments were tested for significance using analysis of variance (ANOVA). Duncan's post hoc multiple range test was used to separate significantly different means and to provide homogeneous groups for the means ($P < 0.05$). All data were subjected to statistical analysis using SAS software (version 7.1; SAS Institute, Cary, NC, USA).

RESULTS

The capitulum explants were successfully established on medium culture (Fig.1). New regenerated shoots were multiplicated by subculture on MS containing 0.3 mg L^{-1} BA and 0.05 mg L^{-1} NAA. The applied levels of TDZ, BA and NAA in both establishment and multiplication steps are based on our previously unpublished trials in the lab.

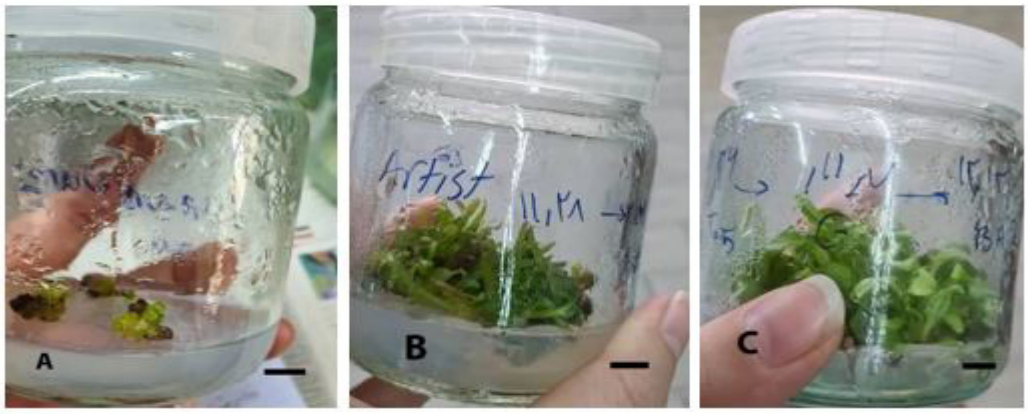

Fig. 1. *In vitro* establishment stage of gerbera capitulum as explants (A); Development of shoots in two gerbera cultivars: Artist (B) and Brilliance (C) (scale bar = 5 mm).

This study was conducted to elucidate the significance of the single effects double, triple, and quadruple interactions of KNO_3, NH_4NO_3, and mesos on multiplication parameters (shoot number, shoot length, root number, and root length) of two gerbera cultivars, Brilliance and Artist. Thus, there were four main factors in analysis of variance. Interaction effects result from the combined effects of the factors on the dependent variable. The interaction effect is significant when the impact of one factor depends on the level of the other factor. Part of the power of ANOVA is the ability to estimate and test interaction effects (Vittoz, 2021). A summary of ANOVA results is presented in table 2.

Table 2. Two-way ANOVA test for shoot number, shoot length, root number, and root length in *Gerbera jamesonii*.

S.o.V	Shoot number	Shoot length	Root number	Root length
Cultivar	0.0004**	0.0032**	<.0001**	0.0074**
KNO_3	<.0001**	<.0001**	<.0001**	<.0001**
NH_4NO_3	<.0001**	<.0001**	0.04*	0.133^{ns}
Mesos	0.5862^{ns}	<.0001**	0.3796^{ns}	0.2608^{ns}
Cultivar × KNO_3	0.4101^{ns}	0.4854^{ns}	0.0003**	0.4701^{ns}
Cultivar × NH_4NO_3	0.7382^{ns}	0.7566^{ns}	0.2716^{ns}	0.464^{ns}
Cultivar × Mesos	0.1177^{ns}	0.7538^{ns}	0.8051^{ns}	0.5493^{ns}
KNO_3 × NH_4NO_3	0.4704^{ns}	0.0182*	<.0001**	<.0001**
KNO_3 × Mesos	0.3144^{ns}	0.1327^{ns}	0.27^{ns}	0.2752^{ns}
NH_4NO_3 × Mesos	0.0003**	0.0051**	0.759^{ns}	<.0001**
Cultivar × KNO_3× NH_4NO_3	0.0784^{ns}	0.3214^{ns}	0.0682^{ns}	0.002**
Cultivar × KNO_3 × Mesos	0.8497^{ns}	0.7629^{ns}	0.0410^{ns}	0.1480^{ns}
KNO_3 × NH_4NO_3 × Mesos	0.0003**.	0.0174**	0.0007**	0.0062**
Cultivar × KNO3 × NH_4NO_3 × Mesos	0.2032^{ns}	0.2660^{ns}	0.2725^{ns}	0.0871^{ns}

*Significant with p-value < 0.05; **Significant with p-value < 0.01.

Shoot number

As demonstrated by statistical analysis, the effects of KNO_3 × cultivar, NH_4NO_3 × cultivar, mesos × cultivar, KNO_3 × NH_4NO_3 × cultivar, KNO_3 × mesos × cultivar, NH_4NO_3 × mesos × cultivar, and KNO_3 × NH_4NO_3 × mesos × cultivar were not significant at the 0.05 level of probability for shoot number (table 2). However, the effect of the cultivar was statistically significant for this parameter. As shown in table 2, the main effects and interactions are significant. Single effects of KNO_3 and NH_4NO_3 significantly affected shoot number, but mesos did not. In contrast, the triple interaction, KNO_3 × NH_4NO_3 × mesos, was statistically significant for shoot number (Table 2). This means that each factor independently accounted for variability in the dependent variable in its own right. However, they interact synergistically to explain the variance in the dependent variable. Together, these two factors do something else beyond their separate independent main effects. The significance of the KNO_3 × NH_4NO_3 × mesos effect versus the insignificance of the KNO_3 × NH_4NO_3 × mesos × cultivar effect is considered an advantage for optimizing tissue culture protocols. In fig. 1, the means of this significant triple interaction were compared by using Duncan's multiple range test. Shoot number was highly influenced by low concentrations of KNO_3 (0.5X) and NH_4NO_3 (0.5X), and increased mesos (1.5X) (Fig. 2 and 3). Using this combination, 8.75 shoots per explant were produced. Increasing KNO_3 to 1X led to a sharp decrease in shoot number. At 0.5X KNO_3 and by increasing NH_4NO_3 to 1X or 1.5X, the shoot number was gradually reduced.

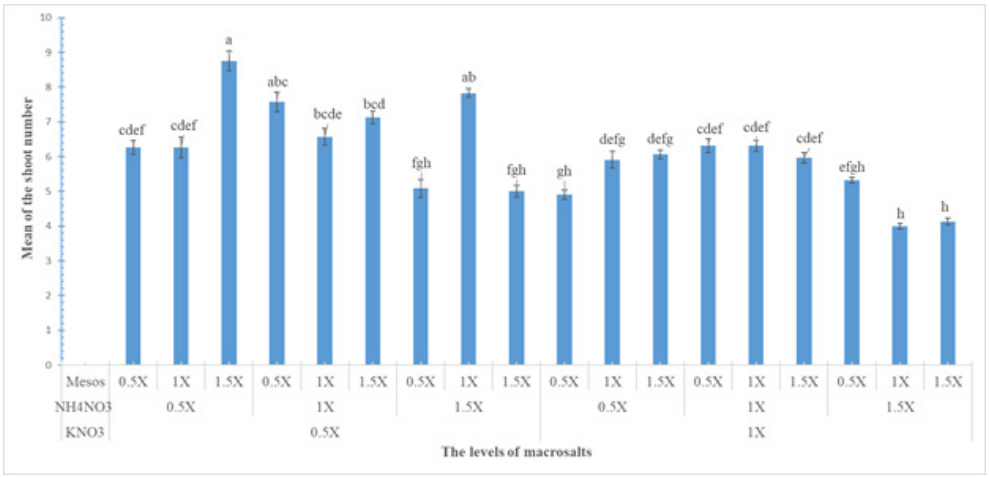

Fig. 2. Comparison of the means of the KNO_3 × NH_4NO_3 × mesos interaction effect on the number of regenerated shoots in G*erbera jamesonii* using Duncan's multiple range test. Horizontal axis: KNO_3 at 0.5X and 1X levels; NH_4NO_3 at 0.5X, 1X, and 1.5X levels; mesos at 0.5X, 1X, and 1.5X levels.

Fig. 3. High numbers of regenerated shoots in two *Gerbera* cultivars (left: Brilliance, right: Artist) on multiplication medium containing 0.5 × KNO_3, 0.5 × NH_4NO_3, and 1.5 × mesos (scale bar = 5 mm).

Shoot length

Shoot length was also affected by the KNO_3 × NH_4NO_3 × mesos interaction independent of the cultivar effect (Table 2). According to the results of the mean comparison analysis, higher levels of KNO_3 and NH_4NO_3 produced shorter shoots. However, at low KNO_3 and/or NH_4NO_3 concentrations and low mesos, shorter shoots were produced in both cultivars (Fig. 4).

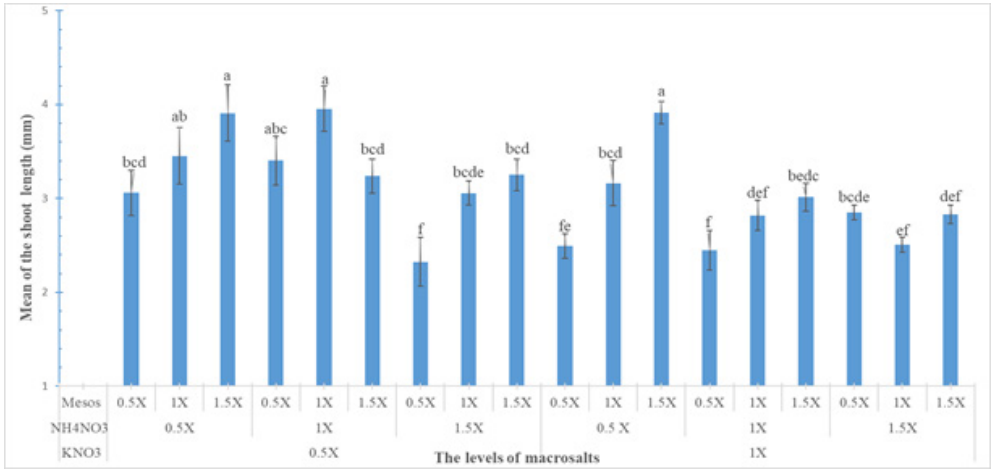

Fig. 4. Comparison of the means of the KNO_3 × NH_4NO_3 × mesos interaction effect on the length of regenerated shoots in *Gerbera jamesonii* using Duncan's multiple range test. Horizontal axis: KNO_3 at 0.5X and 1X levels; NH_4NO_3 at 0.5X, 1X and 1.5X levels; mesos at 0.5X, 1X and 1.5X levels.

Root number

Root number was also influenced by the KNO_3 × NH_4NO_3 × mesos interaction instead of the KNO_3 × NH_4NO_3 × mesos × cultivar interaction (Table 2). Mean comparison analysis showed that the root number decreased with low KNO_3 (0.5X), low NH_4NO_3 (0.5X), and high mesos (1.5X) (Fig. 5). In contrast, high KNO_3 (1X), low NH_4NO_3 (0.5X) and high mesos (1.5X) concentrations produced a greater number of roots.

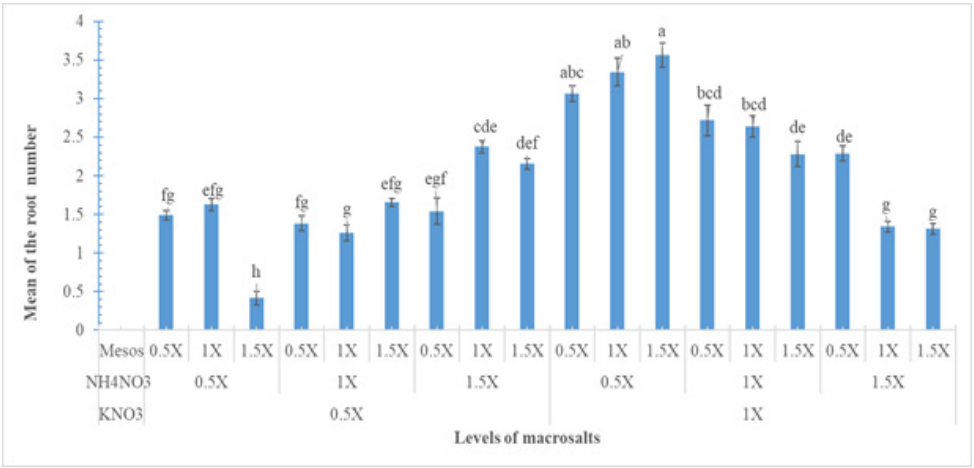

Fig. 5. Comparison of the means of the KNO_3 × NH_4NO_3 × mesos interaction effect on the number of regenerated roots in *Gerbera jamesonii* using Duncan's multiple range test. Horizontal axis: KNO_3 at 0.5X and 1X levels; NH_4NO_3 at 0.5X, 1X and 1.5X levels; mesos at 0.5X, 1X and 1.5X levels.

Root length

Root length showed the same response as root number, and was influenced by the KNO_3

× NH_4NO_3 × mesos interaction (Table 2). Root length decreased by low KNO_3 (0.5X), low NH_4NO_3 (0.5X) and high mesos (1.5X) as shown by mean comparison test (Fig. 6). In contrast, high KNO_3 (1X), low NH_4NO_3 (0.5X) and low mesos (0.5X) produced greater number of roots.

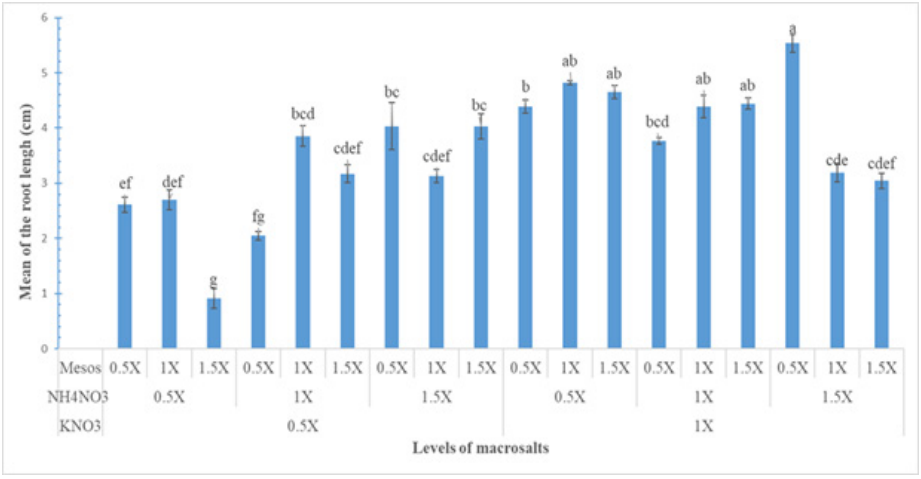

Fig. 6. Comparison of the means of KNO_3 × NH_4NO_3 × mesos interaction effect on length of regenerated roots in *Gerbera jamesonii* using Duncan's multiple range test. Horizontal axis: KNO_3 at 0.5X and 1X levels; NH_4NO_3 at 0.5X, 1X and 1.5X levels; mesos at 0.5X, 1X and 1.5X levels.

One of the main results in this research was the insignificance of the KNO_3 × NH_4NO_3 × mesos × cultivar interaction effect on shoot number, shoot length, root number, and root length. This was in contrast to KNO_3× NH_4NO_3 ×mesos interaction effect, which was significant for all four traits.

Acclimatization

In vitro-rooted shoots were transferred to the soil for *ex vitro* establishment. As a result, 80% of plantlets were successfully established and survived (Fig. 7).

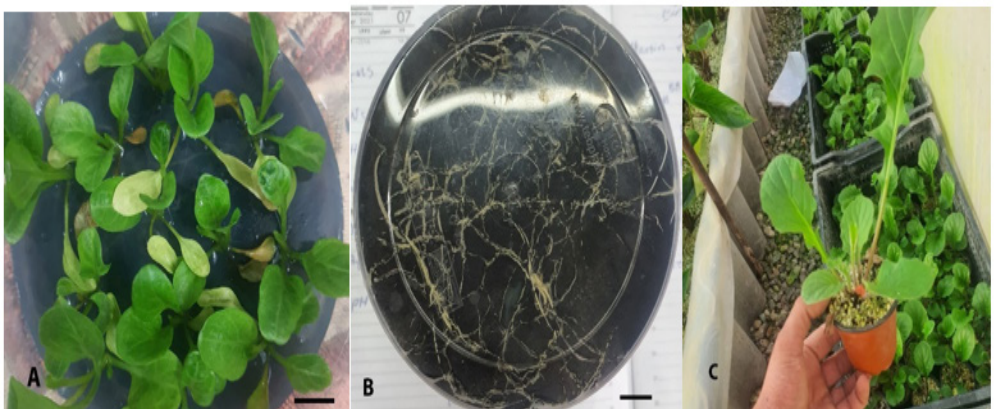

Fig 7. *In vitro* rooted gerbera shoots (A) and (B): Acclimatized plants grown in a greenhouse (C) (scale bar = 10 mm).

DISCUSSION

In gerbera, the effects of mineral nutrients on micropropagation efficiency have mostly been studied based on modification of the MS medium. Mineral availability and uptake in the

culture medium affect the growth, development, and quality of gerbera during *in vitro* culture, and their optimized concentrations in the medium are crucial for improving the proliferation efficiency and plant quality. Despite many reports in the literature, there are no useful universal protocols for commercial laboratories. Additionally, most studies have examined the effects of different strengths of MS medium on the *in vitro* multiplication of gerbera, instead of focusing on the components of the medium. In this regard, we disclosed the alterations in the macronutrient levels of the MS medium and their interaction effects at the multiplication stage of gerbera.

In our investigation, the first remarkable finding was the significance of the interaction among KNO_3, NH_4NO_3, and mesos, which is independent of the genotype effect. This result is supported by previous studies that used a half-strength MS medium for different cultivars. Rezende *et al*. (2008) used ½ MS to regenerate 'Jaguar Cream' cultivar. Cardoso and Teixeira da Silva (2012) used ½ MS for proliferation of several gerbera cultivars.

In the current study, we concluded that the combination of low KNO_3 (0.5X), low NH_4NO_3 (0.5X) and high mesos (1.5X) improved the number of shoots produced per explant. According to the statistical analysis, using moderate KNO_3 (1X), shoot number decreased significantly. Moreover, by increasing the NH_4NO_3 concentration, the shoot number began to decrease perceptibly. In a recent study, gerbera shoot culture at MS medium with 50% of salts (MS ½) and 25% of salts (MS ¼) resulted in higher shoot formation (3.34 and 3.32 shoots/plants, respectively) (da Silva *et al*., 2020). Nitrate ions are an essential source of nitrogen for most plant cultures; however, they must first be converted into ammonium inside the cell. Due to its toxicity, ammonium cannot be the sole available source of nitrogen; therefore, nitrate is added to the culture medium. The proportion of NH_4^+ nitrogen is high in MS medium and growth of plant cultures may also be hindered in media due to high concentrations of NH_4^+ even in the presence of high concentrations of NO_3^- at the same time. The free ammonium ion can lead to a rise in ethylene production, at least in whole plants (Ma *et al*., 2023). High amounts of ammonium ions in the culture medium can cause regeneration of stunted or hyperhydric plants. In *Bryophyllum*, using machine learning based tool, ammonium was identified as a significant factor affecting shoot number, as low concentrations promoted shoot multiplication (Lozano-Milo *et al*., 2022). In three varieties of Malus domestica Borkh, NH_4NO_3, $CaCl_2$, and $MgSO_4$ at 0.5× MS, was significantly better for two of the three cultivars (Kabylbekova *et al*., 2020).

We also investigated the best concentration of mesos, and in contrast to low amounts of KNO_3 and NH_4NO_3, high levels of mesos increased the propagation efficiency. Our result was in accordance with da silva *et al.* (da Silva *et al*., 2020). They reduced the MS concentration up to one-fourth in the multiplication medium and added 250 mg L^{-1} calcium silicate resulted in 3.32 to 6 shoots per plant. A previous study demonstrated the positive effect of increasing the concentration of mesos salts of MS medium ($MgSO_4$, $CaCl_2$, KH_2PO_4) to improve the number of shoots (Poothong and Reed, 2014). Hunková *et al*. (2020) indicated that a treatment of MSx3 mesos components ($MgSO_4$, $CaCl_2$, KH_2PO_4) increased *in vitro* growth of several berry fruits and Amelanchier alnifolia compared to MSx4. Using machine learning to optimize culture medium of *Actinidia argute*, Hameg *et al*. (2020) demonstrated that low K^+ with high concentration of SO_4^{2-} increased shoot number. In addition, they reduced nitrogen content to 20% but increased mesos up to 200% compared to MS (Hameg *et al*., 2020). In a previous study, the highest multiplication rate for *Amelanchier alnifolia* was achieved with tripled mesos, whereas Rubus fruticosus reacted positively to a lower (1 – 2X) concentration of mesos. Decreasing the concentration of mesos to half led to worse quality in both blackberry and blueberry shoots (Hunková *et al*., 2020).

Another observation in our study was the growth of longer shoots after the application of higher meso concentrations. A similar effect in micropropagated red raspberries, *Rubus ideaus*, was reported by previous study (Poothong and Reed, 2014). In *Salvia santolinifolia,* MS medium modification with NH_4NO_3 (412 mg L^{-1}), KNO_3 (475 mg L^{-1}) and $CaCl_2.2H_2O$ (880 mg L^{-1}) was found the best medium to increase multiplication rate (Jan *et al*., 2023).

CONCLUSIONS

This study presents a significant triple interaction effect among macronutrients in the culture medium on the multiplication rate of two gerbera cultivars. We recommend low concentrations of KNO_3 and NH_4NO_3, in addition to high levels of mesos compared to MS medium, to improve the propagation efficiency of gerbera *in vitro*. Additional detailed analysis could provide new insights into the role of each component of mesos group in the micropropagation of gerbera plants.

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Humic Acid and Iron Fertilizers Enhance the Growth Responses and Antioxidant Enzyme Activity of Cineraria (*Pericallis* × *hybrida* L.)

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Abstract

Cineraria (*Pericallis hybrida* L.) has faced growth hindrance and chlorosis. Humic acid, iron sulfate and iron chelate fertilizers were investigated for cineraria cultivation in alkaline soil. The study was arranged in a factorial experiment based on a completely randomized design with three replications and 4 samples in each replication. The media were enriched using humic acid (0, 0.5, 1 g/kg soil) and iron fertilizers (5 and 10 mg/kg iron sulfate and 5 and 10 mg/kg iron chelate). The plants that were treated with soil supplemented with 1 g/kg humic acid in combination with 10 mg/kg iron chelate exhibited improvements in plant height (86%), stem diameter (100%), fresh root weight (170%), flowering period (166%), flower number (182%), inflorescence number (252%), flower diameter (59%) and total chlorophyll content (300%). The application of 1 g/kg humic acid and 10 mg/kg iron chelate increased the mineral element content of potassium, nitrogen and phosphorous by 179%, 193% and 675%, respectively. Combinations of 0.5 g/kg humic acid and 10 mg/kg iron chelate enhanced the anthocyanin (131%), leaf area (140%), TSS (332%) and starch (642%). Fertilization with 0.5 g/kg humic acid in combination with 5 mg/kg iron chelate resulted in the highest activity of the antioxidant enzymes SOD (238%), POD (324%), and CAT (667%), and reduced ion leakage by 60%. 1 g/kg humic acid as a biofertilizer in combination with 0.5 mg/kg iron chelate are suggested for use in the production of plants in soils with stressful high pH conditions.

Keywords: Antioxidant enzymes activity, Cineraria, High soil pH, Leaf chlorosis, *Pericallis* × *hybrida* L.

INTRODUCTION

Cineraria (*Pericallis hybrida*) is a flowering plant that belongs to the Asteraceae family and is full of numerous small daisy-like flowers surrounded by large green foliage (Kasem and El-baset, 2016). It is a popular New Year's Day and Spring Festival decorative potted flower and bedding plant (Jin-gang *et al*., 2014). The main advantage of this plant is its wide range of flower colors (white, pink, blue, red, purple, etc.). The capitulum sizes and types of flowers also vary widely (single, semidouble and double) (Kasem and El-baset, 2016). Although, cineraria is a perennial plant, it is used in commercial production as an annual plant where it needs a neutral to slightly acidic medium for optimal growth and development (Dole and Wilkins, 1995).

One of the main hurdles for plant growth and development in alkaline soils is microelement shortage, especially iron deficiency, which results in leaf chlorosis. Even though micronutrient elements are only required in trace amounts for sufficient plant development and production, their absence can result in micronutrient shortages during physiological and metabolic processes. As a result, micronutrient fertilizer applied in cultivation zones may not fulfil crop needs for root growth and nutrient absorption (Pirzad and Shokrani, 2012).

Plants require iron for growth and development. Biochemical processes such as respiration, photosynthesis, and symbiotic nitrogen fixation all require iron as an activator. Iron is a crucial element in the production of chlorophyll, and a lack of iron causes chlorosis. Many ideal landscape and agricultural plants are affected by iron shortages and thus exhibit chlorosis symptoms on their leaves. Iron deficiency in plants causes chlorophyll to be produced in insufficient quantities, resulting in pale green and yellow leaves (Pirzad and Shokrani, 2012; Nemati Lafmejani *et al*., 2018; Ali *et al*., 2021). Ferrous sulfate, which contains 98% ferrous sulfate heptahydrate, is a regularly utilized substance for this purpose. Instead, an equivalent amount of chelated iron can also be utilized (Mousa *et al*., 2015). Plants can absorb and use the iron chelator Fe-EDTA through the tissues of their leaves or through the roots if it is supplied to the soil. However, soil conditions, notably pH, affect nutrient absorption (Nemati Lafmejani *et al*., 2018; Gabra, 2021). Furthermore, the efficacy of spraying leaves with inorganic and chelated Fe fertilizers (Fe-SO_4, Fe-EDTA, Fe-DTPA, Fe-EDDHA, and Fe-citrate) for overcoming Fe deficiency is highly variable and depends on the solubility, stability, leaf cuticle penetration ability, mobility, and translocation of the fertilizers following diffusion into leaf tissues (Ghafari and Razmjoo, 2013).

On the other hand, biofertilizers are replacing chemical fertilizers due to environmental concerns. Humic acid can be used as a direct replacement for synthetic fertilizers to boost crop yields, or it can have an indirect effect by altering soil structure (Memon *et al*., 2014). Humic acid is a natural polymer with carboxyl and phenolic groups and is derived from various sources, such as humus, peat, oxidized lignite and coal (Adam, 2021). Humic acid forms stable and soluble complexes with micronutrients as a result of chemical and biological interactions in the soil, known as humification. Humic acid has a molecular weight of 3,000 to 30,000 Daltons and forms stable and soluble complexes with micronutrients (Boogar *et al*., 2014). It has also proven to be useful in mineral nutrient absorption. Humic substances provide support to growing plants, increase soil fertility and productivity, increase the water holding capacity of soil, and assist plants in drought resistance and seed germination. These advantages are attributed to humic acid. It improves nutrient accessibility, enhances root system expansion, enhances soil aeration and drainage, boosts protein and mineral contents in most crops, and creates a favourable environment for microorganism development (Memon *et al*., 2014; Rasouli *et al*., 2022). Previous studies have revealed the beneficial effects of humic acid fertilizer on yield increases in ornamental plants (Mirzaee *et al*., 2020; Adam, 2021), vegetables (Noroozisharaf

and Kaviani, 2018; Abbass *et al*., 2020; Kamali Omidi *et al*., 2022), medicinal plants (Hourani, 2022; Rasouli *et al*., 2022), fruits (Mohamed *et al*., 2020) and nuts (Li, 2019).

This study is the first to report the effects of humic acid, a biofertilizer, an iron chelate and iron sulfate, which are chemical fertilizers, on the morphological, physiological and biochemical characteristics of cineraria (*P. hybrida*). Additionally, the antioxidant enzyme activity of the fertilized plants was evaluated under alkaline soil conditions. The findings of this study could be useful in the floriculture industry for cineraria production under unfavourable high-pH conditions in the soil and for alleviating iron deficiency.

MATERIALS AND METHODS

Plant materials and treatments

The authors confirm that the present study complies with national regulations and international guidelines and legislations for research on plants. This study adheres to the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. The seeds of cineraria (*Pericallus x hybrida* cv. Early perfection Red) were purchased from Takii Seed Company (Takii, Japan). The plants were subsequently grown for 15 days in seed trays containing germination peat substrate, after which the plants were transplanted into plastic pots containing a mixture of leaf mold, sand, and soil (2:1:1). The study was carried out in a greenhouse with an average day/night temperature of 28 ± 2/20 ± 2°C and a relative humidity of 55±5%.

This research was conducted as a factorial experiment based on a completely randomized design (CRD) with three replications and 4 sub replicates. As the plants reached the six fully expanded leaf stage, the soil was treated with humic acid (0, 0.5, or 1 g/kg) and iron fertilizers (5 mg/kg iron sulfate, 10 mg/kg iron sulfate, 5 mg/kg iron chelate, or 10 mg/kg iron chelate). It is worthy to note that as the content of the iron in the tested soil was 4 (mg/kg), by adding 1 (mg/kg) iron sulfate and 6 (mg/kg) iron chelate, the final iron concentration of the soil was reached to 5 (mg/kg) and 10 (mg/kg), respectively. The treatments were applied monthly, and soil fertilization continued for six months.

Plant height and stem diameter

At the end of the experiment, the length of the stem was measured from the crown to the end of the last meristem. The stem diameter was measured using a digital calliper.

Flowering characteristics

The flower diameter was measured using a digital calliper. The petal, flower and inflorescence numbers were counted in each pot. The number of days from the beginning of flowering of the first flower to the full opening of the last flower on each plant was calculated as the flowering duration.

Root fresh weight

At the end of the experiment, the roots were weighed using a digital balance.

Leaf area

Leaf area was measured (Delta-T-Devices LTD England) on 3 leaves that had been collected from the 3rd, 5th and 7th leaves from the bottom to the top of the plant. The results are presented as the means of three leaves.

Chlorophyll determination

Chlorophyll and carotenoid contents were measured according to the methods described by Hiscox and Israelstam (1979). Chlorophyll contents were determined by a spectrophotometer (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., USA) at wavelengths of 645 and 663 nm for chlorophylls (a, b and total). DMSO was used as the extraction solvent for the pigments. Chlorophyll concentrations were calculated by the following equations, and the results are expressed as mg/g FW:

$$\text{Chlorophyll a (mg/g FW)} = \frac{12.7(A_{663}) - 2.69(A_{645}) \times \text{Volume made}}{\text{Wt of the sample}}$$

$$\text{Chlorophyll b (mg/g FW)} = \frac{22.9(A_{645}) - 4.68(A_{663}) \times \text{Volume made}}{\text{Wt of the sample}}$$

$$\text{Total Chlorophyll (mg/gFW)} = \frac{20.2(A_{645}) + 8.02(A_{663}) \times \text{Volume made}}{\text{Wt of the sample}}$$

where Wt is the weight of the sample and A λ is the absorption at wavelength λ (nm).

Total soluble solids and starch

To determine the soluble solids content, 0.1 g of leaf powder was extracted with ethanol (80%). The samples were centrifuged at 5000 rpm for 20 min, after which the volume of the supernatant was adjusted to 10 ml. The soluble solid content was measured according to the method described by Fox and Robyt (1991). The starch content of the samples was determined according to the method described by McCready *et al*. (1950) using anthrone reagent. The starch content was read by a spectrophotometer (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., USA) at a wavelength of 630 nm.

Anthocyanin content

The anthocyanin content of the petals was measured according to the method of Wang *et al*. (2017). One hundred milligrams of fresh petals were extracted with 1 ml of methanol containing 1% HCl and placed on a shaker at 150 rpm at 4°C overnight. Then, the extract was centrifuged at 10500 rpm for 10 min. The content of anthocyanin was measured according to the following formula. The results are expressed as micrograms per gram of fresh weight.

$$\text{Anthocyanin content (mg/L)} = (A530\text{-}0.25) \times A\ 657$$

$$\mu\ g/(g\ F.W.) = ((\text{Anthocyanin content (mg/L)} \times \text{Volume of solvent})/(\text{Sample weight})) \times 1000$$

Phosphorous, nitrogen and potassium contents

The dry ash method was used to measure phosphorous (P) and potassium (K). Leaf samples were dried at 70°C for 48 hrs in an oven. The dried samples were ashed at 500 °C for 4 hr and subsequently dissolved in 10 ml of 2 N hydrochloric acid. The phosphorous concentration was determined using spectrophotometry according to the method of Murphy and Riley (1962). Potassium was measured via flame emission spectroscopy. Nitrogen (N) uptake was determined according to the Kjeldahl method, described by Bremner (1996).

Ion leakage

Ion leakage from the leaves was measured using the method of Gulen and Eris (2004). A total of 0.1 g of fresh leaf disc was washed with distilled water and placed in a test tube filled with 15 ml of distilled water. After shaking for 24 h, the electrolytic conductivity (EC1) of the samples was measured using a digital conductivity meter. The samples were then autoclaved at 115°C for 20 min. Then, the solutions were cooled, and the conductance was measured (EC2). The extent of ionic leakage (EC%) was calculated as the percentage of the final reading via the following equation:

$$EC\% = EC1/EC2 \times 100$$

Antioxidant enzyme activity

Leaf samples were subjected to enzyme extraction according to the protocol described by Ozden *et al.* (2009). The CAT activity was measured as described previously (Dhindsa *et al*., 1981). and the absorbance of 1 mL of the reaction mixture (50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 50 µL of extracted enzyme) was read at 240 nm. CAT activity was assessed as the reduction in H_2O_2 with an extinction coefficient of 39.4 mM^{-1} cm^{-1} and is expressed as units/mg FW. For the SOD assay, the decrease in light absorbance of the nitrobuterazolium chloride (NBT) superoxide complex under the influence of enzyme activity was measured at 560 nm. The activity of the SOD enzyme was measured using the method of Giannopolitis and Ries (1977) and is expressed as units/mg FW. For the POD assay, 50 µl of enzyme extract, 2.9 ml of 10 mM potassium phosphate buffer (pH = 7) and 0.05 ml of 20 mM guaiacol were used as the reaction mixture. The reaction was started by adding 20 µL of 40 mM H_2O_2. Using the extinction coefficient of 26.6 mM^{-1} cm^{-1}, the activity of the enzyme was calculated and reported as micromoles of oxidized guaiacol in one minute per gram of fresh weight of the sample.

Statistical analysis

The data were analysed using SAS software (ver. 9.4), and the means were compared using the least significant difference (LSD) test at $P < 5\%$.

RESULTS

Shoot and root growth

The application of humic acid increased the leaf area (60%), stem diameter (61%), plant height (81%) and fresh weight of roots (92%) of the cineraria plants compared with those of the control (Fig. 1). When humic acid was used in combination with iron fertilizer, shoot and root growth nearly doubled. Combining 0.5 g/kg humic acid with 10 mg/kg iron chelate increased the leaf area by 140%. Treatment of plants with 1 g/kg humic acid in combination with 10 mg/kg iron chelate increased the plant height, stem diameter and fresh weight of roots by 86%, 100%, and 170%, respectively (Fig. 1).

Flowering characteristics

Treatment of *P.* × *hybrida* with humic acid increased the flower diameter (35%), flowering duration (86%), flower number (115%) and inflorescence number (155%) of cineraria plants compared with those of the control (Fig. 2). When humic acid was used together with iron fertilizer, the flowering characteristics exhibited a multifold increase. The combination of 1 g/kg humic acid and 10 mg/kg iron chelate increased the flower diameter, flowering duration, flower number and inflorescence number by 59%, 166%, 182% and 252%, respectively (Fig. 2).

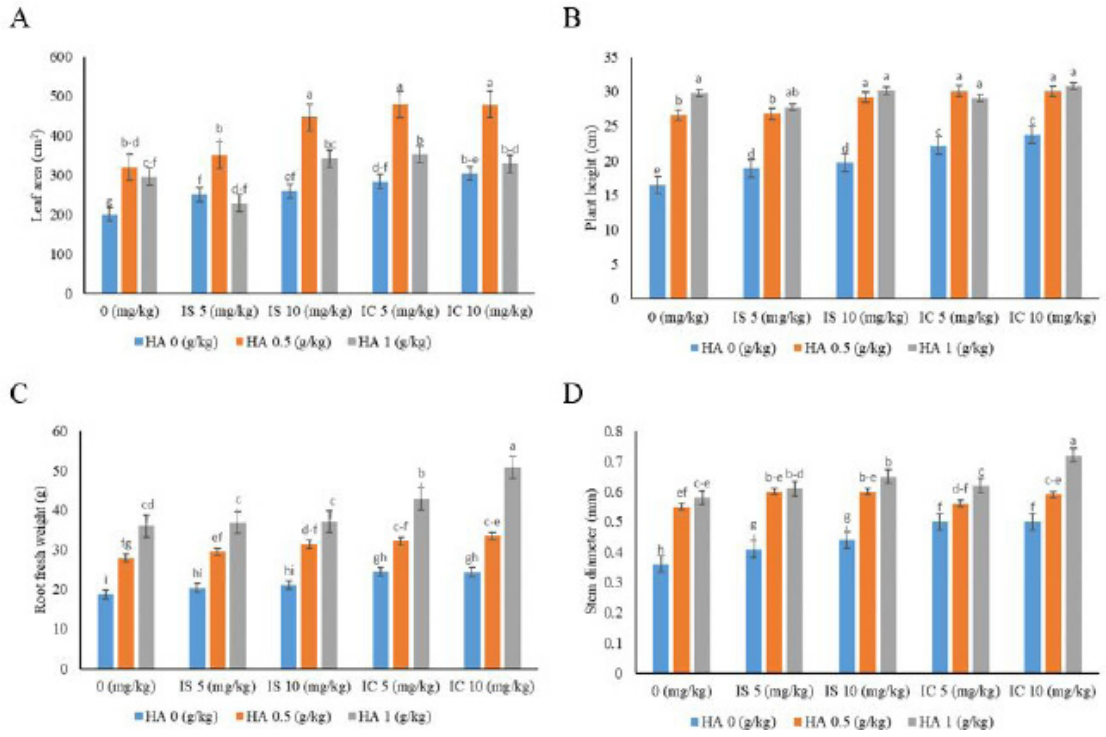

Fig. 1. Shoot and root growth enhancement of *P.* × *hybrida* by application of humic acid and iron fertilizers. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

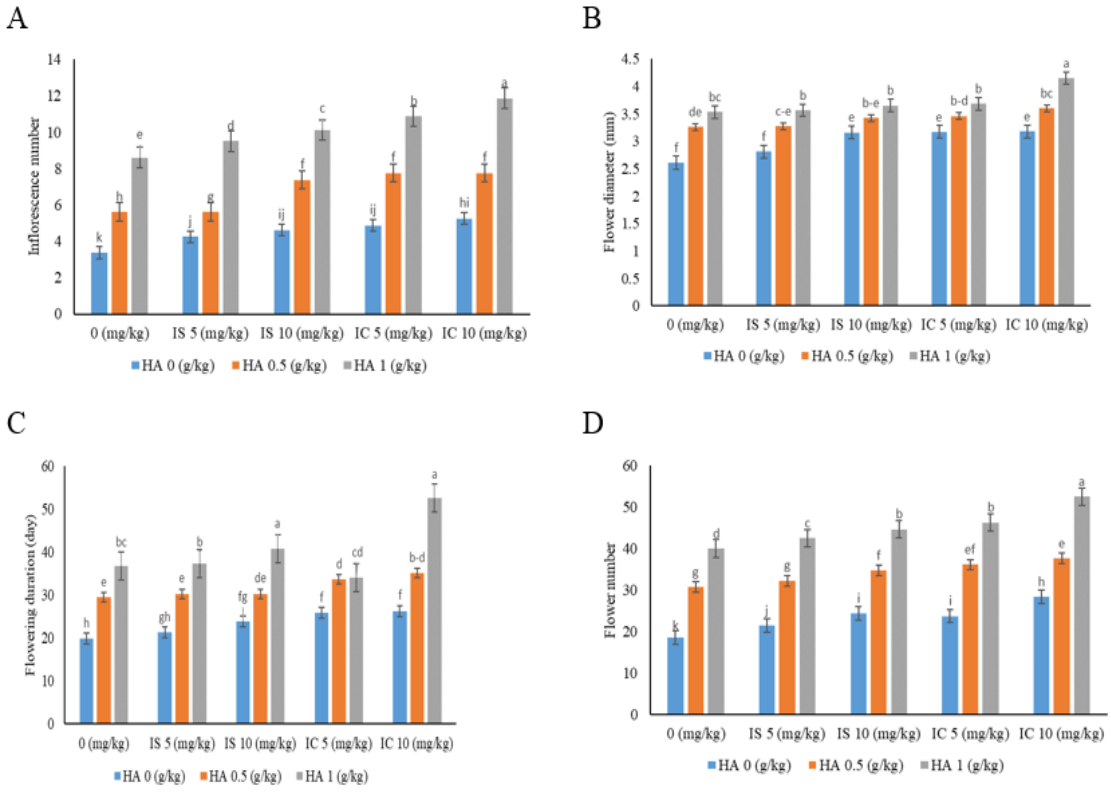

Fig. 2. Improvement of cineraria flowering by application of humic acid and iron fertilizers. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

Chlorophyll content

Compared with those of the control, the fertilization of cineraria plants with humic acid doubled the chlorophyll content of the leaves (Fig. 3). When humic acid was used in combination with iron fertilizer, the chlorophyll content tripled. The combination of 1 g/kg humic acid and 10 mg/kg iron chelate improved the chlorophyll content by 3-fold (Fig. 3).

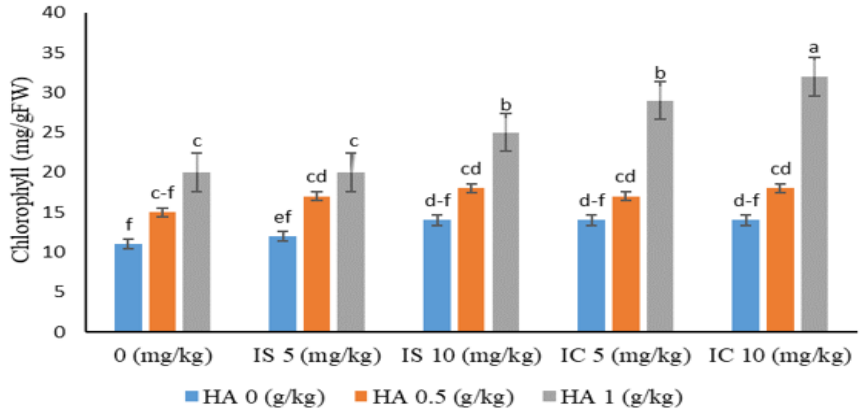

Fig. 3. The effect of humic acid and iron fertilizers on the chlorophyll content of cineraria leaves. HA= Humic acid, IS=Iron sulfate, IC=Iron chelate.

Total soluble solids, starch and anthocyanin content

Humic acid increased the anthocyanin (26%), total soluble solids (68%) and starch (190%) contents of the cineraria leaves compared with those of the control (Fig. 4). When humic acid was used in combination with iron fertilizer, the anthocyanin, total soluble solids and starch contents greatly increased. Coapplication of 0.5 g/kg humic acid with 10 mg/kg iron chelate amplified the amount of anthocyanin, total soluble solids and starch content by 131%, 332% and 642%, respectively (Fig. 4).

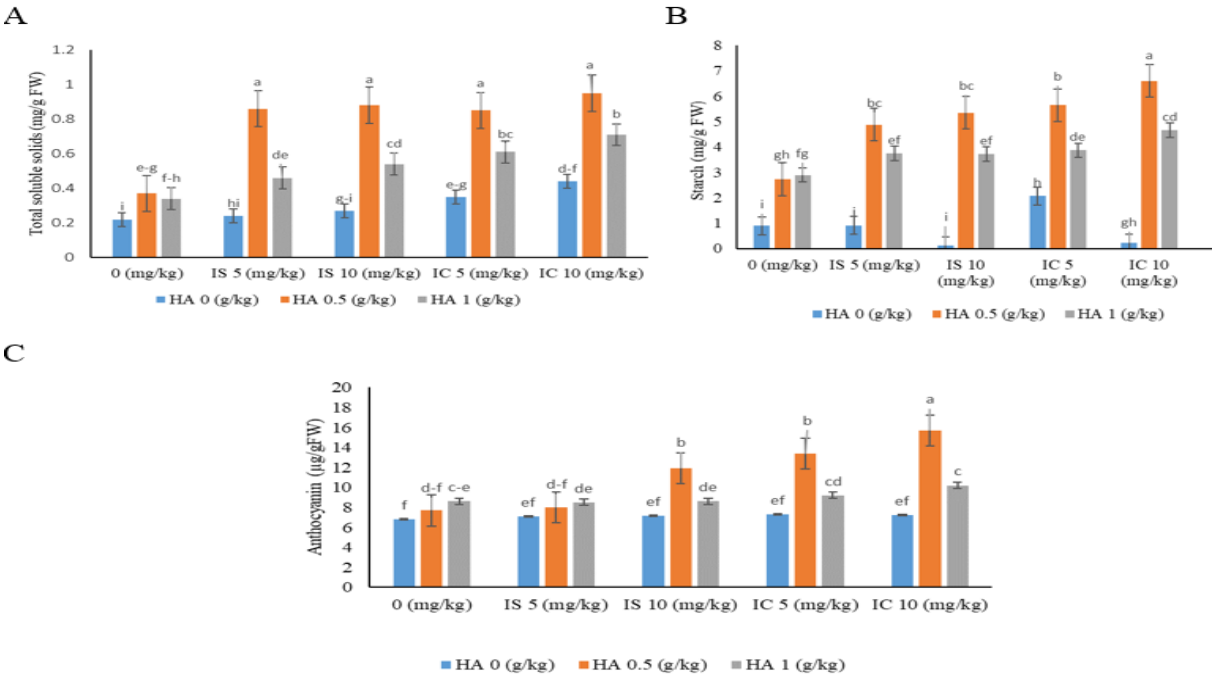

Fig. 4. The effect of humic acid and iron fertilizers on total soluble solids, starch and anthocyanin content of cineraria. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

Phosphorous, nitrogen and potassium contents

Treatment of cineraria with humic acid increased the potassium (13%), nitrogen (101%) and phosphorous (308%) contents of the leaves compared with those of the control (Fig. 5). When humic acid was used together with iron fertilizer, slight increases in the phosphorous, nitrogen and potassium contents of the plants were observed. The combination of 1 g/kg humic acid and 10 mg/kg iron chelate increased the potassium, nitrogen and phosphorous contents of the plants by 179%, 193% and 675%, respectively (Fig. 5).

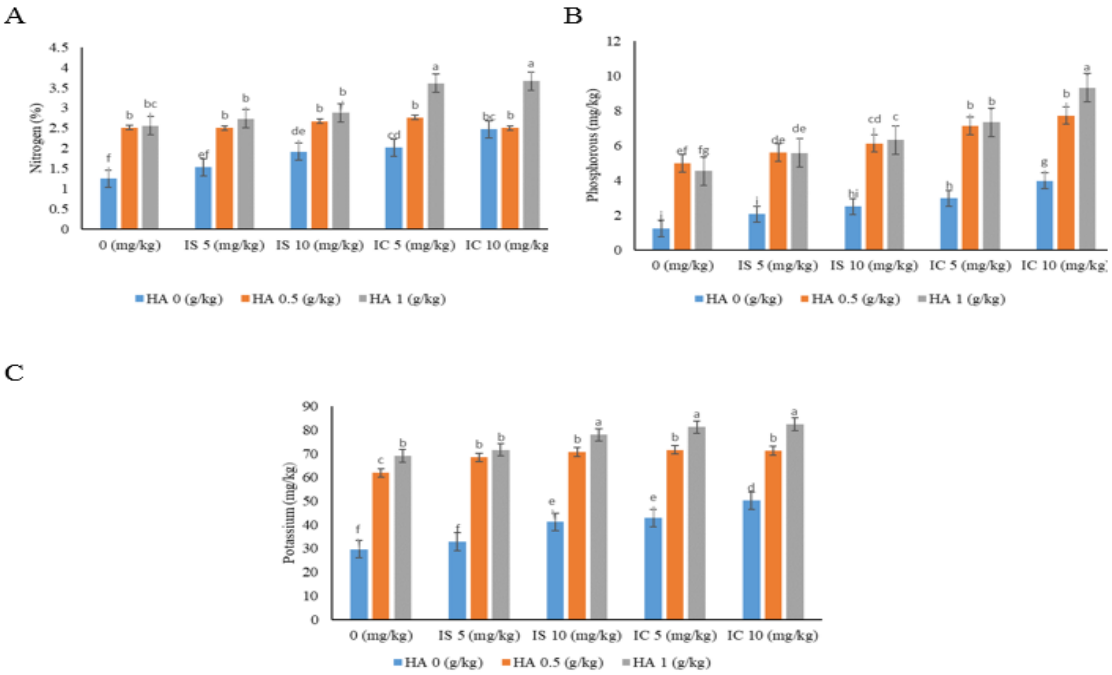

Fig. 5. Effect of different levels of iron and humic acid on phosphorous, nitrogen and potassium content of *Pericallis* × *hybrida*. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

Ion leakage

Compared with that in the control treatment, humic acid application to the cineraria plants decreased the ion leakage of the plants by 13% (Fig. 6). When humic acid was used in combination with iron fertilizer, the ion leakage drastically diminished. The combination of 0.5 g/kg humic acid and 5 mg/kg iron chelate had the least ion leakage and a 60% reduction (Fig. 6).

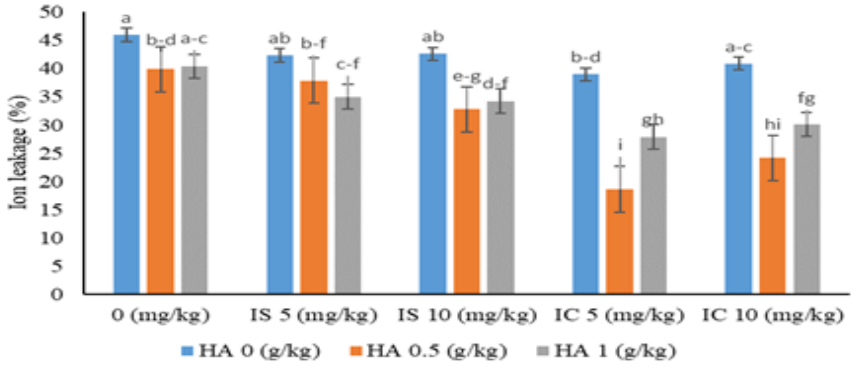

Fig. 6. Effects of different levels of iron and humic acid on the ion leakage of *Pericallis* × *hybrida*. HA= Humic acid, IS=Iron sulfate, IC=Iron chelate.

Antioxidant enzyme activity

Humic acid increased the antioxidant enzyme activity of SOD (59%), POD (172%) and CAT (267%) in *Pericallis* × *hybrida* plants compared with those in the control group (Fig. 7). When humic acid was used in combination with iron fertilizers, the antioxidant enzyme activity strongly increased. Cotreatment with 0.5 g/kg humic acid in combination with 5 mg/kg iron chelate increased the activity of the antioxidant enzymes SOD, POD and CAT by 238%, 324% and 667%, respectively (Fig. 7).

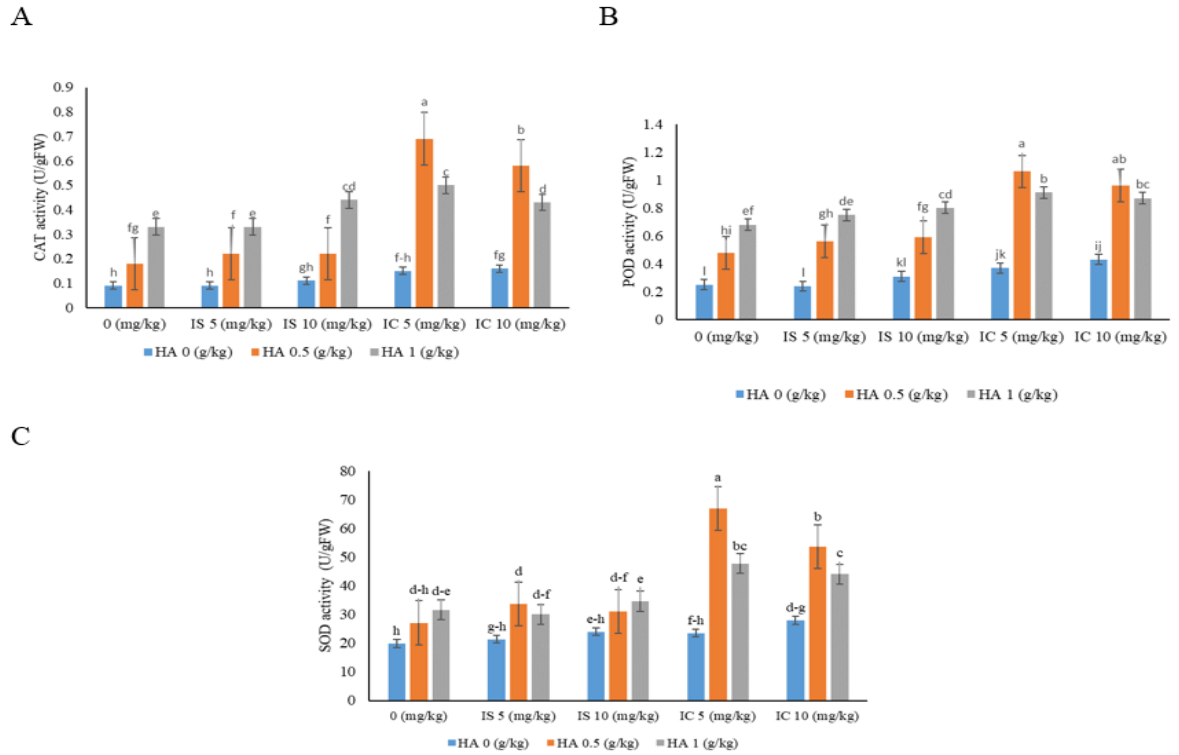

Fig. 7. Effect of different levels of iron and humic acid on antioxidant enzymes activity of cineraria. HA= Humic acid, IS= Iron sulfate, IC= Iron chelate.

DISCUSSION

Iron is required for enzyme systems, chlorophyll synthesis, enzyme activity, and the regulation of respiration, photosynthesis, nitrate and sulfate assimilation, which are important for plant development and reproduction (Salem *et al*., 2019). When the cineraria plants in the present study were treated with the highest concentrations of humic acid and iron chelate fertilizers, the plants presented the most photosynthetic pigments, which is consistent with previous observations (Haghighi *et al*., 2012; Fan *et al*., 2014; Salem *et al*., 2019). Iron is involved in the synthesis of chlorophyll and is required for the maintenance of chloroplast structure and function; therefore, utilizing appropriate treatments will improve chlorophyll synthesis in leaves (Salem *et al*., 2019). On the other hand, humic acid application could enhance NO_3 uptake, N metabolism, and protein production and increase chlorophyll content (Haghighi *et al.*, 2012).

The impact of iron on increasing plant growth and leaf area has been confirmed in previous reports, including for safflower plants (Ibrahim, 2019). In addition, humic acid is absorbed by plant roots and translocated to shoots, which in turn enhances plant growth responses. The current research revealed that humic acid and iron fertilizer improved vegetative

growth in cineraria plants, which was in agreement with the findings of Ahmad *et al*. (2013) in *Gladiolus* and Hembrom and Singh (2015) in *Lilium.* The increase in fresh weight of cineraria roots due to humic acid consumption can be attributed to the important role of humic acid in the formation of lateral roots through enhancing the root surface and subsequently increasing nutrient absorption. Similar results were found by Cordeiro *et al.* (2011) in *Zea mays* plants. In accordance with the results of the present study, humic acid application prominently improved gladiolus growth due to its impact on photosynthetic activity, nitrogen metabolism and protein synthesis (Baldotto and Baldotto, 2013). The use of humic acid by plants enhances photosynthetic activity and increases leaf area. Given the role of humic acid in hastening protein synthesis, enhancing water and nutrient absorption, and increasing fertilizer use efficiency (Rasouli *et al*., 2022), increasing plant growth is expected.

The use of humid acid and iron chelate fertilizer increased cineraria height, which could be related to the role of iron in activating the vegetative growth rate, cell division, and elongation of ornamental plants, resulting in greater stem height (Salem *et al*., 2019). Cineraria stem length also greatly increased when various doses of humic acid were used. These findings validated the significance of humic acid in increasing stem length and overall plant quality by enhancing nitrogen uptake. Ahmad *et al.* (2013) reported similar findings of improved nutrient absorption (i.e., N, P, and S) as a result of humic acid uptake. Additionally, the increase in flowering stem diameter and length can be attributed to the auxin-like action of humic acid (Mirzaee Esgandian *et al*., 2020).

Moreover, an investigation of humic acid and iron chelate fertilizers applied to *Cineraria* plants in this study revealed increases in the total soluble solids (TSS), starch and anthocyanin contents of the plants, which directly impact flower quality. These findings are in accordance with those of Abbass *et al*. (2020), where the application of humic acid and iron chelate increased the total soluble solids in *Freesia* plants by 51%, although cineraria plants exhibited a much greater increase (by 332%). A previous study in strawberry plants showed that humic acid and iron chelate could increase leaf area, dry matter, carbohydrate and anthocyanin content (Mohamed *et al*., 2020). Moreover, similar results were found for *Hibiscus sabdariffa* (Ibrahim, 2019) and *Cymbopogon citrus* (Ghatas and Mohamed, 2018).

Iron has been shown to increase the formation of healthy green leaves, resulting in more assimilates being distributed to floral parts (Hembrom and Singh, 2015; Salem *et al*., 2019). In the present research, the flower diameter, flower number, inflorescence number, and flowering period of cineraria increased following coapplication of iron chelates and humic acid fertilizers. Other findings were also reported in roses, where iron use enhanced metabolic activity, cell wall loosening, cell elongation, and cell expansion, resulting in an increased flower diameter (Poornima *et al.*, 2018; Salem *et al.,* 2019). Similarly, humic acid exerts a positive effect on flower characteristics through direct quasihormonal activity or indirect influence on Ca uptake, which improves cell wall mechanical resistance and cell membrane integrity (Nikbakht *et al.*, 2008).

The application of iron and humic acids to ornamental plants results in increased flowering duration by enhancing protein synthesis and further chlorophyll formation. The results of the current study in cineraria showed that flowering time was prolonged. Previous studies have shown that iron application effectively leads to extended flowering in *Rosa* plants (Salem *et al.,* 2019). Iron treatment has been shown to prolong flowering time in both *Calendula officinalis* (Izadi *et al.,* 2020) and chrysanthemum (Bhute *et al*., 2017). Ahmad *et al.* (2013) previously showed that humic acid application at relatively high concentrations resulted in increased duration of gladiolus flowering.

The cineraria plants in this study fertilized with iron chelate and humic acid exhibited a great increase in potassium (179%), nitrogen (193%), and phosphorus (675%) absorption. Ngan *et al*. (2020) also demonstrated that iron fertilization could enhance mineral uptake in carnation plants, although the present research showed greater absorption of essential elements. Previous studies on rose (Nikbakht *et al*., 2008) and pumpkin (Kamali Omidi *et al.*, 2022) plants confirmed the findings in cineraria, where humic acid treatments increased the absorption of macronutrients (i.e., nitrogen, phosphorous, and potassium).

Humic acid and iron chelate fertilizers decreased electrolyte leakage in cineraria plants by 60%. These findings showed that their coapplication could help maintain cell membrane integrity. The positive effect of these compounds could be partially explained by the strong antioxidant capacity induced by their use. Earlier reports on canola plants under environmental stress indicated that cell membrane damage and subsequent ion leakage are relieved by iron application (Shokri-Gharelo and Ghader, 2017).

This study revealed that coapplication of humic acid with iron chelate in cineraria plants exponentially increased the activity of the antioxidant enzymes SOD, POD and CAT by 238%, 324% and 667%, respectively. Researchers have previously shown that the application of humic acid and iron chelate fertilizers enhances the activity of antioxidant enzymes in azalea (Elmongy *et al.,* 2018) and pot marigold (Izadi *et al.,* 2020), respectively. This could be attributed to the fact that humic acid acts as an antioxidant, auxin activator and scavenger of reactive oxygen species (Elmongy *et al.,* 2018). Iron is a vital element that is well known as a main structural component of many enzymes and plays an important role as a functional and regulatory cofactor of enzyme activity (Ribeiro *et al.*, 2015; Ibrahim, 2019). Okra plants grown in low-iron media had lower catalase activity than control plants (Kabir *et al.*, 2015), which demonstrated that iron deficiency results in reduced catalase activity. Increased antioxidant enzyme activity has been proven to be a plant adaptation strategy for reducing ROS damage (Ribeiro *et al.*, 2017).

CONCLUSION

In conclusion, the application of humic acid as a biofertilizer in soil is an effective substitute and a financially sustainable solution for enhancing plant growth and production. Although the application of humic acid combined with iron chelate in soil has been explored for other crops, this is the first study on this topic in cineraria plants. The combined application of humic acid with iron chelate improved the vegetative and reproductive growth of cineraria. Therefore, the use of humic acid and iron chelate fertilizers could be recommended for the production of cineraria and possibly other ornamental plants, especially in calcareous soils and challenging soil pH conditions.

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The Relationship between Vermicompost Biofertilizer and Growing Substrates with *Calendula officinalis* L. Yield and Its Components

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Abstract

The cultivation of marigold (*Calendula officinalis* L.) has become increasingly significant due to its medicinal benefits and ornamental value. The widespread use of agrochemicals has adversely impacted soil quality, crop yields, and the environment. As a viable alternative, organic amendments and biofertilizers have been suggested to improve both soil and plant health. Vermicompost presents a sustainable approach for providing plant nutrition and enhancing production, thereby promoting soil health and fertility. This experiment was carried out under field conditions to investigate the effects of vermicompost (0, 1, 2, and 3 kg/m^2) and growing substrates (field soil, field soil and sand, and field soil, sand, and vermicompost) on some parameters of marigold. Shoot number, flower number, flower diameter, plant dry weight, and flower dry weight were measured. Results showed that the higher levels of vermicompost increased all measured parameters. Comparison between various substrates and different levels of vermicompost showed that substrate including field soil, sand, and animal manure induced more yield and its component than other treatments of the experiment except for 3.0 kg/m^2 vermicompost. These results underline the potential of bio- and organic fertilizer in improving flower induction of the *C. officinalis* with the possibility of avoiding chemical fertilization.

Keywords: Biofertilizers, Cultivation beds, Marigold, Organic fertilizers, Ornamental and medicinal plants.

INTRODUCTION

Marigold (*Calendula officinalis* L.) is a prominent flowering plant belonging to the Asteraceae family, renowned for its vibrant yellow and orange blooms that attract a lot of attention in urban green space, as well as for its medicinal properties (Shaabani *et al*., 2022). Although, *C. officinalis* is originally from the Mediterranean region, it is cultivated globally, including in countries such as China, Europe, and the United States (Soliman *et al*., 2024). This species is classified as a winter annual herb. The characteristics of the soil and the environmental conditions significantly influence the quantity and size of the flowers produced each season (Shaabani *et al*., 2022).

The challenges associated with the overuse of chemical inputs in the agricultural sector, particularly the significant effects on soil pH and the common agricultural production practices, have led to a growing interest in the application of organic and biofertilizer amendments in recent years (Libutti *et al.*, 2020; Shaabani *et al*., 2022). To achieve optimal yields, it is crucial to improve soil health through the incorporation of organic matter and biofertilizers (Hasan *et al*., 2024). Although, mineral fertilizers play a vital role, they should be complemented or substituted with accessible organic resources and biofertilizers, especially in light of increasing costs and concerns regarding soil health, environmental sustainability, and human well-being (Hasan *et al.*, 2024). The impact of these amendments on agricultural soils is influenced by various factors, including the characteristics of the feedstock, processing conditions, application rates, soil types, environmental factors, and the specific crop species involved (Urra *et al.*, 2019; Libutti *et al*., 2020). The adoption of biofertilizers among farmers is rapidly increasing, as these products are effective in enhancing microbial activity, thereby improving nutrient availability for plant uptake (Shaabani *et al.,* 2022). Biofertilizers consist of various combinations of microbes that promote plant growth (Nada *et al*., 2024). Given that organic amendments and biofertilizers positively influence crop yields without causing environmental harm, their implementation and advocacy represent a viable and promising alternative to chemical fertilizers (Shaabani *et al*., 2022). Furthermore, organic amendments and biofertilizers contribute to improved plant nutrition, enhanced crop yield and quality, preservation of soil fertility, and the promotion of sustainable agricultural practices (Libutti *et al*., 2020).

Vermicompost is an organic fertilizer resembling peat, characterized by its high nutritional value, excellent aeration, porosity, and capacity to retain water, produced through the collaborative efforts of earthworms and microorganisms. As an efficient organic fertilizer and biocontrol agent, vermicompost positively influences plant growth, yield, and quality (Joshi *et al*., 2015). Beyond its role in organic waste management, it is acknowledged as a potent promoter of plant growth (Rehman *et al*., 2023). The microbial activities within vermicompost enhance the availability of numerous macro- and micronutrients (Rehman *et al*., 2023). This nutrient-rich fertilizer has gained popularity for the rehabilitation of agricultural soils contaminated with metals (Wang *et al*., 2018; Zhang *et al.*, 2020). Vermicompost acts as a biofertilizer, comprising a biologically active mix of bacteria, enzymes, plant residues, and earthworm casts that facilitate the decomposition of soil organic matter and enhance microbial activity in the planting medium (Shaabani *et al.*, 2022; Rehman *et al.*, 2023). It is not only environmentally sustainable but also a non-toxic amendment that enriches soil with vital nutrients and growth-enhancing substances. A systematic review conducted by Oyege and Balaji Bhaskar (2023) indicated that incorporating vermicompost into agricultural practices improves soil quality, including increased permeability, aeration, drainage, and water retention, while also enhancing

soil pH and microbial diversity, ultimately leading to higher crop yields. Similar conclusions have been drawn by other researchers (Iqbal *et al*., 2024; Terefe *et al.*, 2024). Furthermore, vermicompost has demonstrated the ability to convert unavailable nutrients into accessible forms, supplying both micro- and macronutrients to plants, and it has been found to contain higher levels of sulfur (S) compared to mineral fertilizers, which can further promote plant growth (Hoque *et al.*, 2022; Shen *et al*., 2022; Iqbal *et al*., 2024). Additionally, vermicompost is more effective than traditional plant compost in lowering heavy metal concentrations in soil and reducing their uptake by plants (Li *et al.*, 2021).

The application of soil amendments, particularly animal manures rich in organic matter and nutrients, serves as a cost-effective approach to enhance both crop yield and soil quality (Antonious *et al.*, 2023). The reprocessing of animal manures can diminish the reliance on synthetic inorganic fertilizers while providing beneficial amendments that improve soil structure and nutrient content. Animal manures are a significant source of ammonia (NH_3), which, upon reacting with water, forms ammonium ions (NH_4^+). These ions readily bind to negatively charged soil organic matter and clay particles, facilitating absorption by plant roots (Antonious *et al*., 2023). The incorporation of organic amendments, including cattle manure, biochar, and compost, constitutes an environmentally sustainable approach for mitigating heavy metal contamination (Gu *et al*., 2019; Hamid *et al.*, 2020; Mashur *et al.*, 2021). Nonetheless, these practices are frequently considered impractical due to their costs and the risk of introducing additional pollutants (Pramanik *et al.,* 2018). Organic fertilizers play a crucial role in sustainable agriculture by maintaining nutrient availability, enhancing soil organic matter, and improving the physical and chemical properties of the soil, ultimately contributing to increased crop productivity (Hasan *et al.*, 2024).

Some studies have investigated the impact of vermicompost (Libutti *et al.*, 2020; Shaabani *et al.*, 2022) and animal manure (Antonious *et al.*, 2023; Nada *et al.*, 2024) on the morpho-physiological characteristics and their variations in *C. officinalis*. The application of vermicompost, whether used alone or in combination with other organic amendments, has been shown to enhance growth and yield in some species (Libutti *et al.,* 2020; Makhtoumi *et al.*, 2022; Terefe *et al*., 2024). The use of biofertilizers resulted in significant improvements in plant height, total biomass yield, seed weight, and harvest index in *C. officinalis*; however, parameters such as flower diameter, number of shoots, dried flower yield, flower number, and seed yield remained unaffected by the treatments (Rezae and Baradaran, 2013). Furthermore, a higher application rate of poultry manure demonstrated notable advantages over chemical fertilizers in terms of plant height, shoot number, plant dry weight, chlorophyll content, carbohydrates, flower number, fresh weight of flower, and carotenoid levels in *C. officinalis* (Nada *et al.,* 2024).

Fertilizer management plays a crucial role in the successful cultivation of marigold plants, and identifying appropriate fertilizers can significantly influence both quantitative and qualitative outcomes (Onofrei *et al.*, 2017). While the impact of fertilizers on crop growth has been thoroughly investigated, research focusing on the response of medicinal herbs to organic fertilizers in Iran remains limited (Shaabani *et al.,* 2022). Most existing studies have been conducted in greenhouse settings, with field studies in open environments being relatively rare. Consequently, this research aimed to examine the effects of varying levels of vermicompost biofertilizer and different growing substrates, particularly animal manure, on the floral characteristics of *C. officinalis*, a valuable ornamental and medicinal plant, in a field setting.

MATERIALS AND METHODS

Plant sample and geographical and climatic coordinates of the experiment site

The seed of ornamental-medicinal *Calendula officinalis* L., from the family Asteraceae, was used as the sample. In order to investigate the effect of different levels of vermicompost biofertilizer and growing substrates on the yield and yield components of this plant in Guilan province, an experiment was conducted in the crop year 2020-2021 at 15 km from Rasht city at an altitude of 70 m above sea level and at a latitude of 37° 10′ 5.5″ north and longitude 45° 33′ 12.6″ east. This region has an annual rainfall of 1348 mm and an average annual temperature of 15°C with a humid climate.

Field preparation and plotting

After field preparation and tillage operation, the desired field was divided into 36 plots with dimensions of 1.8 x 3.5 m and an area of 6.3 m^2 by means of peaking. The distance between experimental plots was 0.5 m and the distance between blocks was considered to be 1 m. Therefore, the total area of the plan, including the buffers (barriers in agricultural lands), was 376.2 m^2.

Soil characteristics of the experiment site and experimental plan map

In order to prepare the soil sample, 10 one-kg soil samples were prepared from 10 points of the field and from a depth of 0 to 30 cm, and after complete mixing, a mixed sample was transferred to the Water and Soil Laboratory, Islamic Azad University, Rasht Branch for analysis. The result of soil analysis is presented in table 1. The map of the experimental design, having a total of 2 factors, 7 levels, 12 treatments and 36 plots are presented in Fig. 1.

Table 1. The results of the soil test at the experiment site.

Soil depth (cm)	pH	EC (ds/m)	Texture	Organic matter (%)	Potassium (ppm)	Phosphorus (ppm)	Nitrogen (%)
0-30	6.33	0.59	Clay	0.7	84.0	7.0	0.3

R1	F1	F3	F4	F2	F2	F2	F3	F3	F1	F1	F4	F4
	S1	S3	S2	S3	S1	S2	S2	S1	S3	S2	S3	S1
R2	F1	F3	F2	F4	F4	F1	F3	F2	F3	F1	F2	F4
	S3	S1	S2	S2	S1	S1	S3	S3	S2	S2	S1	S3
R3	F1	F4	F2	F4	F3	F2	F3	F2	F1	F4	F1	F3
	S3	S2	S1	S1	S2	S3	S3	S2	S1	S3	S2	S1

Fig. 1. Plan implementation design. F: biofertilizer, S: substrate, and R: replication.

Planting method

By hookah (manual plow of agricultural lands), in each plot, furrow and ridges were created with a distance of 40 cm from each other. After preparing the modified seed, in the required quantity and randomly selecting the experimental plots, it was planted on the ridges with high density. After two weeks, the bushes were thinned.

Irrigation method

After planting the seeds, each plot was irrigated separately by furrow and ridges method. Depending on weather conditions, if effective rainfall did not occur, irrigation was repeated every 8 days. In case of rain, irrigation was done with delay.

To weed

Weeding was done manually in three stages after planting according to different planting dates. The first, second, and third weeding was done 15, 30, and 45 days after planting, respectively.

Measurement of parameters

To measure the number of shoots in each plant, in each experimental plot, 5 plants were selected and the number of shoots in them was counted. To measure the number of flowers per plant, 5 plants were selected in each experimental plot and the number of flowers produced in them was counted. To measure the flower diameter, it was measured with a digital caliper after the flowers bloomed and before they were harvested. The average diameter of flowers in each sample was calculated as flower diameter. To measure the dry weight of the plant, in each experimental plot, 5 plants were selected and cut from the collar. After weighing each plant separately, drying them in an oven at 72°C for 6 h. Finally, the average dry weight of the plant was recorded in each plot. To measure the dry weight of flowers, 5 flowers were selected in each experimental plot and dried in an oven with a temperature of 45°C for 6 h. Finally, the average flower dry weight was recorded as flower yield per plant.

Experimental design and data analysis

A split plot experiment with a randomized complete block design (RCBD) was used in three replications. Vermicompost biofertilizer at three levels of 0, 1, 2, and 3 kg/m^2 (added to the farm soil), as the first factor, and substrate growing including farm soil, farm soil combined with sand (in ratio of 1:1), and farm soil combined with sand and animal manure (in ratio of 1:1:1) were used as the second factor. The significance of differences between mean values was calculated using LSD test performed at $P < 0.05$. All data were presented as means ± SE for at least three replications for each sample. The statistical analysis and means comparison were done using SPSS Statistics software.

RESULTS

There was significant difference (at $P < 0.01$) in all measured parameters (shoot number, flower number, flower diameter, plant dry weight, and flower dry weight) among the seven individually treatments when analyzed by an ANOVA test (Table 2). Statistically significant difference was not observed in the amount of all measured parameter during interaction effect of different levels of vermicompost and various substrates (Table 2).

Table 2. Analysis of variance of the effect of different levels of vermicompost biofertilizer and different growing substrates on *Calendula officinalis* L. yield and its components.

S.o.V	df	MS				
		Shoot number per plant	Flower number per plant	Flower diameter	Plant dry weight	Flower dry weight
Replication	2	0.65	2533.55	0.69	4.90	0.27
Substrates (A)	2	1.87**	271.86**	2.55**	582.13**	9.44**
Biofertilizer (B)	3	2.75**	38.63^{ns}	2.07**	635.56**	9.66**
A × B	6	0.15^{ns}	10.09^{ns}	0.14^{ns}	75.13^{ns}	0.66^{ns}
Error	18	0.24	43.12	0.33	30.01	0.57
CV (%)	-	8.24	18.58	13.28	9.80	9.89

** and ns: Significant at $P < 0.01$ and insignificant based on the LSD test, respectively. df: degree of freedom, CV: coefficient of variations.

Shoot number

The highest number of shoot per plant (6.50) was obtained in the plants produced from the seeds grown in the substrate containing 3 kg/m^2 of vermicompost. The difference in the number of shoots obtained in this treatment with the number of shoots obtained in substrate containing field soil together with sand and animal manure (with 6.35 shoots), 2 kg/m^2 of vermicompost (with 6.15 shoots), and 1 kg/m^2 of vermicompost (with 6.03 shoots), was not significant. The lowest number of shoots per plant (5.15) was obtained in plants produced from seeds grown in the substrate without vermicompost (Table 3).

Table 3. Mean comparison of the effect of different levels of vermicompost biofertilizer and different growing substrates on *Calendula officinalis* L. yield and its components.

Treatments	Shoot number per plant	Flower number per plant	Flower number per plant	Plant dry weight (g)	Flower dry weight (g)
Vermicompost (kg/m^2)					
0.0	5.19^{b} ± 0.13	22.60^{c} ± 2.06	22.60^{c} ± 2.06	46.55^{d} ± 5.28	6.36^{c} ± 1.03
1.0	6.03^{a} ± 0.32	22.74^{c} ± 1.80	22.74^{c} ± 1.80	52.92^{c} ± 4.75	7.41^{b} ± 1.33
2.0	6.15^{a} ± 1.02	24.38^{b} ± 1.57	24.38^{b} ± 1.57	57.51^{b} ± 6.20	7.85^{b} ± 1.24
3.0	6.50^{a} ± 1.21	27.60^{a} ± 1.78	27.60^{a} ± 1.78	66.53^{a} ± 5.04	8.86^{a} ± 1.14
Substrates					
Field soil	5.56^{c} ± 0.63	18.74^{b} ± 3.10	18.74^{b} ± 3.10	47.98^{c} ± 4.68	6.63^{c} ± 1.32
Field soil + sand (1:1)	5.99^{b} ± 0.82	26.20^{ab} ± 1.54	26.20^{ab} ± 1.54	58.53^{b} ± 5.55	7.90^{b} ± 1.19
Field soil + sand + animal manure (1:1:1)	6.35^{a} ± 0.39	27.60^{a} ± 1.84	27.60^{a} ± 1.84	61.13^{a} ± 4.73	8.33^{a} ± 1.05

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

Flower number

The plants obtained from the seeds grown in the substrate containing 3 kg/m^2 of vermicompost and substrate field soil together with sand and animal manure produced the highest number of flowers per plant (27.60). The difference in the number of flower obtained in these two treatments with the number of flower obtained in the other treatments was significant. The lowest number of flowers per plant (18.74) was obtained in the plants produced from the seeds grown in the field soil substrate (Table 3).

Flower diameter

The flowers produced in the plants obtained from the seeds grown in the substrate containing 3 kg/m^2 of vermicompost and field soil together with sand and animal manure had the largest diameter (4.63 cm). The difference between the diameter of the flower obtained in this treatment and the diameter of the flowers obtained in the treatments of 2 and 1 kg/m^2 of vermicompost (with diameters of 4.61 and 40.4 cm, respectively) was not significant. The smallest flower diameter (with 3.61 and 3.80 cm) was calculated in the plants obtained from the seeds grown in the substrate without vermicompost and the field soil substrate, respectively (Table 3).

Plant dry weight

Table 3 shows that the highest dry weight of the plant (66.53 g) was calculated by weighing the plants obtained from the seeds grown in the substrate containing 3 kg/m^2 of vermicompost. The difference between the dry weight of the plant obtained in this treatment and the dry weight of the plant obtained in the treatment of field soil together with sand and animal manure (with 61.13 g) was not significant. The lowest dry weight of the plant (with 46.55 and 47.98 g) was obtained in the plants produced from the seeds grown in the substrate without vermicompost and the field soil substrate, respectively (Table 3).

Flower dry weight

The data obtained from the mean comparison (Table 3) revealed that the highest dry weight of the flower (8.86 g) was obtained by weighing the flowers produced in the plants from the seeds grown in the substrate containing 3 kg/m^2 of vermicompost. The difference in the dry weight of the flower obtained in this treatment with the dry weight of the flower obtained in the field soil together with sand and animal manure (with 8.33 g) was not significant. The lowest dry weight of flowers (with 6.36 and 6.63 g) was obtained from the weighing of flowers produced in plants from seeds grown in vermicompost-free substrate and field soil substrate, respectively (Table 3).

DISCUSSION

Marigold (*Calendula officinalis* L.) is a notable flowering plant that garners significant interest in urban green spaces, primarily due to its vibrant yellow and orange blooms. A key objective within the floriculture sector, particularly concerning ornamental flowers, is to enhance both the quantity and size of these flowers. The current study revealed that the application of 3 kg/m^2 of vermicompost, as well as a substrate composed of field soil mixed with sand and animal manure, resulted in an increase in both the number and size of flowers in marigold.

In marigold cultivation, the application of vermicompost biofertilizer and organic fertilizers, such as animal waste, has been demonstrated to significantly enhance both plant yield and its components (Khalid and da Silva, 2012; Rezae and Baradaran, 2013; Shaabani *et al*., 2022; Nada *et a*l., 2024). The combination of vermicompost with humic acid as an organic fertilizer has been shown to increase the number of flowers produced in marigold (Shaabani *et al.*, 2022). Our research indicated that both vermicompost and animal manure, when applied individually, resulted in an increase in the number and size of flowers. The incorporation of organic amendments into the soil, particularly those derived from animal waste (Mashur *et al*., 2021), as well as vermicompost (Makhtoumi *et al*., 2022; Hoque *et al*., 2022; Shen *et al*., 2022; Oyege and Balaji Bhaskar, 2023; Iqbal *et al*., 2024; Terefe *et al*., 2024; Rehman *et al*., 2023), and their combined use (Libutti *et al*., 2020; Antonious *et al*., 2023; Hasan *et al*., 2024), has been associated with enhanced growth and yield in various other plant species.

The impact of vermicompost on the growth and flowering of marigolds in greenhouse conditions was assessed (Sardoei, 2014). The application of vermicompost fertilizer was found to have the most significant influence on the biomass of basil (*Ocimum basilicum* L.) (Makhtoumi *et al*., 2022). According to Sultana *et al.* (2015), the use of vermicompost enhanced various growth and flowering metrics of *Zinnia elegans*, including shoot and root lengths, leaf number, flower number, flower diameter, and both fresh and dry weights of flowers, in comparison to NPK fertilizer (Nada *et al.*, 2024). In the case of *Foeniculum vulgare*, the application of biofertilizers, especially a mixture of bacteria and fungi, led to increases in total plant fresh weight, dry weight, and shoot numbers when compared to the control group

(Nada *et al.*, 2022). In alignment with our findings, it was noted that the use of organic and biofertilizers significantly improved the number and diameter of flowering heads in marigold plants compared to those that were not fertilized (EL-Zawawy *et al*., 2021). A higher level of organic fertilization was associated with the greatest flower number and yield (Nada *et al*., 2024). A meta-analysis indicated that the incorporation of vermicompost into the soil markedly enhances shoot biomass in plants (van Groenigen *et al*., 2014). The beneficial effects of vermicompost on various medicinal plants, including garlic (*Allium sativum* L.) (Verma *et al*., 2013), coriander (*Coriandrum sativum* L.) (Godara *et al*., 2014), *German chamomile* (*Matricaria chamomilla* L.) (Ansarifar *et al*., 2012), and basil (*Ocimum basilicum*) (Sirousmehr *et al*., 2014), have been documented. Experimental results demonstrated that the application of vermicompost significantly improved growth parameters such as shoot biomass and flower quantity in African marigold compared to mineral fertilizers (Joshi *et al*., 2015).

The utilization of animal manure as an organic fertilizer possesses significant characteristics that are unattainable through synthetic inorganic fertilizers. The microorganisms present in animal manures promote the gradual release of the primary plant nutrients—nitrogen (N), phosphorus (P), and potassium (K)—from soil organic matter, thereby minimizing their offsite movement into natural water bodies and mitigating the risk of eutrophication (Antonious *et al*., 2023). In contrast to our findings, previous research indicated that the application of poultry manure had a more substantial effect on marigold growth than biofertilizers (Nada *et al*., 2024). The results of this study indicated that the application of organic fertilizers and plant growth-promoting microbes had a beneficial impact on the growth and flowering of marigold when compared to the control treatment.

The literature indicates that the impact of incorporating organic fertilizers into cultivated soil on crop growth exhibits considerable variability and inconsistency. This variability may originate from factors such as the types of fertilizer feedstock, pyrolysis conditions, the structure and composition of the fertilizers, soil characteristics, and the specific crops being tested (Meschewski *et al*., 2019; Libutti *et al.*, 2020). The effective provision of N through biological fertilizers, particularly vermicompost, which is rich in biologically active substances that function as growth regulators, can elucidate the positive effects of vermicompost on both vegetative and reproductive growth (Makhtoumi *et al*., 2022). The application of biological fertilizers to medicinal plants such as chamomile (*Matricaria chamomilla* L.) and marigold has been shown to enhance flower yield (Makhtoumi *et al.*, 2022). Furthermore, when organic materials are introduced into the soil, they improve its physical properties, including aeration, aggregation, permeability, and water retention capacity (Yadav *et al.,* 2023), all of which are conducive to plant growth and development (Nada *et al.,* 2024). The ability of organic manure to retain moisture and maintain adequate pore spaces for effective air circulation and drainage of excess water may contribute to its beneficial effects on shoot numbers and plant dry weight (Nada *et al*., 2024). It is likely that the application of suitable quantities of vermicompost enhances soil microbial activity and the production of plant growth regulators by these microorganisms, leading to improved nutrient absorption, increased photosynthesis, and greater dry matter accumulation, ultimately resulting in enhanced flowering (Rezae and Baradaran, 2013).

CONCLUSION

It is crucial to develop strategies that improve both the yield and the components contributing to the yield of ornamental-medicinal plants. Research highlights the potential of bio- and organic fertilizers in promoting flower induction in the *C. officinalis* species, which

may facilitate a reduction or complete removal of synthetic and chemical fertilizers, thereby fostering a more sustainable and ecologically sound agricultural framework. The incorporation of vermicompost and organic fertilizers into the soil likely enhances the availability of essential nutrients for the plants, improves the physical and chemical properties of the soil, aids in N fixation, promotes plant growth regulators, produces antibiotics, and facilitates the decomposition of organic matter, all while creating an optimal environment for root development. This, in turn, contributes to the growth and development of the plant's aerial parts. Additional studies are necessary to clarify the specific mechanisms by which organic and biofertilizers affect the growth and flowering of marigolds. Gaining insight into these mechanisms will assist in optimizing the application of these interventions to maximize their advantages. Future research that integrates biological and organic fertilizers is expected to further enhance flowering in marigold plants. The findings suggest that the application of biological and organic amendments to the soil can improve both the quantitative and qualitative characteristics of marigold. However, the varied responses of plants to these fertilizers indicate the need for further experimentation.

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Enhancing Callus Growth in Calla Lily (*Zantedeschia* 'Sun Club') through Fipexide, Activated Charcoal, and Ascorbic Acid in *In Vitro* Cultivation

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The potted calla lily (*Zantedeschia* spp.) is a popular ornamental plant in the global flower market. The most important problem of calla lily in indirect organogenesis is callus production, low durability and growth of callus due to the phenolic and alkaloidal compounds present. For this purpose, an experiment was conducted to investigate the effect of fipexide (FPX) compared with activated charcoal and ascorbic acid in the culture medium to improve the quality of the callus of the potted calla lily (*Zantedeschia* 'Sun Club'). FPX is one of the chemical compounds used in the pharmaceutical industry. Recently, for *in vitro* conditions FPX was used in the culture media to improve the quality of different stages of plant growth. A factorial experiment in the form of a completely randomized design including FPX at concentrations of 0, 15 and 30 µmol L^{-1}, activated charcoal (0 and 1 g L^{-1}) and ascorbic acid at concentrations of 0 and 2 g L^{-1}, including 12 treatments, 3 replications, 12 samples for each treatment and in the collection with 192 callus samples were implemented. In this research, callus diameter, callus fresh weight, callus growth index and healthy callus percentage were evaluated. The results showed that, FPX treatment, especially at a concentration of 15 µmol L^{-1} with activated charcoal, had the greatest effect on the examined traits such as callus diameter (15.98 mm), healthy callus percentage (90%) compared to the control samples (with an average of 7.36 mm and 54 % respectively). Totally, FPX can be used as an effective and alternative compound in the callus growth medium to increase quality and performance.

Keywords: Crown calli, Geophyte, Ornamental plant, Pot plant, Plant tissue culture.

INTRODUCTION

Callus is used as an important tool for some genetic studies, for example, mutation and gene transfer (Efferth, 2018). Today, many plant researches have focused on callus production under *in vitro* conditions, which is influenced by nutrients and plant growth regulators (Nalousi *et al*., 2019; Yu *et al*., 2021; Chutipaijit and Sutjaritvorakul, 2018). However, the use of the organogenesis method requires callus from many plants cultured in vitro, which can be associated with browning of the explant and production of phenolic compounds (Corduk and Cuneyt Aki, 2011). Browning and possible death of plant tissues during the initial stages of plant tissue culture remains one of the persistent problems (Gerema and Emiru, 2021). Browning occurs through high activities of polyphenol oxidase, peroxidase, and other enzymes that are triggered for their defense in response to wounding (Onuoha *et al*., 2011). When cells are wounded during cutting, with temperature fluctuations and aging of the sample, the browning reaction begins depending on the plant sample's nature. One of the key factors in callus production, preservation, and use is maintaining color stability and callus quality (Pan and Staden, 1999; Onay *et al*., 1996). Browning in tissue culture samples may be dependent on species, cultivar, developmental stage, physiological condition, tissue type, explant size, and age (Mohd Din *et al*., 2016; Ozyigit, 2009). This may decrease the propagation rate, regeneration rate, and viability of plant samples (Parthasarathy *et al*., 2007).

One of the significant problems in producing callus from some plant species involves the browning effect caused by exudation of phenolic compounds into the culture medium, followed by the deterioration and poor quality of the sample (Karolina *et al*., 2024). This could be part of the plant response due to factors such as plant variety, injury, or other factors like physiological aging or fluctuation in environmental conditions (Dias *et al*., 2016).

In some plant species like calla lily, tissue culture callus samples are highly sensitive and produce a significant amount of phenolic compounds (Nery *et al*., 2015; Kulpa, 2016; Xuan *et al*., 2023). Over the past few years, the potted calla lily (*Zantedeschia* spp.) has been one of the most popular tuberous flowers in demand throughout the world. It possesses many cultivars and plenty of colors. It is grown and traded as one of the most popular ornamental plants in several regions all over the world (Xuan *et al*., 2023).

To reduce browning, substances like activated charcoal, ascorbic acid, PVP (polyvinylpyrrolidone) and others are used to absorb the released compounds or prevent quality degradation of callus samples due to their antioxidant properties (Thomas, 2008). Activated charcoal is a tasteless material with an excellent porous system and large internal surface areas that eliminate all non-carbon impurities. This chemical is used for the absorption of phenolic substances that could cause browning of the explants and the culture medium (Huang *et al*., 2007). Other chemicals that might be used to maintain quality and prevent excessive phenolic compounds secretion from the callus are antioxidants such as ascorbic acid (Giri *et al*., 2012).

Numerous reports have demonstrated the use of activated charcoal and ascorbic acid in in vitro conditions to preserve the quality of plant samples against damage caused by phenolic compounds produced by callus (Thomas, 2008; Chaabani *et al*., 2015; Fitriana *et al*., 2019; Chutipaijit and Sutjaritvorakul, 2018). Fipexide is a phytochemical compound which has also served use in the pharmaceutical industry in the treatment of patients who have been suffering from dementia and Alzheimer disease (Missale *et al*., 1983). Recent studies have revealed that this compound induces callus growth and maintains its quality. Nakano *et al*. (2018) reported that FPX acts as a new bioactive compound in the formation and growth of callus in plants. Similarly, Yoshiki *et al.* (2022) conducted a study on the ornamental plant *Matthiola incana,* reporting that the use of FPX in the culture medium enhanced callus formation and quality.

Given the high production rate of phenolic compounds in calla lily, which may influence callus production and quality under in vitro conditions, this study aimed to investigate the effects of FPX—a novel compound—on callus-related indicators and quality. Additionally, we examined the role of ascorbic acid and activated charcoal in relation to the quantitative and qualitative performance of callus in the ornamental potted calla lily (*Zantedeschia* 'Sun Club') under controlled *in vitro* conditions.

MATERIALS AND METHODS

The experiment was conducted in the Plant Tissue Culture Laboratory, National Ornamental Plant Research Institute of Iran, Mahallat city. Plant materials used in this study were the ornamental pot calla lily (*Zantedeschia* spp. cv 'Sun Club'). Freshly formed callus masses, derived from tissue-cultured microtubers of the calla lily plant with a diameter of 6 mm and of similar size, were used for the experiment.

For the experiment, different treatments were applied as outlined in table 1 using the MS medium (Murashige and Skoog, 1962). The treatments included different concentrations of activated charcoal (A0 and 1 g L^{-1}), ascorbic acid (0 and 2 g L^{-1}) and fipexide (0, 15, and 30 µM L^{-1} immediately after inoculation, the treated callus samples were transferred to a growth chamber with 16 hours of light (60 µmol m^{-2} s^{-1}) provided by white fluorescent lamps at a temperature of 23 ± 2°C for 14 days.

Table 1. Different concentrations of fipexide, ascorbic and activated charcoal on the callus parameters of calla lily (*Zantedeschia* 'Sun Club').

Treatments code	Activated charcoal (g/L)*	Ascorbic acid (g/L)	Fipexide (µmol/L)
T_1	-	-	-
T_2	-	-	15
T_3	-	-	30
T_4	-	2	-
T_5	-	2	15
T_6	-	2	30
T_7	1	-	-
T_8	1	-	15
T_9	1	-	30
T_{10}	1	2	-
T_{11}	1	2	15
T_{12}	1	2	30

*1 mol activated charcoal (AC)= 12.01 g, 1 mol ascorbic acid (AA)= 176.124 g, 1 mol fipexide (F)= 388.85 g

In this experiment, the measured traits included callus diameter, fresh callus weight, callus growth index, callus survival percentage, and callus regeneration percentage. Callus diameter was measured using a digital caliper, fresh callus weight using a digital scale, and the callus growth index and healthy callus percentage were calculated using the following formulas.

$$\text{Formula (1): Cell growth index} = \frac{\text{Callus diameter at the beginning of the experiment} - \text{The final diameter of the callus}}{\text{Callus diameter at the beginning of the experiment}} \times 100$$

$$\text{Formula (2): Percentage of healthy callus} = \frac{\text{Number of healthy calli}}{\text{The number of calluses at the begining of the expripent}} \times 100$$

This study was conducted as a factorial experiment based on a completely randomized design with a total of 12 treatments, each with 3 replications and 12 samples per replication. Statistical analysis of the data was performed using SAS 9.4 software, and the mean comparisons were done using Duncan's multiple range test. The charts were drawn using Excel software.

RESULTS

The effects of different treatments on the measured traits were evaluated after 14 days. Based on the results, it appears that among the three examined factors, the treatments containing FPX were the most effective on the measured indices.

Callus diameter

According to the results presented in Fig. 1 and 6, the treatment with 15 µM L^{-1} FPX combined with 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_1$) had the greatest effect on callus size, with an average diameter of 15.98 mm, compared to the other treatments. However, no significant difference was observed between the $AC_1.AA_0.F_1$ and the $AC_1.AA_0.F_2$ treatment (15 µM L^{-1} FPX + 1 g L^{-1} activated charcoal), which shared the same average diameter of 15.98 mm. Furthermore, the application of 2 g L^{-1} ascorbic acid alone ($AC_0.AA_1.F_0$) gave a higher value in terms of callus diameter, with an average of 9.86 mm, compared to the application of 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_0$), which gave a mean of 6.87 mm in the culture medium. However, in combination with FPX, the factor of activated charcoal ($AC_1.AA_0.F_1$= 15.98 and 15.68= $AC_1.AA_0.F_2$) had a higher effect on this trait compared to ascorbic acid (11.75= $AC_0.AA_1.F_1$ and 11.73= $AC_0.AA_1.F_2$). Also, in those two levels (15 and 30 µM L^{-1}) of application of FPX alone in the culture medium ($AC_0.AA_0.F_1$ and $AC_0.AA_0.F_2$), it is more effective on callus diameter compared to 2 g L^{-1} ascorbic acid ($AC_0.AA_1.F_0$) or 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_0$) alone (11.75= $AC_0.AA_0.F_1$ and 11.53= $AC_0.AA_0.F_2$). Moreover, the use of all three factors in the culture medium, at both levels of FPX (12.86= $AC_1.AA_1.F_1$ and 13.12= $AC_1.AA_1.F_2$), had a greater effect compared to the use of ascorbic acid with different levels of FPX ($AC_0.AA_0.F_1$ and $AC_0.AA_0.F_2$), but less effect compared to the use of 1 g L^{-1} activated charcoal with both levels (15 and 30 µM L^{-1}) of FPX ($AC_0.AA_0.F_1$ and $AC_0.AA_0.F_2$).

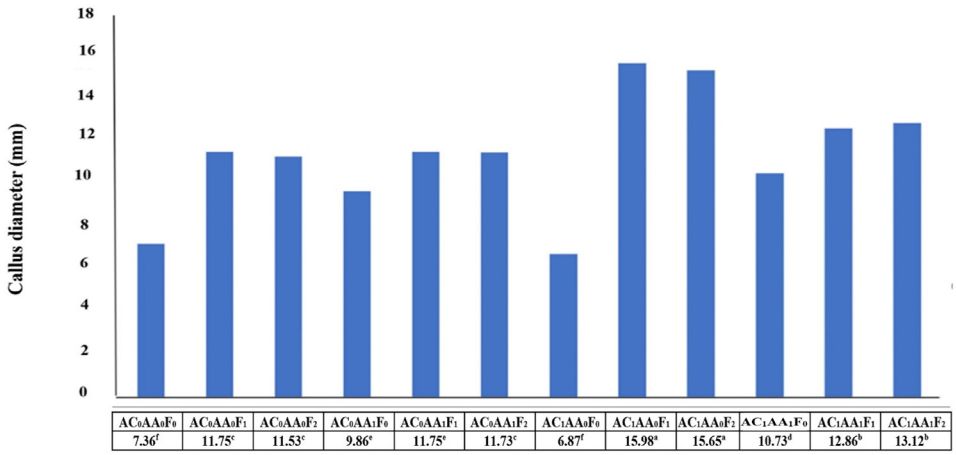

Fig. 1. The effect of different treatments of fipexide, ascorbic acid and actived charcoal on the callus diameter of calla lily (*Zantedeschia* 'Sun Club').

Callus weight

Based on the result, the treatment with 15 Mm L^{-1} FPX in combination with 1 g L^{-1} activated charcoal was the highest effect for callus weight with an average of 284 mg as compared with other treatments. Similar to callus diameter, FPX treatments at both concentrations combined with 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_1$= 284 mg and $AC_1.AA_0.F_2$= 276 mg) had a more significant effect than when used with 2 g L^{-1} ascorbic acid ($AC_0.AA_1.F_1$= 223 mg and $AC_0.AA_1.F_2$=219 mg) in the culture medium. Even when all three factors were used in the culture medium ($AC_1.AA_0.F_1$= 284 mg and $AC_1.AA_0.F_2$= 276 mg), this resulted in higher callus weight (Fig. 2).

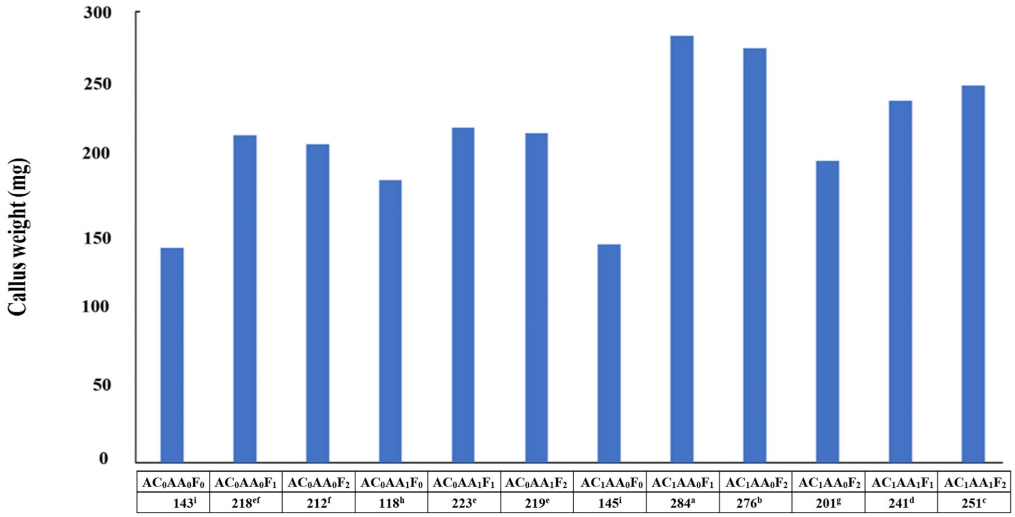

Fig. 2. The effect of different treatments of fipexide, ascorbic acid and actived charcoal on the callus weight of calla lily (*Zantedeschia* 'Sun Club').

Callus growth index

The term cell growth index has been used to determine the magnitude of callus growth from the initiation of the culture up to the end of the experiment. Based on the analyzed results, it appears that the $AC_1.AA_0.F_1$ treatment had a strong correlation with the evaluated traits, as all traits showed significant relationships with each other. According to the results in Fig. 3 and 6, treatments that included FPX (15 and 30 µM L^{-1}) showed a notable performance for this trait compared to other treatments. Treatments $AC_1.AA_0.F_1$ and $AC_1.AA_0.F_2$ demonstrated significant increases in the growth index, with averages of 136 and 130, respectively, compared to the control treatment ($AC_1.AA_0.F_2$= 19).

Percentage of healthy callus

Maintaining health without the necrosis of callus is among the main factors for callus quality. This factor has a direct impact on the division and multiplication of callus and sample regeneration, enhancing the chances of success in callus production and propagation projects, whatever the aim may be. The treatment with 15 µM L^{-1} FPX along with 1 g L^{-1} activated charcoal ($AC_1.AA_0.F_2$=90%) had the most significant effect on the health and quality of the callus. The most interesting finding within this experiment was the significant increase in callus quality while using various FPX concentrations (15 and 30 µM L^{-1}) in comparison with the control treatment (54%). According to the obtained results, it can be assumed that FPX not only reduced necrosis and increased the lifespan of callus but also had the higher effect on callus growth and size (Fig. 4 and 5).

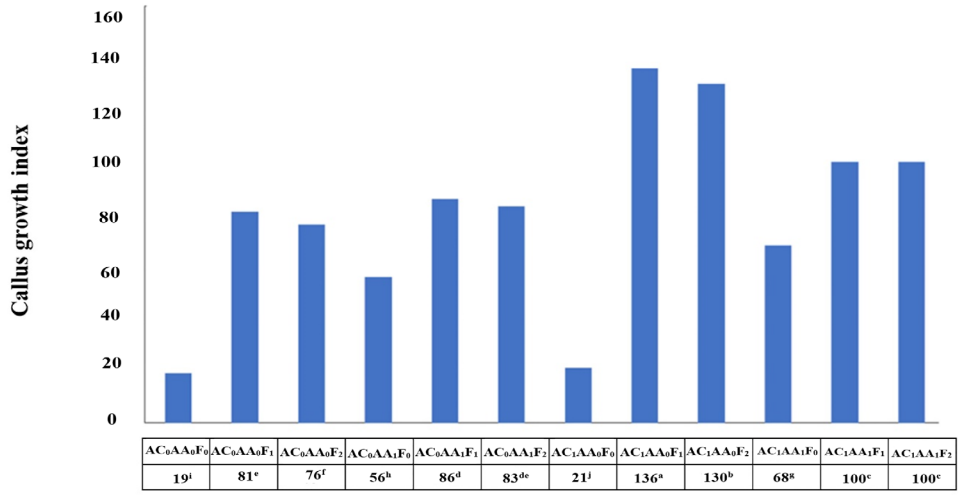

Fig. 3. The effect of different treatments of fipexide, ascorbic acid and actived charcoal on the callus growth index of calla lily (*Zantedeschia* spp. cv 'Sun Club').

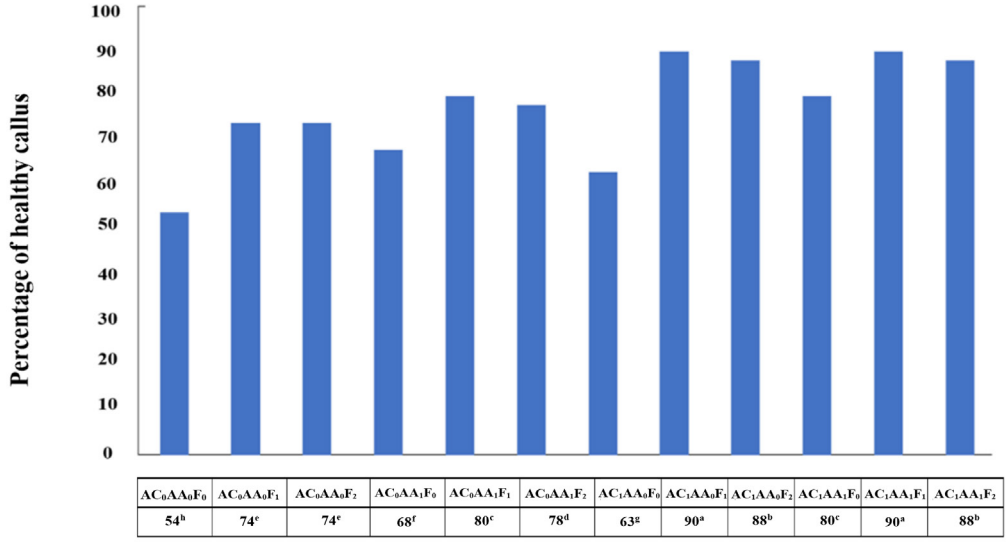

Fig. 4. The effect of different treatments of fipexide, ascorbic acid and actived charcoal on the percentage of healthy callus of calla lily (*Zantedeschia* 'Sun Club').

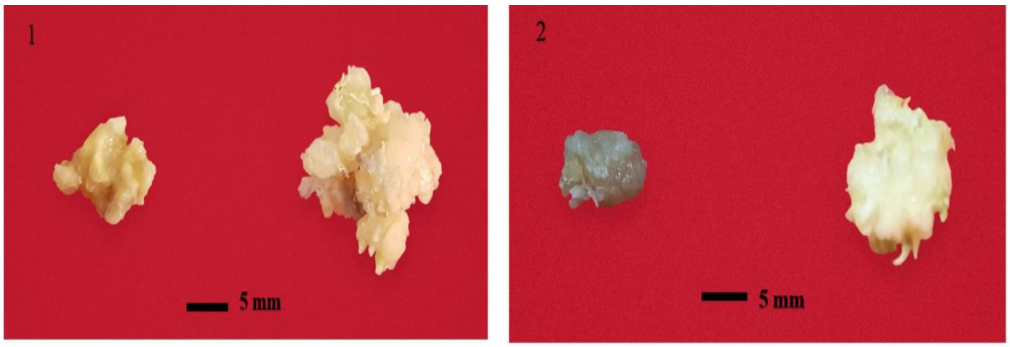

Fig. 5. (1). Callus size increase in $C_1A_0F_1$ treatment containing FPX (15 Mm L^{-1}) and activated charcoal (2 g L^{-1}) compared to the control. (2): Appearance of health and severity of necrosis of calluses, with $C_1A_0F_1$ treatment compared to control.

DISCUSSION

Based on previous research, there are numerous reports on the application of compounds like activated charcoal and ascorbic acid on the quality and growth of callus. As the results showed, ascorbic acid and activated charcoal treatments decreased browning and improved the quality of callus to some extent compared with the control; however, this difference was not significant. Due to its antioxidant properties, ascorbic acid can significantly reduce substances that negatively affect callus quality and the culture medium. Supplementation of ascorbic acid into the culture medium was reported by Amente and Chimdessa (2021) to reduce browning of callus quickly and improve their coloration. However, this activity is ceased after some time because of the loss of antioxidant activity of ascorbic acid (Huang *et al*., 2007). Elmore *et al.* (1990) did identify ascorbic acid as one of the antioxidant chemicals responsible for inhibiting the browning of plant tissues in tissue culture. Using activated charcoal in the culture medium increased callus growth and quality compared to the control, although its effects were less pronounced compared to ascorbic acid across all traits.

A striking observation of the results showed that the use of activated charcoal in combination with ascorbic acid in the culture medium proved more effective than the use of ascorbic acid alone. In addition, the treatment consisting of the interaction of activated charcoal with FPX proved more effective with regard to the characteristics evaluated in callus. Activated charcoal has a positive effect on growth as a result of its being able to adsorb phenolic compounds present in the culture medium (Chutipaijit and Sutjaritvorakul, 2018).

In a study conducted on date palm callus under tissue culture conditions, it was found that the application of activated charcoal in the culture medium, by reducing callus browning, created favorable conditions for the growth and survival of callus, and when combined with other growth-promoting substances, it had better performance on callus growth and quality (Fitriana *et al*., 2019). Activated charcoal exerts an indirect stimulatory effect by providing a better cultural condition, thus enhancing the bioactivity of other active agents in the medium (Chutipaijit and Sutjaritvorakul, 2018). However, it should be noted that in high concentrations, activated charcoal can absorb not only phenolic compounds but also other active substances in the culture medium, which may render them unavailable to the plant samples. Therefore, using the minimum effective amount of activated charcoal in the medium, along with other growth-promoting supplements, can help achieve the highest callus quality (Sakularat *et al*., 2015).

Moreover, using these compounds in high concentrations may have the opposite effect and exacerbate callus browning. Thus, selecting the optimal minimum concentration of compounds in the culture medium is crucial to achieving maximum quality (Thomas, 2008). A study showed that the combination of activated charcoal with ascorbic acid in the culture medium had a higher effect in inhibiting phenolic compounds in the culture medium and promoting the growth of plant samples compared to using each of these compounds alone (Nisyawati and Kariyana, 2013; Priyanka and Alok, 2015).

In the present work, FPX has been used as an additive for both enhancement of growth and improvement in quality of callus. Nakano *et al*. (2018) stated that FPX is an effective compound that enhances the intake of active agents in plants, thus enhancing callus induction and proliferation during tissue culture. Additionally, the researchers noted that the physiological, biochemical, morphological, and even gene expression mechanisms of plant samples in callus production with FPX differ significantly from those of plant hormones, and further studies are needed to investigate its effects on plants. Our research showed that FPX is effective in enhancing callus growth and quality of calla lily *in vitro*, acting as an effective elicitor for induction and development. Identification of FPX as an inducing molecule can add significant

understanding and also open ways for further studies related to plant science. Yoshiki *et al*. (2022) introduced FPX as a compound effective in callus growth, induction, and quality, which leads to faster callus formation and growth compared to plant hormones. It was reported by the researchers that applying FPX at a concentration of 15 µmol L^{-1} showed higher efficacy in callus growth for plants such as soybeans, tomatoes, and Matthiola incana. Furthermore, the authors indicated that higher concentrations of FPX would inhibit or reduce growth and quality-related parameters, and the degree of effect depended on plant species (Nakano *et al*., 2018; Yoshiki *et al*., 2022).

CONCLUSION

In this study investigated the effects of FPX—a novel compound—on callus-related indicators and quality. Additionally, we examined the role of ascorbic acid and activated charcoal in relation to the quantitative and qualitative performance of callus in the ornamental potted calla lily (*Zantedeschia* 'Sun Club') under controlled *in vitro* conditions. In summary, combining FPX with substances such as activated charcoal, which aids in absorbing phenolic compounds in the growth medium, can be helpful in managing problems with callus growth and browning in calla lily. The reduced effectiveness of the mixture with activated charcoal, ascorbic acid, and FPX might be because of the higher levels of these substances in the mixture, could have a lesser effect. Moreover, carrying out additional studies on this substance in plant research and determining the best concentration to enhance its effectiveness in the growth medium for different plant growth phases like callogenesis, regeneration, rooting, and gene transformation based on the specific challenges of the plant in tissue culture procedures could be beneficial for addressing hurdles in all *in vitro* culture related investigations. Our findings pave the way for significant advancements in the improvement of calla lily through the application of *in vitro* mutagenesis, genetic engineering, and genome editing techniques. By harnessing these innovative methodologies, we anticipate the development of more robust and diverse cultivars that are better suited to both commercial cultivation and ornamental use.

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Economic and Botanical Analysis of Ornamental Plants of the Central Iran

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Abstract

In the last few decades, many non-native plants have been introduced to the flora of the Central Plateau, especially in the green spaces. The present study aimed to identify and understand various ornamental members of 62 diverse plant families in the central plateau of Iran. Many medicinal plants have been used in hand-planted green spaces in desert areas due to their tolerance to salinity and adaptability to arid and semiarid climates. Creating urban green spaces with the plants introduced in this research conserves and restores biodiversity and brings many ecological and economic benefits. The concern of the research was on plant identification and exploring the economic importance of these species. Through detail- oriented fieldwork and observation, the identified plants were categorized into their respective families, providing valuable insights into the rich biodiversity of the central Iran. The economic importance of these plants was thoroughly examined, considering their applications in medicine, landscaping, traditional uses, and potential commercial uses. Out of these 158 studied species, in terms of medicinal and edible uses, 116 species have edible uses, 149 have medicinal uses, and 110 have both edible and medicinal uses. In this study, a model was designed to evaluate aesthetic values and economic services (food provisioning; natural medicines, pharmaceuticals; and wood, fiber production) for 158 ornamental studied species. Using the Ecosystem Services Evaluation Model presented in this study to compare the aesthetic values and economic benefits of ornamental species provides a guide for selecting plants in green spaces. The model ranking Aesthetic Value and Economic Benefits in four level: Low (<1.5), medium (<2.5, >1.5), high (<3.5, >2.5), and very high (>3.5). The main motto of selecting the ornamental flowering plants is to highlight the uses of the plants and their species in various industries rather than just beautifying the gardens and landscapes.

Keywords: Economic plants, Green spaces, Medicinal and industrial plants, Resistance to aridity, Salt tolerance.

INTRODUCTION

Urban biodiversity has a broad definition that is useful in a multidisciplinary approach to biodiversity conservation. Hahs and McDonnell (2014) describe two ideologies at play in managing urban biodiversity for creating biodiversity-friendly cities, namely the conservation of an area's local native biodiversity and managing biodiversity for the benefit of people, i.e. ecosystem services. These two ideologies need to be balanced to achieve long-term success. Management is required to achieve win-win situations that neither overemphasize conservation by creating areas wherein people are largely excluded (nature wins) nor de-emphasize conservation by managing solely for ecosystem service delivery regardless of its effect on other fauna and flora (people win) (Siebert *et al*., 2017).

At present, two types of strategies have been adopted for sustainable landscaping under adverse conditions of drought and salinity. The first strategy is environmental engineering, which manages the increase in salt levels in the soil and reduces water losses by managing irrigation and drainage. Another is plant engineering to increase plant tolerance to salt and drought. However, large areas of saline land cannot be managed this way. Many possible solutions are very expensive in terms of money, energy, and time. Therefore, the effective long-term method is to use plant species resistant to salinity and drought, which can be the most practical and economical solution (Alam *et al*., 2017).

Plants are drought-resistant in two ways. The first is genetically resistant to drought. The second is escape from arid periods. The resistance of drought and cold in plants supports each other. In other words, a plant resistant to drought also has resistance against cold. Therefore, the identification and use of drought-resistant plants and the economic benefit of water are essential in choosing plants for landscape design, especially medicinal plants (Tulukcu, 2020). With an increase in population and a decrease in per capita available arable land (particularly in developing countries), it has become difficult to find fertile land for cultivating medicinal and aromatic plants (MAPs). The results reported in this paper indicate that the cultivation of MAPs on degraded lands through bio saline agriculture is feasible and profitable (Dagmar *et al*., 2011).

Trade-offs exist among the multiple ecosystem services generated by forests. Wood production conflicts with public-good ecosystem services such as carbon storage, nutrient retention, and biodiversity conservation. Recognizing that forests generate both private- and public-good ecosystem services implies that forestry should be optimized to maximize the contribution of forests to societal welfare. Therefore, welfare would improve through the expansion of continuous cover forestry. We anticipate that this approach will contribute to sustainable forestry development by informing decision-makers of the impacts of alternative forestry practices on social welfare (Zanchi and Brady, 2019).

Plant species differ in tolerance to total salts and to specific ions. Certain species are highly tolerant to the shortage or excessive supply of one or more ecological factors, while others are sensitive. However, when water availability is limited, plants struggle to survive, and producing a wide array of secondary chemical metabolites is considered a survival strategy. Medicinal and aromatic plants are important sources of these chemicals used as pharmaceuticals. These appear to be protective agents for plants against biotic and abiotic stresses, including salinization (Qasem, 2015).

Medicinal plants have low water requirements due to their adaptation to arid and semi-arid climates, and their cultivation and development can play an effective role in preserving limited water resources. Therefore, changing the cultivation pattern and replacing common crops with drought-resistant medicinal plants with low water requirements can play a significant

role in reducing water consumption and be an effective step toward achieving sustainable agriculture (Al-Ebrahim Dehkordi and Azad Ghahfarkhi, 2021). Many medicinal plants can be used in hand-planted green spaces in desert areas due to their resistance to salinity and adaptation to arid and semi-arid climates.

Nowadays, awareness has increased about medicinal plants, their importance in life, and the benefits of growing them. People are coming forward to cultivate medicinal plants not only as organized farms or large plantations but also as home gardens which are easier to access at home. This paves the way for gardening and landscaping services as an investment and income-generating venture based on our plant wealth (Haridasan *et al.*, 2017). This research aims to introduce medicinal and industrial plants resistant to salinity and drought for the design of hand-planted green spaces in the hot and dry regions of the Iranian plateau.

MATERIALS AND METHODS

Study area

The Central Iranian Plateau lies between 1000 and 2000 m a.s.l. The climate is generally arid, with an annual rainfall of ca. 170-230 mm, falling from January to March or April. The characteristic plateau vegetation consists of a plateau steppe with Artemisia maritima and the grass Stipa holosericea, with occasional trees such as *Pistacia khinjuk, P. terebinthus, Prunus scoparia*, and *Juniperus excelsa*. Poorly drained plateaus support low halophytic communities. There, the specific zonation related to the salinity and depth of the water table is often seen (Wickens, 1998).

Central Iran is bounded by the Alborz Mountains in the north, the Zagros Mountains in the west and south, and the Khorasan Mountains in the east. Most of the central regions of Iran have a hot and dry climate, which is more moderate and humid in the highlands. In terms of forest cover, Iran is considered one of the countries with low forest cover (around 7% of the country's surface) (Foolad and Erfanifard, 2009). Therefore, to increase forest cover the creation of artificial forests is one of the trustee's programs in the development plans.

The area of the central plateau of Iran is 824,400 Km2, and its average surface runoff coefficient is 10%.

The total volume of water spilled in the catchment area of the Central Plateau is 111,210 million m3 according to official statistics. In other words, the average annual rainfall in the region is 135 mm. The annual rainfall in this region is the lowest compared to other parts of the country (Statistical Center of Iran, 2016). Regarding climate classification based on the UNEP aridity index, most of the central plateau areas of Iran are classified as "Arid" environment, and its aridity index is between 0.05 and 0.2 (Marani-Barzani *et al*., 2017).

Djamali *et al.* (2012) demonstrated that climate is a primary determinant of phytogeographic regionalization. Topographic context, geologic history, and climatic history are also important factors in determining the floristic features and the nature of boundaries of floristic regions. The Irano-Turanian region forms a distinct bioclimatic area in South West and Central Asia. It is defined by a small ensemble of climatic parameters (continentality index, winter temperature, precipitation seasonality). The west-central part of the Irano-Turanian region (Iran-Anatolian province or IT2 subregion) is the best representative of the Iran-Turanian territory, with both climatic and floristic aspects that at least overlap with the surrounding regions. While phytogeographic and bioclimatic regionalizations should be determined independently, we suggest that the term "Irano-Turanian bio climate" can be used to describe the climate of the region as well as approximately circumscribing the Irano-Turanian floristic region.

List of studied plants

In the central desert area, there are saline water agricultural lands, with nearby underground water, which have been left barren due to climate change and drought. Furthermore, farmers and investors need to introduce plants that are compatible with these lands and have good economic returns. Also, considering the trend of decreasing water reserves and lowering the level of underground water tables in most of the arid and semi-arid regions of the country, there is a need for a change of attitude in the selection of common agricultural species and the introduction of alternative species with less water requirement and higher economic efficiency. The selection and introduction of salinity and drought-resistant medicinal plants can be a practical step towards solving these problems. Therefore, there was a need for a comprehensive survey regarding the flora of plant species that have entered the flora of the Central Plateau during the last few decades, especially in the green space sector.

A combination of multiple sources, including field visits, and expert opinions have been used to prepare this checklist. As a result, 158 species of medicinal and industrial plants resistant to salinity and drought were identified in the central plateau of Iran, which are either hand-planted or naturally scattered in different regions. In the initial investigation, the edible or medicinal use, resistance to salinity and drought, and the vegetative form of 158 studied ornamental species were investigated through scientific sources. The families, genera, and scientific names of the species are listed in table 1. Then, the global chorotype and regional distribution of each species were studied. The characteristics of nativeness (endemic, indigenous, exotic) and the life forms of 158 studied species were investigated.

To check the global chorotype and geographical distribution of 158 studied species from scientific sources, books and articles, and reliable scientific websites, including the Iran herbal network website (Netplant.ir, 2024), The North Carolina extension gardener plant toolbox (Plants.ces.ncsu.edu, 2024), Plants of the world online (Powo.science.kew.org, 2024), Open online galleries and plant identification guide (Plantarium.ru, 2024), The global biodiversity information facility (GBIF.org, 2024) were used.

The priority of using native medicinal plants in landscaping the green spaces of desert areas should be considered in each region. Although, there is no universally accepted definition of native plants, regardless of the variation in the term, native plants usually include plants found in distinct natural locations without the help or introduction of humans. Naturally, native plant species adapted to local climate conditions are best when designing a landscape in arid areas, as they are adapted for high water efficiency and minimal maintenance time and cost.

Non-native ornamentals are usually hard to adapt, require more care, and use large amounts of irrigation water in addition to other production inputs. Unlike native plants that are best adapted to local climate and soil conditions, using native plants in landscape projects can be very beneficial in conserving limited resources. Natural landscaping is an opportunity to restore and create a diverse native ecosystem while providing a natural look to parks and gardens that reflect national heritage and culture.

Based on the investigations carried out in the central plateau of Iran, of these 158 studied Ornamental species in terms of salinity and drought, 156 species are drought resistant, and 67 species are salinity tolerant (Imanian *et al*., 2023).

Out of these 158 ornamental species, in terms of medicinal and edible uses, 116 species have edible uses, 149 species have medicinal uses, and 110 species have both edible and medicinal uses (Imanian *et al*., 2023). Among these 158 ornamental species in terms of vegetative form, 46 are tree species (29 deciduous trees and 17 evergreen trees), 55 shrub species (26 deciduous shrubs and 29 evergreen shrubs), 10 bush species, 43 herbaceous species, and four succulent species (Imanian *et al*., 2023).

Table 3 presents a list of 158 ornamental, medicinal, and aromatic plants (MAP) cultivated in the green spaces of arid and semi-arid areas of the central plateau of Iran.

In this research, the edible or medicinal use, and vegetative form of 158 studied species were investigated, the results of which are summarized in table 3.

Research methodology

In this study, a model was designed to evaluate aesthetic services and economic services (food, pharmaceutical, and timber production) for 158 ornamental studied species. The proxy indicators of Ecosystem Services Evaluation Model (ESEM) for scoring each of the four ecosystem services: Aesthetic values (d), food provisioning (a), natural medicines, pharmaceuticals (b), and wood, fiber production (c) are presented in table 1.

Table 1. The proxy indicators of the Ecosystem Services Evaluation Model (ESEM) for scoring each of the four plant ecosystem services.

Row no.	Proxy indices	Qualitative expression of index score	Quantitative expression
1	Plant density	Low density	0 - 1.50
		Medium density	1.51 - 2.50
		High density	2.51 - 3.50
		Very high density	3.51 - 4
2	The height of vascular plants	<1.5 meters	0 - 1.50
		1.5 to 3 meters	1.51 - 2.50
		3 to 5 meters	2.51 - 3.50
		>5 meters	3.51 - 4
3	Soil type	Soil fertility	0 - 4
4	Age of plants	0 to 10 years	0 - 1.50
		10 to 25 years	1.51 - 2.50
		25 to 40 years	2.51 - 3.50
		More than 40 years	3.51 - 4
5	Bed depth	0 to 1 meter	0 - 1.50
		1 to 2 meters	1.51 - 2.50
		2 to 3 meters	2.51 - 3.50
		More than 3 meters	3.51 - 4
6	Type of vegetation	Grass(Lawn) or tree or bush	0 - 2.50
		A combination of G and T, or G and B, or B and T	2.51 - 3.50
		A combination of three types of grass, bush and tree	3.51 - 4
7	Fertilizer use	No	0 - 2.50
		Yes, chemical fertilizer	2.51 - 3.50
		Yes, organic fertilizer	3.51 - 4
8	Selection of plant species in terms of beauty	Stimulator of touch / smell / taste / hearing / vision	0 - 4
9	Relative prevalence (RP) of woody species	RP = total population of sample species population of woody species / total population of field species	0 - 4

Table 1. Continued.

Row no.	Proxy indices	Qualitative expression of index score	Quantitative expression
10	Relative prevalence (RP) of edible species (fruits and vegetables)	RP = edible species population / total field species population	0 - 4
11	Relative prevalence (RP) of medicinal species	RP = population of medicinal species / total population of field species	0 - 4
12	Plant species richness	Number	0 - 4
13	Plant diversity index	$H = -\sum_{i=1}^{s} P_i \ln P_i$,Where pi is the fraction of individuals belonging to the i.th species. Pi = Ni/N, Ni = Plant population of i.th species. $N=N_1+N_2+N_3+...+N_n$ where N is the number of species	0 - 4
14	Presence of seasonal variety (fruits and vegetables)	Yes/No	0- 4
15	Presence of wood/fiber/ pulp producing plant species such as maple, sugarcane, etc.	Yes/No	0- 4
16	Desirability of Crop yield per year	Not at all/ low/ medium/high	0- 4
17	Frequency of crop supply	Yearly/seasonally/monthly/weekly/ regularly	0- 4
18	Frequency of use of herbal medicines	Rarely / little / moderate / much	0- 4
19	Selling crops (sharing products for sale)	Yes/no	0- 4
20	Appearance	Tidy/messy	0- 4
21	Frequency of visit by the visitors	Monthly/weekly/regularly	0- 4
22	Mental satisfaction level of the personnel	Very high/high/moderate/low/not at all	0- 4

The formula for calculating the score of four ecosystem services with the relevant indicators is presented in table 2.

Table 2. Calculating the scores of four ecosystem services with relevant indicators.

Economic, and cultural benefits	Four ecosystem services	Arithmetic average formula of related proxy indicators to calculate the score of four ecosystem services
Provisioning services	Food provisioning(a)	$a= (\sum_i 3,4,5,7,10,14,16,17,19)/n_a$
	Natural medicines, Pharmaceuticals (b)	$b=(\sum_i 3,4,5,7,11,16,17,18,19)/n_b$
	Wood, fiber production(c)	$c=(\sum_i 3,4,5,7,9,15,16,17,19)/n_c$
Cultural Service	Aesthetic values(d)	$d=(\sum_i 1,2,4,6,8,12,13,20,21,22)/n_l$

The Ecosystem Services Evaluation Model (ESEM) presented in this study is a composite index method, and is used to score each ecosystem service in question through a rapid assessment checklist tool. The score of each of the four ecosystem services (indicated in this study by the abbreviation (a, b, c, d) is obtained from the arithmetic mean of its proxy indicators. The score for each of the aesthetic values, and economic benefits (food provisioning; natural medicines, pharmaceuticals; wood, fiber production) was calculated for the 158 ornamental plants studied, and the calculated scores are given in columns 5 and 6 of table 3.

Table 3. Ornamental, medicinal, and aromatic plants (MAP) cultivated in the green spaces of arid and semi-arid areas of the central plateau of Iran.

Row no.	Scientific name	Medicinal use	Edible use	Analysis of		Life form/ chorotype
				Aesthetic values	Food/ medicine/ wood values	
Lamiaceae						
1	*Salvia rosmarinus*	Leaves, essential oil	Leaves, spice, herbal tea	2.4	3.00/3.11/1.22	He/ M
2	*Perovskia abrotanoides*	Leaves	Flower	2.3	2.94/2.72/1.16	Ch/ IT(End)
3	*Origanum majorana*	Essential oil, oil	Leaves	2.1	2.78/2.67/1.00	Ch/ Cosm
4	*Ballota nigra*	Leaves	No	2.15	1.78/2.67/1.00	Ch/ M-IT
5	*Salvia sclarea*	Seed essence, oil	Leaves, flowers	2.35	2.78/2.89/1.00	He/ M
6	*Salvia nemorosa*	Leaves, essential oil	Seeds	2.2	2.78/2.83/1.00	He/ ES
7	*Salvia officinalis*	Leaves, essential oil	Leaves	2.05	2.78/2.78/1.00	He/ M
8	*Lavandula angustifolia*	Flowers, leaves, essence, oil	Leaves, flowers	2.4	3.00/3.11/1.22	He/ M
9	*Marrubium vulgare*	Leaves, essential oil	Leaves	2.35	2.83/2.83/1.06	He/ M-IT
Verbenaceae						
10	*Vitex agnus-castus*	Seeds, leaves, flowers, essential oil	Seeds, leaves, flowers	2.56	3.06/3.06/1.28	He/ M-IT
11	*Lantana camara*	Sap, leaves, stem bark	No	2.46	1.11/2.78/1.11	He/ AM
12	*Aloysia citrodora*	Leaves, essential oil	Leaves	2.55	3.12/3.12/1.34	He/ NEO
13	*Phyla nodiflora*	Leaves	Leaves	2.07	2.51/2.51/0.84	He/ AM
Asteraceae						
14	*Artemisia persica*	Essential oil	Leaves	2.4	2.39/2.61/1.06	G/ IT(End)
15	*Artemisia vulgaris*	Leaves, essential oil	Flowering branches	2.45	2.39/2.61/1.06	He/ ES-AM
16	*Gazania krebsiana*	Plant extract	No	2.26	0.89/2.50/0.89	He/ SU
17	*Santolina chamaecyparissus*	Leaves, flowers, essential oil	Leaves, flowers	2.46	2.23/2.68/0.90	He/ M
18	*Artemisia scoparia*	Seeds, essential oil	Leaves	2.2	2.11/2.28/0.94	Ch/IT(End)
19	*Cynara scolymus*	Leaves, plant extract	Buds	2.25	3.11/3.11/1.22	He/ M-ES

Table 3. Continued

Row no.	Scientific name	Medicinal use	Edible use	Analysis of		Life form/ chorotype
				Aesthetic values	Food/ medicine/ wood values	
20	*Artemisia absinthium*	Leaves, flowering branch, extract	Extract	2.45	2.39/2.61/1.06	He/ SU-ES
21	*Achillea millefolium*	Leaves, flowering branch, extract	Leaves	2.42	2.61/2.89/0.89	He/ ES-AM
22	*Achillea filipendulina*	Flowering branch, leaves, essential oil	Leaves	2.47	2.61/2.89/0.89	He/ IT
23	*Anthemis nobilis*	Flowers, leaves, essential oil	Flowers	2.27	2.33/2.72/0.72	He/ ES
24	*Tagetes erecta*	Plant extract	Flowers	2.51	2.62/3.06/1.06	Th-He/ NEO
25	*Calendula officinalis*	Plant extract	Leaves, flowers	2.46	2.73/3.17/1.06	Th/ M
26	*Senecio cineraria*	Plant extract	No	2.36	1.12/2.84/1.12	He/ ES- SU
Rubiaceae						
27	*Rubia tinctorum*	Roots	No	2.40	1.28/3.28/1.28	G/ IT(Ind)-M-SU
Apiaceae						
28	*Foeniculum vulgare*	Seeds, leaves, essential oil	Seed, leaf, root, stem	2.20	3.06/3.17/1.17	He/ M
Poaceae						
29	*Cymbopogon schoenanthus*	Leaves, oil, essential oil	Leaves	2.35	2.28/2.83/0.94	He/ SS-SU
30	*Chrysopogon zizanioides*	Root, essence, oil	No	2.55	1.11/3.00/1.11	He/ PAL
31	*Cymbopogon citratus*	Leaves, essence, oil	Leaves	2.25	2.39/2.83/0.83	He/ PAL
32	*Stipa barbata*	No	No (fodder)	2.10	2.39/0.83/0.83	He/ IT(Ind) -SS
33	*Pennisetum orientale*	Plant extract	No (fodder)	2.20	2.39/1.39/0.83	G/ IT, PAL
Onagraceae						
34	*Oenothera glazioviana*	Flowers	Leaves, roots, oil	2.40	2.56/3.00/1.11	He/ NEO
Ephedracea						
35	*Ephedra sinica*	Root and branch extract	Fruits	2.25	2.50/2.89/1.17	Ch/ IT-ES
Oleaceae						
36	*Fraxinus excelsior*	Seed, bark, young branch, extract	Seeds, manna, tea, oil	3.15	3.22/3.28/3.11	Ph/ ES
37	*Jasminum mesnyi*	Essential oil	No	2.71	1.28/3.11/1.28	Ph/ PAL
38	*Jasminum grandiflorum*	Bud, flower, leaf oil, root	No	2.77	1.28//3.11/1.28	Ph/ PAL
39	*Olea europaea*	oil	Fruit, leaves,oil	3.05	3.67/3.61/2.06	Ph/ M, PAL
40	*Jasminum nudiflorum*	Flowers	No	2.71	1.33/3.17/1.33	Ph/ ES
41	*Ligustrum lucidum*	Wax, seed extract	Leaves, seed powder	3.05	3.40/3.46/2.07	Ph/ PAL

Table 3. Continued

Row no.	Scientific name	Medicinal use	Edible use	Analysis of		Life form/ chorotype
				Aesthetic values	Food/medicine/ wood values	
Salicaceae						
42	*Populus euphratica*	Bark, extract of branches	No	2.91	1.44/2.50/2.00	Ph/ SS-IT
Rosaceae						
43	*Rubus hyrcanus*	Fruit, leaf, root, stem	No	2.55	1.06/2.78/1.06	Ph/ IT(End)
44	*Rosa foetida*	Flower extract	Petal, fruit	2.65	3.00/3.17/1.39	Ph/ IT(Ind)-ES
45	*Pyrus boissieriana*	Young leaves, stem bark, seeds, fruit	Fruits	2.97	3.40/3.18/2.12	Ph/ IT(End)
46	*Amygdalus lycioides*	Fruit, root and stem extract	Fruits	2.71	2.63/2.79/1.34	Ph/ IT(End)
47	*Amygdalus scoparia*	Resin	Fruits	2.61	2.74/2.62/1.46	Ph/ IT(End)
48	*Crataegus monogyna*	Flower, leaf	Leaves, flowers, fruits	3.22	3.37/3.46/1.79	Ph/ M-ES
49	*Rosa canina*	Leaves, flowers, essential oil	Seed, flower, fruit	2.93	3.14/3.52/1.47	Ph/ IT(Ind)-M-ES
50	*Eriobotrya japonica*	Flowers	Fruit, seed	2.73	3.03/2.69/1.24	Ph/ PAL
51	*Rhaphiolepis umbellata*	No	Seed, flower	2.96	2.72/1.30/1.30	Ph/ PAL
52	*Cotoneaster salicifolius*	Resin	Fruits	2.73	2.62/3.01/1.22	Ph/ PAL
53	*Pyracantha coccinea*	No	Fruits	2.91	2.68/1.28/1.44	Ph/ M-IT(Ind)
Lythraceae						
54	*Punica granatum nana*	Flowers, leaves, oil	Flowers	2.96	3.06/3.67/1.72	Ph/ IT(End)
55	*Punica granatum*	Fruit, seed oil	Fruits	2.95	3.61/3.28/1.72	Ph/ IT(End)
Moraceae						
56	*Maclura pomifera*	Fruit, extract	No	3	1.44/3/2.33	Ph/ AM
Fabaceae						
57	*sophora mollis*	No	Leaf	2.76	2.56/150/1.50	Ph/ IT(Ind)-PAL
58	*Robinia pseudoacacia*	Essential oil	Flower, seed, seed pod, oil	3.01	2.61/2.50/2.00	Ph/ AM
59	*Halimodendron halodendron*	Flower, root	No	2.75	1.22/2.56/1.22	Ph/ IT(Ind)-ES
60	*Cercis siliquastrum*	Flowers, bark, roots, young leaves	Seed pod, flower	2.92	2.28/1.83/1.56	Ph/ IT(Ind)-M
61	*Acacia farnesiana*	Essential oil, resin	Flowers	2.97	2.34/2.34/1.84	Ph/ NEO
62	*Amorpha fruticosa*	Fruit extract	Flowers	2.67	2.17/1.94/1.17	Ph/ AM
63	*Spartium junceum*	Flowers, young branches, seeds, roots	Flower, essential oil	2.72	2.00/2.33/1.28	Ph/ M
64	*Acacia victoriae*	No	Seed	2.66	2.33/1.17/1.11	Ph/ AUS
65	*Gleditsia caspica*	Fruit extract	Seed	3.05	2.78/2.06/1.84	Ph/ ES(End)
66	*Erythrostemon gilliesii*	Root	No	3.01	1.44/2.17/1.78	Ph/ NEO
67	*Leucaena leucocephala*	Bark, root, seed	Seeds, green pods	2.96	2.91/2.61/2.28	Ph/ NEO
68	*Albizia Julibrissin*	Bark, flowers, gum	Leaves, flowers	3.11	2.17/2.50/1.89	Ph/ ES(Ind)-PAL
69	*Sophora japonica*	Flower buds	Leaves, flowers	3.26	2.72/3.11/1.89	Ph/ PAL

Table 3. Continued

Row no.	Scientific name	Medicinal use	Edible use	Analysis of		Life form/ chorotype
				Aesthetic values	Food/medicine/ wood values	
Rhamnaceae						
70	*Ziziphus jujuba*	Fruit	Leaves, fruits, coffee substitute	3.2	3.07/3.07/1.83	Ph/ ES-PAL
71	*Ziziphus lotus*	Fruit, leaf, flower	Fruits	3.16	2.83/2.90/1.72	Ph/ SS-M
72	*Paliurus spina-christi*	Flowers	Fruits	2.92	2.31/2.37/1.24	Ph/ ES(Ind)-M
Convulvulaceae						
73	*Cressa cretica*	Leaf, extract	Fruit oil	2.55	1.72/1.72/0.83	He/ IT(Ind)-M-SS-SU
Ulmaceae						
74	*Ulmus boissieri*	Leaves, the bark of branches	Leaves	3.10	2.07/2.26/1.73	Ph/ IT(End)
75	*Ulmus Umbraculifera*	Leaves, bark, roots	Leaves	3.10	2.07/2.26/1.73	Ph/ IT(End)
76	*Zelkova carpinifolia*	Fruit, extract	Leaves	3.21	2.39/2.34/2.17	Ph/ ES(End)
Meliaceae						
77	*Melia azedarach*	Leaves, root bark	No	2.96	1.34/2.26/1.62	Ph/ PAL- AUS
Amaranthaceae						
78	*Halothamnus subaphyllus*	Plant extract	No	2.46	0.83/1.17/0.83	Ch/ IT(End)
79	*Seidlitzia rosmarinus*	Stem, leaf, extract	No	2.56	1.36/1.92/1.12	Ch/ IT(Ind)-SS-M
80	*Suaeda aegyptiaca*	Leaf-stem	No	2.11	0.78/1.36/0.5	Th/ IT(Ind)-SS
81	*Salsola abarghuensis*	Leaf, stem, extract	No	2.67	1.29/1.81/1.07	Ph/ IT(End)
82	*Salsola dendroides Pall.*	Leaf-stem	No	2.50	1.18/1.70/0.96	He/ IT(End)
83	*Haloxylon recurvum*	Leaf-stem	No	2.51	1.12/1.86/0.90	Ph/ SS
84	*Anabasis aphylla*	Annual branches	No	2.40	1.01/1.59/0.73	He/ IT(Ind) -ES
85	*Atriplex halimus*	Leaf extract	Seed, leaf	2.92	2.06/2.12/1.17	Ph/ M- PAL
86	*Salicornia Europaea*	Plant extract	Stem, leaf, seed, oil	2.08	1.89/1.50/0.50	Th/ ES
87	*Eurotia ceratoides*	No	No	2.41	1.18/0.84/0.84	Ch/IT(Ind)-ES-SS
Arecaceae						
88	*Phoenix dactylifera*	No	Fruits	3.17	3.40/1.89/2.07	Ph/ SS(End)
89	*Nannorrhops ritchiana*	Leaf, flower	Fruits	2.91	2.80/2.57/1.97	Ph/ SS(End)
Tamaricacea						
90	*Tamarix aphylla L*	Leaves extract, bark, gall	No	3.06	1.00/1.90/1.39	Ph/ IT, SS(Ind)
91	*Tamarix ramosissima*	Leaf extract, stem bark	No	3.02	0.91/1.81/0.91	Ph/ IT(Ind) -ES
Nitrariacea						
92	*Nitraria schoberi*	Fruits	Fruits	2.72	1.52/1.52/0.97	Ph/ IT(Ind) -ES
Polygonaceae						
93	*Calligonum aphyllum*	Fruit, essential oil, extract	No	2.67	1.01/1.81/1.01	Ph/ ES

Table 3. Continued

Row no.	Scientific name	Medicinal use	Edible use	Analysis of		Life form/ chorotype
				Aesthetic values	Food/medicine/ wood values	
Berberidaceae						
94	*Berberis thunbergii*	Root and stem extracts, leaves, flowers, fruits	Fruits, leaves	2.78	2.37/2.48/1.47	Ph/ PAL
95	*Berberis khorasanica*	Fruits	Fruits, leaves	2.97	2.52/2.48/1.47	Ph/ IT(End)
Bignoniaceae						
96	*Chilopsis linearis*	Seed pod, flower	Seed pod, flower	3.07	2.12/2.18/1.62	Ph/ AM
Sapindaceae						
97	*Dodonaea viscosa*	Leaf extract	Seed	2.86	2.00/2.12/1.67	Ph/ AUS-PAL-NEO-SU -SS(Ind)
98	*Koelreuteria paniculata*	Flower	Seed, leaf	3.21	2.06/2.28/1.44	Ph/ ES-PAL
99	*Acer negundo*	Sap, inner bark	Seed, leaf, skin, sap	3.21	2.18/2.57/1.90	Ph/ AM
100	*Acer pseudoplatanus*	Sap	Leaves, sap, seed pods	3.21	2.18/2.57/1.90	Ph/ M-ES
Acanthaceae						
101	*Ruellia simplex*	No	No	2.82	1.11/1.11/1.11	He/ NEO
Nyctaginaceae						
102	*Bougainvillea glabra*	Leaves and bracts	No	3.07	1.46/2.37/1.46	Ph/ NEO
Bignoniaceae						
103	*Tecoma radicanstrumpet*	Flower, leaf, branch, root	No	3.27	1.39/2.40/1.39	Ph/ AM
Simaroubaceae						
104	*Ailanthus altissima*	Leaf, fruit, root bark	Leaf	3.16	1.78/2.11/1.44	Ph/ PAL
Caprifoliaceae						
105	*Lonicera caprifolium*	Flowers, leaves, essential oil	Fruits	2.96	1.96/2.39/1.17	Ph/ ES
106	*Symphoricarpos albus*	Fruits	Fruits	2.66	1.67/1.83/0.83	Ph/ AM
Solanaceae						
107	*Datura stramonium*	Leaf, seed	No	2.46	1.07/2.34/1.07	Ph-Th/ NEO-AM
108	*Withania coagulans*	Seed, leaf, root	Seed, fruit	2.35	2.07/2.23/1.17	Ph/ IT-SS(Ind)
109	*Lycium ruthenicum*	Plant extract	Fruit, leaf	2.61	2.29/2.23/1.07	Ph/ IT(Ind) -ES
Pinaceae						
110	*Pinus mugo*	Leaf, fruit, essential oil	Leaf	3.06	2.11/2.44/1.56	Ph/ ES
111	*Pinus nigra*	Extract	Fruits	3.11	2.00/2.06/1.94	Ph/ M
112	*Cedrus deodara*	Essential oil, stem bark	No	3.22	1.47/2.47/2.19	Ph/ PAL- IT
Boraginaceae						
113	*Cordia myxa*	Fruit, sap, leaf, root	Fruits	3.06	2.51/2.48/1.84	Ph/ PAL-SS(Ind)
114	*Cynoglossum officinale*	Leaf, root, oil	Leaves	2.11	1.61/1.72/0.61	He/ M-ES(Ind)
115	*Symphytum officinale*	Leaf, root, gum	Leaves	2.27	1.40/2.62/1.01	He/ M-ES
116	*Echium amoenum*	Flower, leaf	Flowers	2.27	1.86/2.29/0.68	He/ IT(Ind)-ES

Table 3. Continued

Row no.	Scientific name	Medicinal use	Edible use	Analysis of		Life form/ chorotype
				Aesthetic values	Food/medicine/ wood values	
Moringaceae						
117	*Moringa oleifera*	Leaves, roots, seeds, bark, fruits, flowers, and unripe pods	Pods, young leaves	2.85	2.63/2.73/1.61	Ph/ PAL
Paulowniaceae						
118	*Paulownia tomentosa*	Leaf and fruit extract	Honey production	3.10	2.37/2.91/2.62	Ph/ PAL
Myrtaceae						
119	*Melaleuca citrina*	Root extract	Seed, leaf	3.01	1.86/2.01/1.51	Ph/ AUS
120	*Eucalyptus camaldulensis*	Leaves, resin	Seed	3.21	1.89/2.34/2.39	Ph/ AUS
121	*Myrtus communis*	Leaf, stem, essential oil	Fruit, leaf, flower	2.91	2.39/2.62/1.73	Ph/ M-IT-SS(Ind)
Fagaceae						
122	*Quercus ilex*	Fruit, skin, oak apple, leaf	Acorn	3.17	2.87/2.89/2.18	Ph/ M-ES
123	*Quercus brantii*	Fruit, skin, oak apple, leaf	Acorn	3.17	2.87/2.89/2.12	Ph/ IT(Ind)
124	*Quercus longipes*	Fruit, skin, oak apple, leaf	Acorn	3.17	2.87/2.89/2.18	Ph/ IT(Ind)-ES
Cucurbitaceae						
125	*Luffa acutangula*	Fruit, seed	Unripe fruits	2.47	2.49/2.91/1.36	Th/ PAL
Lauraceae						
126	*Laurus nobilis*	Leaves, oil, essential oil	Dried leaf	3.18	2.91/3.09/1.73	Ph/ M
Asparagaceae						
127	*Agave americana*	Leaf, sap, root	Seed, leaf, stem, sap	2.50	2.02/2.34/1.28	He/ AM
128	*Yucca gloriosa*	Fruit extract	Flower, fruit, root, stem	2.82	2.42/2.01/1.40	Ph/ AM
129	*Danae racemosa*	Leaf extract	No	2.83	1.40/2.18/1.29	Ph/ IT(Ind)
130	*Ruscus aculeatus*	Root, stem	Stem	2.62	2.12/2.62/1.29	G/ M-ES-IT(Ind)
131	*Ophiopogon japonicus*	Root, root extract	Root	2.57	1.90/2.12/0.96	He/ PAL
Aizoaceae						
132	*Lampranthus spectabilis*	Leaves	No	2.45	1.00/1.68/0.83	He/ SU
Araliaceae						
133	*Hedera helix*	Leaf extract, leaf	No	3.06	1.50/2.39/1.33	Ph/ M-ES(Ind)
Gesneriaceae						
134	*Streptocarpus ionanthus*	Flower, oil	No	2.52	1.13/2.20/1.13	He/ SU
Cupressaceae						
135	*Platycladus orientalis*	Seed, leaf	Seed	3.10	2.22/2.56/1.78	Ph/ ES-PAL-IT(Ind)
136	*Cupressus sempervirens*	Leaves, essential oil	No	3.05	1.22/2.11/2.00	Ph/ M-IT(Ind)

Table 3. Continued

Row no.	Scientific name	Medicinal use	Edible use	Analysis of		Life form/ chorotype
				Aesthetic values	Food/medicine/ wood values	
137	*Juniperus excelsa*	Leaves	Fruits	3.10	2.28/2.123/2.11	Ph/ M- ES-IT(Ind)
138	*Taxodium distichum*	Essential oil	No	3.15	1.46/2.26/2.68	Ph/ AM
139	*Juniperus sabina*	Essential oil	No	2.91	1.28/2.11/1.33	Ph/ M-ES-IT(Ind)
Celasteracea						
140	*Euonymus japonicus*	Leaf, plant extract	Leaves	3.10	2.54/2.48/1.52	Ph/ PAL
Buxaceae						
141	*Buxus sempervirens*	Leaves, wood, bark	Leaves	3.10	2.37/2.47/1.87	Ph/ M-ES-IT(Ind)
Plantaginaceae						
142	*Veronica chamaedrys*	Aerial part	Leaves	2.47	1.74/1.84/0.89	He/ M-ES-IT
Elaeagnaceae						
143	*Elaeagnus angustifolia*	Flower, essential oil	Seed, fruit	3.05	2.57/2.13/1.73	Ph/ ES-IT(Ind)
Portulacaceae						
144	*portulaca grandiflora*	Aerial branch, extract	Seed, leaf, root	2.31	156/1.94/0.61	Th/ NEO
Ginkgoaceae						
145	*Ginkgo biloba*	Fruit, leaf extract	Seed, oil	3.11	2.51/2.67/2.39	Ph/ PAL
Scrophulariaceae						
146	*Verbascum thapsus*	Leaf, fruit, oil	Flowers	2.67	1.92/2.30/0.84	He/ M-ES-IT(Ind)
Malvaceae						
147	*Alcea rosea*	Flower, leaf, root	Flower, root	2.61	2.34/2.50/1.06	He/ IT
148	*Hibiscus syriacus*	Oil, flower, root	Leaf, root, flower	2.98	2.68/2.78/1.42	Ph/ PAL
Caryophyllaceae						
149	*Saponaria officinalis*	Flower, root	No	2.42	0.96/2.51/0.96	G/ ES- IT
Paeoniaceae						
150	*Paeonia lactiflora*	Flower, root	Seed, root, stem	2.76	2.26/2.80/1.40	G/ ES
Anacardiacea						
151	*Cotinus coggygria*	Essential oil, leaves, bark	Leaves	3.06	1.96/2.08/1.84	Ph/ ES-IT(Ind)
Apocynacea						
152	*Nerium oleander*	Flower, bark	No	3.12	1.34/2.52/1.34	Ph/ M- SS-IT(Ind)
Cannabacea						
153	*Celtis australis*	Leaf, fruit	Fruits	3.17	2.46/2.50/2.29	Ph/ M- ES(Ind)
154	*Celtis caucasica*	No	Fruits	3.17	2.46/1.22/2.29	Ph/ ES-IT(Ind)
Asphodelaceae						
155	*Hemerocallis fulva*	Root, stem, oil	Leaf, root, flower	2.81	2.40/1.97/1.01	G/ PAL-ES

Table 3. Continued

Row no.	Scientific name	Medicinal use	Edible use	Analysis of		Life form/ chorotype
				Aesthetic values	Food/medicine/ wood values	
Calycanthaceae						
156	*Chimonanthus fragrans*	Flower, essential oil, leaf, root	Flowers	2.93	2.24/2.08/1.30	Ph/ ES-PAL
Tropaeolaceae						
157	*Tropaeolum majus*	Flower, oil	Seed pods, seeds, leaves, flowers	2.57	2.13/1.79/0.57	Th/ NEO
Altingiacea						
158	*Liquidambar styraciflua*	Gum	Gum	3.27	2.46/2.57/2.51	Ph/ AM

Life form: Th (Therophyte), Ch (Chamaephyte), He (Hemicryptophyte), Ph (Phanerophyte), and G (Geophyte); Chorotype: SS (Saharo-Sindian); IT (Irano-Touranian); M (Mediterranean); ES (Euro-Siberian); Cosm (Cosmopolitan); AM (American); SU (Sudano-Zambezian); TR (Tropical); NEO (Neotropical); AUS (Australian); PAL (Paleotropic); End (Endemic); Ind (Indigenous).

RESULTS AND DISCUSSION

In arid and semi-arid areas, water and soil resources are salty for many reasons. And the development of vegetation is facing serious problems. In addition, due to population growth, water sources that can used for irrigation are limited. Therefore, the introduction and selection of ornamental species that can tolerate salty conditions is of particular importance and can contribute to the stability of the created green covers. In other words, in the sustainable design of urban green spaces in arid environments, it is necessary to choose plant species that can tolerate water and salinity stress in addition to their aesthetic value (Christoforidi *et al*., 2022). Based on the investigations carried out in this research, 158 ornamental species resistant to salinity and drought have been cultivated in the central plateau of Iran, of which 156 species are resistant to drought, and 67 species are tolerant to salinity.

The selection of native species that have already adapted to the environmental conditions of the region outclass the exotic species in landscape design. Introduced ornamental plants are usually difficult to acclimatize and use large amounts of irrigation water and production inputs. Some native species are more salt tolerant than exotic species, attract and retain greater numbers of natural enemies, are used as habitat management in biological control, and are best adapted to local climatic and soil conditions (Alam *et al*., 2017). Therefore, understanding whether these species are native or non-native and how they are distributed in Iran and the world can help in the optimum exploitation of arid environments. Of the 158 studied species, only 60 are native species, 19 of which are endemic, and 41 are indigenous and there are 98 exotic species (Fig. 3).

The best way to utilize the degraded land is to domesticate the wild native species rather than to increase the salt tolerance of plant species. The successful approach is to select the wild species that have genetic tolerance to salt stress and have some economic and landscape potential. This new policy is proposed to promote arid landscaping and maximum use of water for conserving amenity planting. This approach has been successful in saving water in the arid cities of southwestern UAE. It also helped to increase the beauty and aesthetic value of desert cities. The adoption of an arid landscape policy would reduce the energy requirements by more than half and the maintenance costs of the landscape design. (Alam *et al*., 2017).

Final results on the importance of ornamental plants in creating beautiful and sustainable gardens has shown that these ornamental flowering plants not only enhance the beauty of gardens and landscapes, but also have applications in the pharmaceutical, aromatic, timber, food production, and other industries. In this research, out of 158 studied ornamental species, 116 species have edible uses, 149 species have medicinal uses, and 110 species have both edible and medicinal uses.

The silvomedicinal system is the new paradigm of integration of trees and medicinal plants, which can provide an array of products ranging from food, fodder, fruit, fiber, pulp, medicinal plants, etc for consumption and trade. Moreover, conserves biodiversity and reduces the pressure on natural resources.

Most medicinal plants grow in the under-forest layer and are shade-tolerant. Therefore, the agroforestry system offers a convenient strategy for promoting their cultivation and conservation. In the silvomedicinal system shade tolerant medicinal plants would be integrated as lower-strata species in the multistrata system. It would be cultivated in a short cycle in the existing stands of the plantation crops and the medicinal trees as shade providers and boundary markers. Another way is to grow medicinal trees as shade providers and boundary markers. Tall and perennial medicinal trees are planted at wide spacing in this system (Kalaichelvi and Arul Swaminathan, 2009). The interspaces in between the trees are utilized for growing green spaces or medicinal crops.

In terms of global chorotype, there are 60 species of Irano-Touranian region, 17 species of Saharo-Sindian, 39 species of Mediterranean, 51species of Euro-Siberian, 16 species of American, nine species of Sudano-Zambezian, 32 species of Paleotropic, 12 species of Neotropical, five species of Australian, and one species of cosmopolitan (Fig. 1)

Life form spectra of the 158 studied species included 96 phanerophyte species, eight chamaephyte species, 40 hemicryptophyte species, seven geophyte species, and seven therophyte species (Fig. 2).

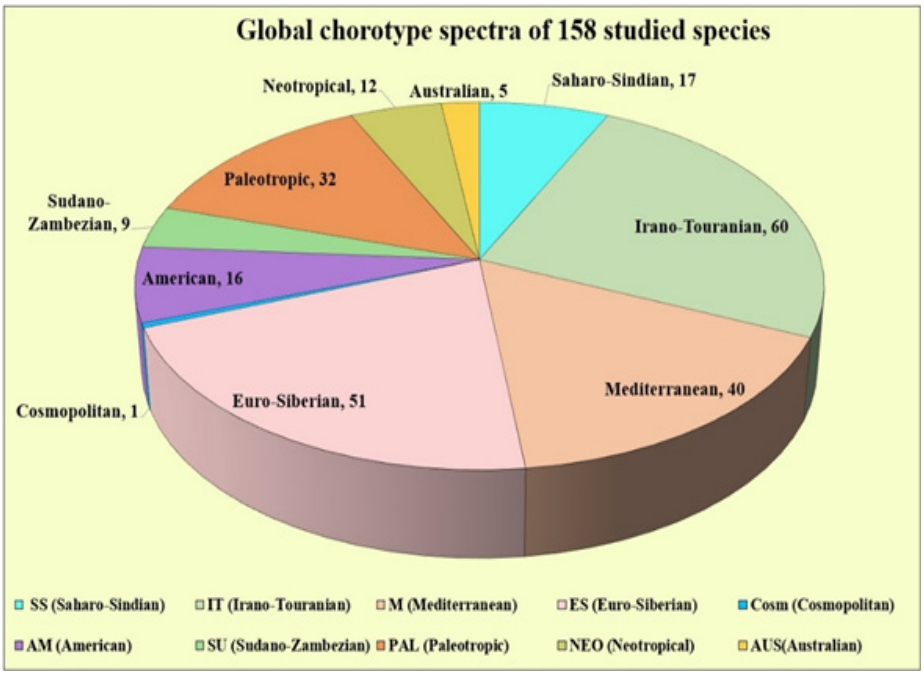

Fig. 1. Global chorotype spectra of the 158 studied species.

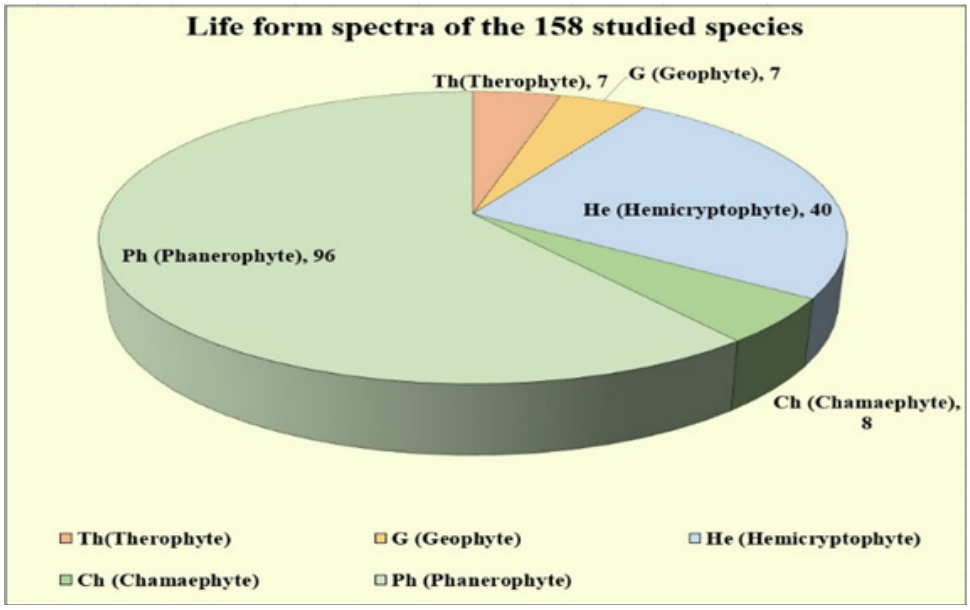

Fig. 2. Life form spectra of the 158 studied species included 96 Phanerophyte species, eight Chamaephyte species, 40 Hemicryptophyte species, seven Geophyte species, and seven Therophyte species.

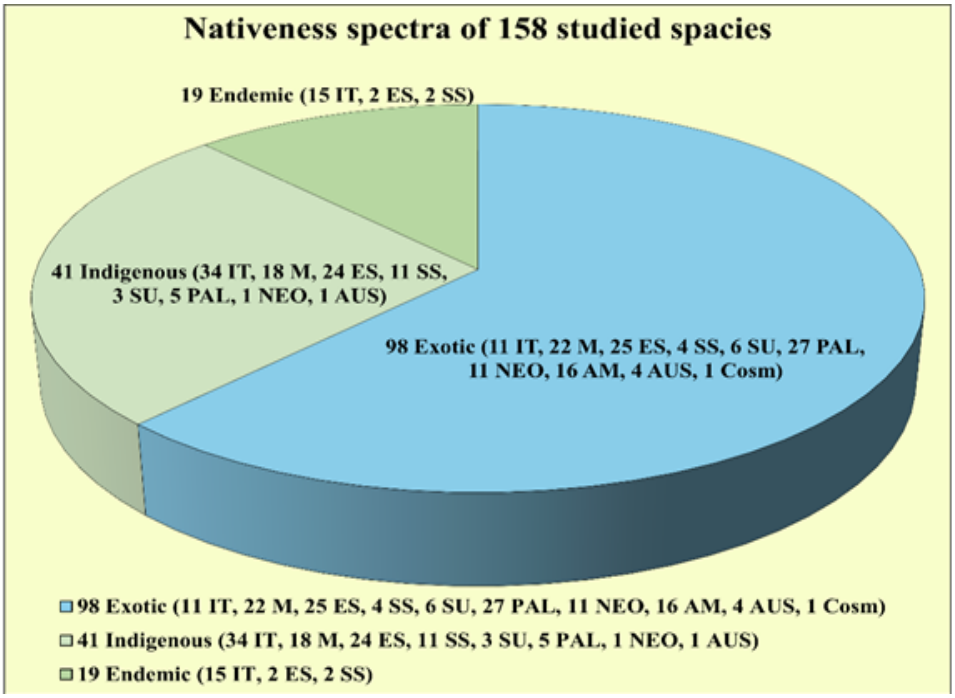

Fig.3. The nativeness spectra of the 158 studied species included 19 endemic species, 41 indigenous species, and 98 exotic species.

In this study, by Ecosystem Services Evaluation Model (ESEM) evaluated aesthetic values and economic benefits (food provisioning; natural medicines, pharmaceuticals; wood, fiber production) for 158 ornamental studied species. The scores of each service are presented in column 5,6 in table 1.

Fig. 4 shows the ranking of aesthetic value and economic benefits in 158 studied ornamental species.

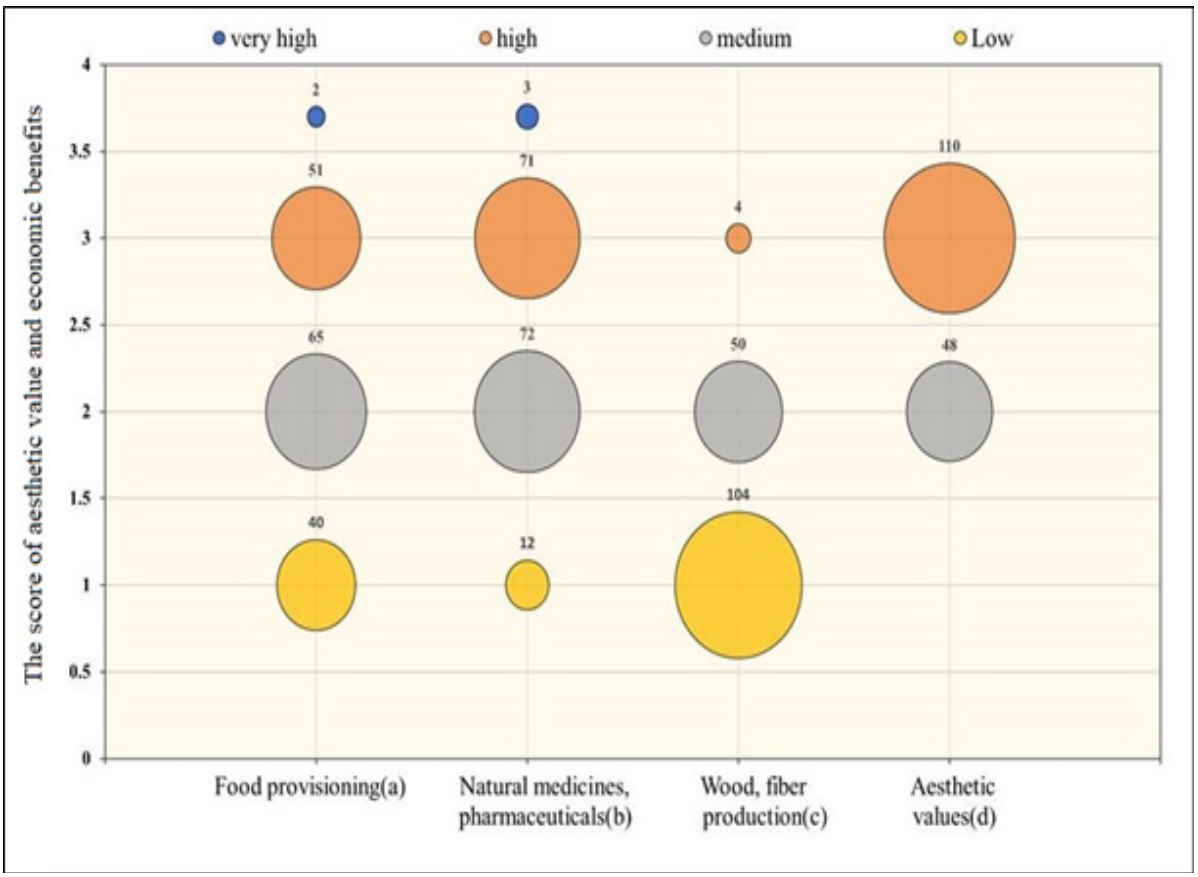

Fig.4. Ranking of aesthetic value and economic benefits in 158 studied ornamental species.

Medicinal plants have a low water requirement due to their adaptability to arid and semi-arid climates, and their cultivation can play an influential role in preserving limited water resources. Therefore, changing the cultivation pattern and replacing drought-resistant medicinal plants can reduce water consumption and be a practical step toward achieving sustainable agriculture. It will also increase the biodiversity of the region.

Nowadays, the introduction and cultivation of new species in Iran has increased, so for better management of dry environments, it is necessary to understand whether this species is native to Iran, what is its life form in Iran, what is its global chorotype, and what is its current geographic distribution in Iran. Investigating these indicators can be used to predict the success or failure of establishing a species in a new environment.

The score for each of the aesthetic values, and economic benefits (Food provisioning; Natural medicines, pharmaceuticals; Wood, fiber production) was calculated for the 158 ornamental plants studied. Then, by comparing the model scores, the species with the highest aesthetic values and economic benefits among the 158 ornamental species studied were identified. Table 4 shows the species with the highest rank of aesthetic values, and economic benefits among the 158 studied ornamental species.

Using the ecosystem services evaluation model presented in this study to compare the aesthetic values and economic benefits of ornamental species provides a guide for selecting plants in green spaces.

Table 4. The species with the highest rank of aesthetic values, and economic benefits among the 158 studied ornamental species.

Rank	Cultural service		Provisioning services					
	Aesthetic values (d)	Score	Food provisioning (a)	Score	Natural medicines, pharmaceuticals (b)	Score	Wood, fiber production (c)	Score
1	*Tecoma radicanstrumpet*	3.27	*Olea europaea*	3.67	*Punica granatum*	3.67	*Fraxinus excelsior*	3.11
2	*Liquidambar styraciflua*	3.27	*Punica granatum*	3.61	*Olea europaea*	3.61	*Taxodium distichum*	2.68
3	*Sophora japonica*	3.26	*Ligustrum lucidum*	3.40	*Rosa canina*	3.52	*Paulownia tomentosa*	2.62
4	*Cedrus deodara*	3.22	*Pyrus boissieriana*	3.40	*Ligustrum lucidum*	3.46	*Liquidambar styraciflua*	2.51
5	*Crataegus monogyna*	3.22	*Phoenix dactylifera*	3.40	*Crataegus monogyna*	3.46	*Ginkgo biloba*	2.39
6	*Zelkova carpinifolia*	3.21	*Crataegus monogyna*	3.37	*Punica granatum*	3.28	*Eucalyptus camaldulensis*	2.39
7	*Koelreuteria paniculata*	3.21	*Fraxinus excelsior*	3.22	*Fraxinus excelsior*	3.28	*Maclura pomifera*	2.33
8	*Acer negundo*	3.21	*Rosa canina*	3.14	*Rubia tinctorum*	3.28	*Celtis australis*	2.29
9	*Acer pseudoplatanus*	3.21	*Aloysia citriodora*	3.12	*Pyrus boissieriana*	3.18	*Celtis caucasica*	2.29
10	*Eucalyptus camaldulensis*	3.21	*Cynara scolymus*	3.11	*Foeniculum vulgare*	3.17	*Leucaena leucocephala*	2.28

CONCLUSION

Indiscriminate use of water for irrigation and an increase in population demand can be one of the reasons for severe water shortage soon. In all the cities located in the central plateau of Iran, they are facing the problem of water shortage for green spaces. This lack of water is the limiting factor for landscaping and greenery. Creating urban green spaces with the plants introduced in this research can be an alternative way for sustainable greenery in severe conditions of water scarcity and salinity, which brings many other ecological and economic benefits. This paper introduces many economic ornamental plants suitable for arid environments. By choosing various species of medicinal and edible plants, we have increased ecosystem services and biodiversity, in addition to benefiting from the aesthetic aspect of these species.

In order to identify and select these species to create green spaces in dry environments, knowing the factors of nativeness, resistance to drought and salinity, and the chorotype of these plants is particularly important for the previously mentioned reasons, and the selection of plant species is the success factor in the expansion of green space.

The bioregion of the Central Plateau of Iran is IT (Irano-Touranian). Out of the 158 species studied, 60 plant species have repeated presence in the flora of Irano-Touranian, of which only 15 plant species are endemic to Iran. There are also four endemic species from the bioregions of ES from the country's north and SS from the south of the country in the central plateau of Iran.

In the success of growing plants in dry environments, attention should be paid to the primary origin of the species; the closer the current conditions of cultivation of the species are to its primary origin, the maximum growth that the plant has in its primary origin will also be in the new habitat. The geographical distribution of 158 studied species in Iran shows that the

regional chorotype spectrum of species transcribes its global chorotype spectrum. A greenspace species is more successful in that habitat closer to its primary origin. For example, there are 60 repetitions of the presence of the species in the Irano-Touranian bioregion. Regarding providing suitable conditions for plant growth, the 158 studied species respectively have the highest environmental affinity with IT, ES, M, PAL, SS, AM, NEO, SU, and AUS bioregions.

In this study, by Ecosystem Services Evaluation Model (ESEM) compared aesthetic values and economic benefits of 158 ornamental studied species. For qualitative expression of Ecosystem Service Evaluation Model (ESAM) score,the scores distinct to four level low (≤1.5), medium (≤2.5 , >1.5), high (≤3.5 , >2.5), and very high (> 3.5).

For example, in terms of qualitative expression, after scoring the natural medicines, pharmaceuticals (b),3 plant species, including *Punica granatum, Olea europaea, Rosa canina*, had very high scores, 71 plant species, including *Ligustrum lucidum, Crataegus monogyna*, etc. had high scores, 72 plant species had medium scores, and 12 plant species had low scores.

Increasing biodiversity is achieved by cultivating medicinal species in green spaces. The aim of this research is to introduce medicinal and industrial plants resistant to salinity and drought for the design of hand-planted green spaces in the hot and dry areas of the Iranian plateau. finally, the number of 158 ornamental species of medicinal and industrial plants for cultivation in the green space of arid and semi-arid regions has been presented. Among the plant species presented, 17 species of evergreen trees, 29 species of deciduous trees, 29 species of evergreen shrubs, 26 species of deciduous shrubs, 10 species of bushes, 43 species of herbs and 4 species of succulents. The largest number of species presented belongs to the Asteraceae family with 13 species, the Fabaceae family with 13 species, the Rosaceae family with 11 species, the Amaranthaceae family with 10 species, the Lamiaceae family with 9 species, and the Oleaceae family with 6 species. the total number of plant families is 62 families.

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Root Dynamics in *Bougainvillea glabra* Choisy Cuttings: Analyzing the Influence of Auxin Type, Concentration, and Cutting Type Over Time in Two Varieties

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Abstract

This study investigated the effects of three types of auxins (indolebutyric acid (IBA), naphthaleneacetic acid (NAA), and dichlorophenoxyacetic acid (2,4-D)) on the rooting of soft, semiwoody, and woody stem cuttings of bougainvillea (*Bougainvillea glabra* Choisy) during early winter and spring under greenhouse conditions. PGRs were prepared at concentrations of 250, 500, and 1000 mg L^{-1}. The stem cuttings were immersed in these solutions for 24 hours before being transferred to the growth media. The experiment was designed as a factorial experiment in a completely randomized design with 10 replications. The results indicated that timing (winter vs. spring) had no significant effect on the rooting percentage (p-value = 0.05). However, significant differences were observed in branching, with the shortest average branch length (2.0 cm) occurring in early winter and the longest (4.7 cm) occurring in early spring. Significant differences were also found between varieties, with the red variety outperforming the white variety (20.44% vs. 10.83%). The woody cuttings produced significantly better results than the soft and semiwoody cuttings did. Among the PGRs, those treated with 250 mg L^{-1} IBA (33.2%) or NAA (32.5%) presented the highest rooting percentages. Increasing concentrations of these auxins resulted in decreased rooting, with the lowest rooting percentage (0%) observed at 1000 mg L^{-1} 2,4-D. This study demonstrated that the type and concentration of auxins significantly affect the rooting and branching of bougainvillea stem cuttings, with optimal results achieved with 250 mg L^{-1} IBA and NAA. These findings suggest that careful selection of the auxin type and concentration, as well as consideration of the cutting type and timing, are crucial for the successful propagation of bougainvillea.

Keywords: Acetic acid, Greenhouse production, Propagation, Seasonal variation, Soft cutting.

INTRODUCTION

Bougainvillea, scientifically known as *Bougainvillea glabra* Choisy, is an ornamental shrub widely recognized across various regions of the world and belongs to the Nyctaginaceae family, which comprises 18 species of shrubs, bushes, and vines (Sattler and Perlin, 1982). In the tropical and subtropical regions of Iran, this plant is commonly used to cover walls, create arbors, and decorate fence walls in houses and parks (Danshur, 2013). Bougainvillea is notablele for its vibrant bracts of various colors and its resistance to salinity and drought, making it suitablele for tropical regions (Singh *et al*., 2011). Recent studies have highlighted the antioxidant and antidiabetic activities of *Bougainvillea glabra*, particularly focusing on the D-pinitol content in various cultivars (Abo-Elghiet *et al*., 2023).

The most prevalent method of propagating bougainvillea is through stem cuttings, although other asexual propagation methods, such as micropropagation via shoot tip cultivation, have also been reported (Lin *et al*., 2024). Propagation by stem cuttings is particularly advantageous because of its simplicity and several benefits, including the preservation of the plant's genetic characteristics, the requirement for fewer mother plants, cost-effectiveness, speed, and the lack of need for specialized techniques or equipment (Hartmann *et al*., 2002). Recent advancements in plant transformation techniques, including nanoparticle methods and protoplast-based approaches, have further expanded the possibilities for plant propagation (Levengood *et al*., 2024).

There is considerable variation in the rooting ability of stem cuttings among different plant species and cultivars. While some species easily root from stem cuttings, others require optimized conditions for successful rooting. The key factors influencing the success of rooting include careful selection of cuttings from the mother plant, proper management of cuttings, and control of environmental conditions during the rooting process (Hartmann *et al*., 2002). Essential considerations for enhancing the rooting of cuttings include selecting the appropriate type of cutting, preparing cuttings at the optimal time, maintaining suitablele temperature and humidity in the rooting environment, using an appropriate planting medium, performing pre- or postpreparation treatments, and applying PGRs at optimal concentrations (Hartmann *et al.*, 2002). Recent research has shown that environmental stress conditions, such as drought and high salinity, significantly affect root development and plant survival (Vives-Peris *et al*., 2020). Given the challenges associated with rooting papaya cuttings, effective propagation is crucial for producers (Asl *et al*., 2012). Gehlot *et al*. (2014) reported that the application of IBA and NAA at specific concentrations improved the rooting percentage and survival of *Azadirachta indica* stem cuttings in an alternating mist system. Further research by Seyedi *et al.* (2014) revealed that the use of IBA in combination with talc powder and potassium nitrate solution increased the rooting percentage, weight, and length of roots. The results of the treatment with 3000–6000 mg L^{-1} IBA demonstrated that thicker cuttings with higher internal auxin concentrations produced more and better roots. Recent studies have also explored the role of phenolic compounds in enhancing the rooting ability of plant cuttings (Santos-Rufo *et al*., 2024). Additionally, some studies on bougainvillea have focused on optimizing callus induction and shoot formation through *in vitro* culture techniques (Aghaali, 2019).

Research indicates that excessive use of PGRs during rooting can disrupt a plant's hormonal balance and increase costs. Therefore, determining the optimal concentration of PGRs, especially for woody species, is critical (Ersoy and Aydin, 2008). The rooting ability of cuttings also depends on their internal auxin content, phenolic compounds, and enzymes (Loreti and Morini, 1985). Additionally, the timing of cutting during the year significantly affects rooting outcomes, as it is more related to the plant's physiological conditions than to a specific calendar time, with genetic and anatomical factors playing a role (Selby *et al*., 1992). Recent

findings have highlighted the importance of hormonal balance and the integration of hormonal signals in shaping root growth and development (Cheng *et al.*, 2023).

The aim of this research is to enhance the propagation of white and red varieties of bougainvillea, which have demonstrated resilience in the tropical and subtropical regions of Iran under adverse conditions. Despite their adaptablility, the current methods of asexual propagation remain suboptimal, leading to inconsistent results and limiting the potential for large-scale cultivation. This research seeks to address this gap by developing more reliable and efficient propagation techniques. Given the increasing demand for aesthetically appealing and environmentally resilient plants to expand green landscapes, it is essential to refine propagation methods, conduct comprehensive tests, and thoroughly study these species. By doing so, this study can contribute to the sustainable development of urban and rural landscapes while promoting biodiversity and ecological balance.

MATERIALS AND METHODS

This research was conducted in the Department of Horticultural Science at Agricultural Sciences and Natural Resources University of Khuzestan between 2019 and 2020.

The stem cuttings used in this experiment were sourced from the mother plants of bougainvillea, which are available at the University. The cuttings were collected twice: Once in the first week of winter (20 December) and once in the first week of spring (20 March). Each cutting was 20.0 cm in length and had a diameter between 0.5 and 1.0 cm. Both the lower and upper ends of the cuttings were cut diagonally, 2.0 cm away from the first bud, and the leaves from the lower two-thirds of the cuttings were removed.

Treatments: Initially, the cuttings were disinfected with a 2% benomyl fungicide solution for 10 min. Subsequently, the last 1.0 cm of the cuttings were immersed in solutions of IBA, NAA, and 2,4-D at concentrations of 0, 250, 500, and 1000 mg L^{-1} for 24 hours. Each experimental treatment consisted of 10 cuttings.

The cuttings were planted in a greenhouse in rooting media composed of equal volumes of washed sand-loam soil and peat. The greenhouse environment was maintained at an average temperature of 25-28 °C, with humidity levels regulated between 60-70 % to simulate optimal growth conditions. Supplemental lighting was provided for 12-14 hours daily to ensure sufficient photosynthesis, while ventilation systems were employed to maintain airflow and prevent fungal diseases. The cuttings were planted diagonally at a 45-degree angle to the horizontal direction and spaced 10.0 cm apart. The planting medium was irrigated daily via a hand-held sprinkler.

Three months after planting, the cuttings were carefully removed from the culture medium. The characteristics studied in this study included the rooting percentage, number of roots, average root length, longest root length and shoot length per cutting (Fig. 1). The rooting percentage was determined by calculating the proportion of cuttings that successfully developed roots out of the total number of cuttings planted. To assess the number of roots, all visible roots on each cutting were carefully counted after a designated growth period. The average root length was measured by summing the lengths of all roots per cutting and dividing the total by the number of roots. The longest root length was determined by identifying and measuring the single root with the greatest length for each cutting. Lastly, the shoot length per cutting was measured from the base of the cutting to the tip of the longest shoot using a ruler, ensuring consistency across all samples.

This study was designed as a factorial experiment with a completely randomized design. Mean comparisons were performed via Duncan's test with SAS software, and graphs were generated via Excel 2019 software.

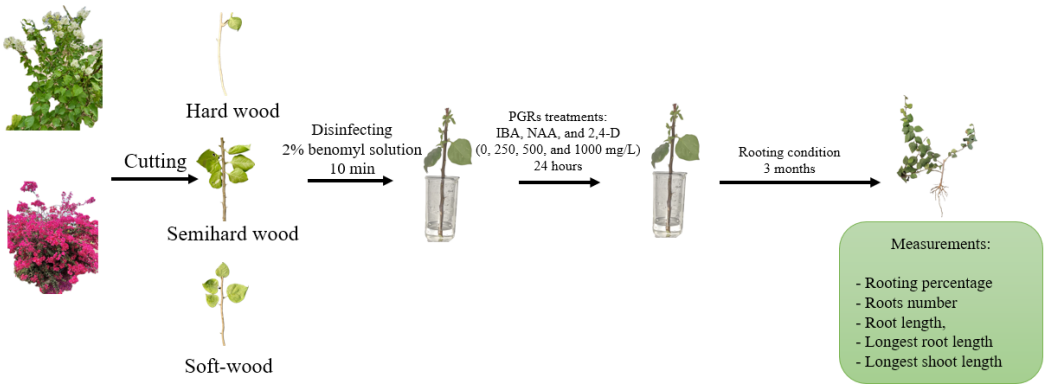

Fig. 1. Overview of the experimental design.

RESULTS

Root percentage

A comparative analysis of the average rooting percentage among cuttings revealed no statistically significant differences (Table 1). However, a significant variation was observed in the response to different plant growth regulators (PGRs), as determined by Duncan's multiple range test at a 5% probability level. Cuttings treated with NAA had the highest rooting percentage, while those treated with 2,4-D had the lowest. The interaction between cultivar and cutting type revealed that red woody cuttings achieved the highest rooting percentage (30.08 %), whereas soft cuttings of the red cultivar had the lowest (6.39 %), with no significant difference from semiwoody cuttings of the white cultivar (Fig. 2-left). Rooting percentages varied with PGR types and concentrations. The lowest rooting percentage occurred at 1000 mg L^{-1} for all PGRs, with no rooting in treatments involving 1000 mg L^{-1} 2,4-D. The highest rooting percentage (32.50%) was achieved using NAA at 250 mg L^{-1}, which was not significantly different from the IBA treatment at the same concentration (Fig. 2-right). The combined effects of time, cultivar, cutting type, PGR type, and concentration identified NAA (250 mg L^{-1}) in red woody cuttings during winter as achieving the highest rooting percentage (90.00%) (Table 1).

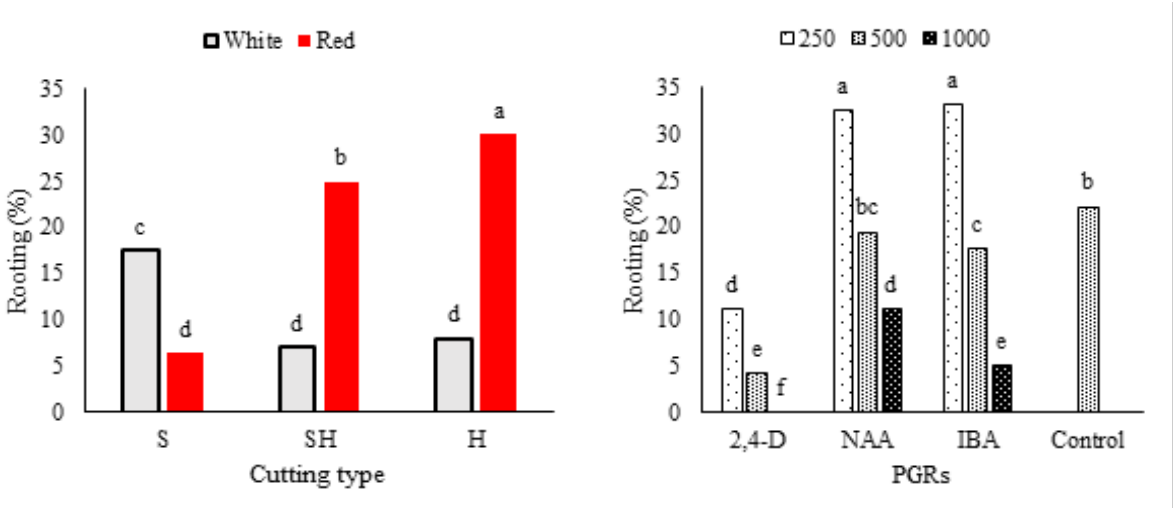

Fig. 2. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (right) on rooting percentage of bougainvillea.

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

Root number

Cutting time in winter and spring had no significant effect on the number of roots produced, as determined by Duncan's multiple range test at a 5% probability level (Table 2). The use of NAA resulted in the greatest number of roots, while 2,4-D produced the lowest. Among PGR types and concentrations, the highest number of roots (4.25) was achieved with 250 mg L^{-1} NAA, whereas the lowest (0.00) was observed with 1000 mg L^{-1} 2,4-D. The 250 mg L^{-1} IBA treatment significantly increased the number of roots compared to the control (Fig. 3-right). The interaction of cultivar and cutting type showed that red woody cuttings had the highest number of roots (2.82 per cutting), while white semiwoody cuttings had the lowest (0.84 per cutting), with no significant difference from white woody cuttings (Fig. 3-left). The soft cuttings of the red cultivar treated with 250 mg L^{-1} NAA in spring produced the highest overall number of roots (20.00 per cutting) (Table 2).

Table 1. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the rooting percentage of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1})										Season
			Control	IBA			NAA			2,4-D			
			0	250	500	1000	250	500	1000	250	500	1000	
Spring	W	H	30.0 ^{gh}	60.0^{d}	20.0^{i}	20.0^{i}	10.0^{j}	0.0^{m}	0.0^{m}	0.0 ^{m}	0.0^{m}	0.0^{m}	14.8^{a}
		SH	41.3^{f}	10.0^{j}	10.3^{j}	0.0^{m}	40.0^{f}	0.0^{m}	0.0^{m}	0.0 ^{m}	0.0^{m}	0.0^{m}	
		S	19.6^{i}	20.0^{i}	10.0^{j}	0.0^{m}	40.6^{f}	30.0^{gh}	20.0 ^{i}	10.0 ^{j}	0.0^{m}	0.0^{m}	
	R	H	6.0^{jk}	0.0^{m}	1.66^{km}	0.0^{m}	20.0^{i}	10.3^{j}	10.6 ^{j}	20.0 ^{i}	0.0^{m}	0.0^{m}	
		SH	40.0^{f}	10.0^{j}	40.0^{f}	0.0^{m}	70.0^{c}	40.0^{f}	0.0^{m}	24.0^{hi}	20.0^{i}	0.0^{m}	
		S	19.9^{i}	50.0^{e}	10.0^{j}	0.0^{m}	30.0^{gh}	40.0^{f}	41.3^{f}	0.0 ^{m}	0.0^{m}	0.0^{m}	
Winter	W	H	30.0^{gh}	60.0^{d}	20.3^{i}	20.0^{i}	40.0^{f}	40.0^{f}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	16.4^{a}
		SH	10.3^{j}	20.0^{i}	10.0^{j}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	
		S	10.0^{j}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	0.0^{m}	
	R	H	9.6^{j}	20.0^{i}	0.0^{m}	0.0^{m}	10.3^{j}	9.6^{j}	10.3^{j}	0.0^{m}	0.0^{m}	0.0^{m}	
		SH	30.0^{gh}	70.0^{c}	10.0^{j}	0.0^{m}	40.0^{f}	33.6^{g}	20.0^{i}	30.0^{gh}	20.0^{i}	0.0^{m}	
		S	20.0^{i}	78.6^{b}	80.0^{b}	20^{i}	90.0^{a}	30.0^{gh}	33.0^{g}	50.0^{e}	10.0^{j}	0.0^{m}	

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the Duncan's test.
The abbreviations W and R denote white and red cultivars, respectively.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

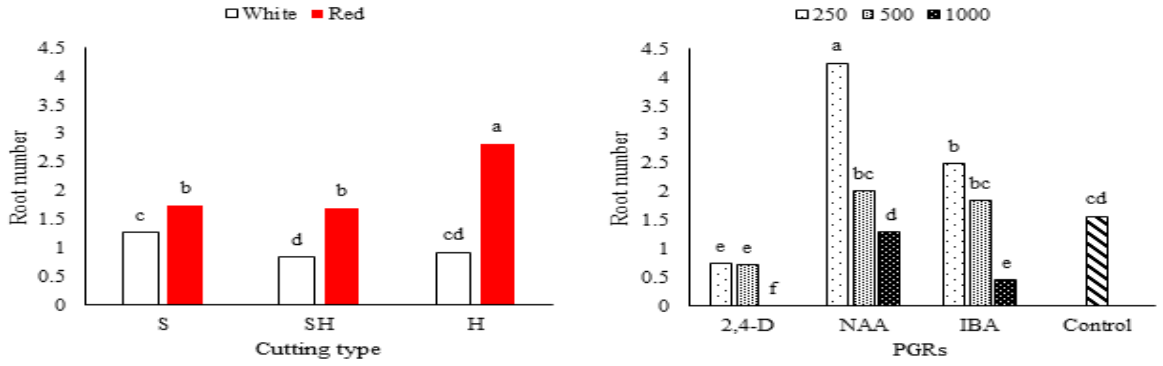

Fig. 3. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (right) on root number of bougainvillea.
*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the Duncan's test.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Table 2. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the number of roots of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1})										Season
			Control	IBA			NAA			2,4-D			
			0	250	500	1000	250	500	1000	250	500	1000	
Spring	W	H	0.5^{ij}	2.9^{c-f}	2.5^{d-g}	3.0^{cde}	3.7^{bcd}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	1.67^{a}
		SH	2.6^{d-g}	4.0^{bc}	2.5^{d-g}	0.0^{j}	2.2^{efg}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	
		S	1.7^{fgh}	2.7^{d-g}	2.5^{d-g}	0.0^{j}	4.0^{bc}	3.5^{cd}	3.0^{cde}	0.5^{ij}	0.0^{j}	0.0^{j}	
	R	H	0.5^{ij}	0.0^{j}	1.0^{hij}	0.0^{j}	20.1^{a}	0.5^{ij}	1.1^{hij}	3.0^{cde}	0.0^{j}	0.0^{j}	
		SH	0.5^{ij}	1.5^{ghi}	4.1^{bc}	0.0^{j}	3.7^{bcd}	3.1^{cde}	0.0^{j}	0.0^{j}	1.7^{fgh}	0.0^{j}	
		S	2.7^{d-g}	2.8^{def}	1.5^{ghi}	0.0^{j}	3.7^{bcd}	3.7^{bcd}	3.5^{cd}	0.0^{j}	0.0^{j}	0.0^{j}	
Winter	W	H	1.0^{hij}	1.0^{hij}	1.0^{hij}	1.5^{ghi}	2.0^{e-h}	3.0^{cde}	0.0^{j}	1.0^{hij}	1.0^{hij}	0.0^{j}	1.40^{a}
		SH	1.0^{hij}	4.0^{bc}	0.5^{ij}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	
		S	0.5^{ij}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	
	R	H	1.5^{ghi}	3.0^{cde}	0.0^{j}	0.0^{j}	1.5^{ij}	1.5^{ghi}	1.5^{ghi}	0.0^{j}	0.0^{j}	0.0^{j}	
		SH	1.5^{ghi}	3.0^{cde}	2.1^{e-h}	0.0^{j}	4.2^{b}	3.2^{cde}	3.0^{cde}	0.0^{j}	2.5^{d-g}	0.0^{j}	
		S	4.8^{b}	5.1^{b}	4.7^{b}	1.0^{hij}	5.9^{b}	5.5^{b}	3.5^{cd}	4.4^{b}	3.5^{cd}	0.0^{j}	

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test. The abbreviations W and R denote white and red cultivars, respectively. The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Root length

Root length showed no significant differences between winter and spring cuttings, as determined by Duncan's multiple range test at a 5% probability level (Table 3). Cultivar type significantly affected root length, with the red cultivar having the highest average root length (Fig. 4-left). Cutting type also influenced root length, with the greatest length observed in hard woody cuttings and the lowest in semihard woody cuttings (Fig. 4-left). For PGR type, no significant differences were found between IBA and NAA, but both were significantly superior to 2,4-D. The interaction of PGR type and concentration indicated the highest root length with 250 mg L^{-1} NAA (Fig. 4-right). The overall greatest root length (13.8 cm) was achieved with hard woody cuttings taken in winter from the red cultivar treated with 250 mg L^{-1} NAA (Table 3).

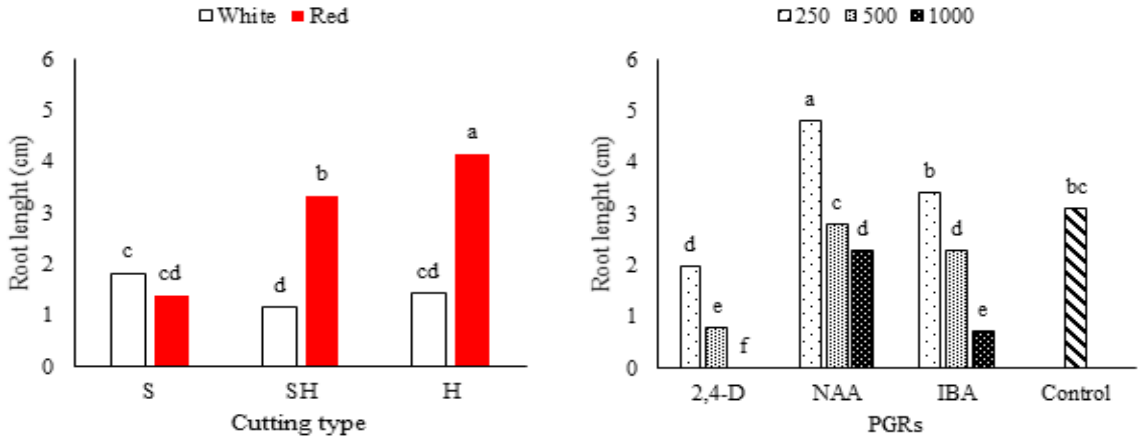

Fig. 4. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (right) on root lenght of bougainvillea.
*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test. The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Table 3. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the length of the roots of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1})										Season
			Control	IBA			NAA			2,4-D			
			0	250	500	1000	250	500	1000	250	500	1000	
Spring	W	H	0.5^{no}	4.6^{fg}	5^{fg}	5.5^{e}	5.2^{f}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	2.1^{a}
		SH	3.3^{hi}	4.2^{g}	2.5^{j}	0.0^{o}	5.0^{fg}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	
		S	3.0^{ij}	2.8^{ij}	3.5^{hi}	0.0^{o}	6.0^{de}	3.5^{hi}	4.5^{fg}	3.0^{ij}	0.0^{o}	0.0^{o}	
	R	H	6.0^{de}	0.0^{o}	1.7^{l}	0.0^{o}	2.7^{ij}	0.5^{no}	1.5^{lm}	4.^{5}^{gh}	0.0^{o}	0.0^{o}	
		SH	2^{8}^{ij}	1.0^{m}	4.0^{h}	0.0^{o}	4.8^{fg}	5.0^{fg}	0.0^{o}	4.1^{gh}	1.5^{lm}	0.0^{o}	
		S	3.5^{hi}	4.7^{fgh}	3.0^{ij}	0.0^{o}	5.7^{e}	5.5^{e}	5.5^{e}	0.0^{o}	0.0^{o}	0.0^{o}	
Winter	W	H	1.3^{lm}	1.3^{lm}	1.6^{lm}	2.0^{kl}	4.0^{h}	4.5^{gh}	0.0^{o}	0.0^{o}	1.0^{m}	0.0^{o}	2.2^{a}
		SH	1.5^{lm}	6.0^{de}	0.5^{no}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	
		S	2.5^{jk}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	0.0^{o}	
	R	H	2.0^{kl}	1.5^{lm}	0.0^{o}	0.0^{o}	3.5^{hi}	2.5^{j}	1.5^{lm}	0.0^{o}	0.0^{o}	0.0^{o}	
		SH	6.5^{de}	5.3^{ef}	4.0^{h}	0.0^{o}	7.0^{c}	4.0^{h}	8.0^{c}	5.3^{ef}	3.5^{hi}	0.0^{o}	
		S	4.4^{gh}	9.5^{b}	1.6^{lm}	1.0^{m}	13.8^{a}	8.2^{bc}	6.5^{d}	6.8^{cd}	3.5^{hi}	0.0^{o}	

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.
The abbreviations W and R denote white and red cultivars, respectively.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Longest root length

The season of cutting preparation significantly influenced the longest root length, with winter cuttings yielding longer roots than spring cuttings (Table 4). Hard wood cuttings of the red variety produced the longest roots, while semihard wood cuttings of the white variety had the shortest (Fig. 5-left). No significant difference was observed between IBA and NAA in terms of the longest root length, but both were significantly superior to 2,4-D. The interaction between PGR type and concentration revealed that 250 mg L^{-1} NAA produced the longest roots (Fig. 5-right). The overall longest root length (20.5 cm) was achieved from soft wood cuttings of the red variety taken in winter and treated with 250 mg L^{-1} NAA (Table 4).

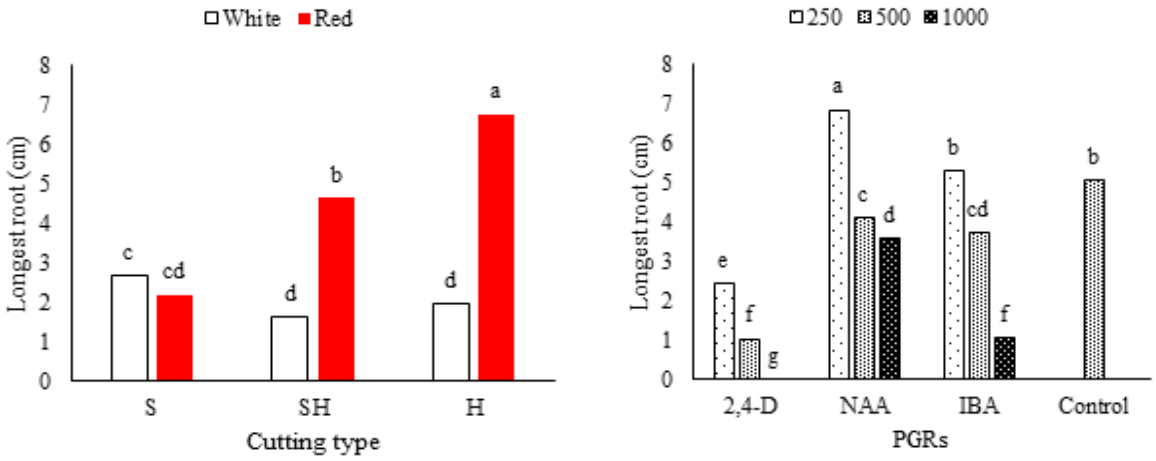

Fig. 5. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (right) on longest root lenght of bougainvillea.
*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test. The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Table 4. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the longest root length of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1})										Season
			Control	IBA			NAA			2,4-D			
			0	250	500	1000	250	500	1000	250	500	1000	
Spring	W	H	3.5^{op}	5.8^{lm}	7.5^{h-k}	9.0^{ef}	6.0^{lm}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	3.2^{b}
		SH	4.5^{no}	5.2^{mn}	3.0^{pq}	0.0^{t}	7.2^{ij}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
		S	3.7^{op}	4.5^{no}	4.5^{no}	0.0^{t}	8.7^{efg}	5.8^{lm}	6.5^{kl}	3.1^{pq}	0.0^{t}	0.0^{t}	
	R	H	8.5^{fgh}	0.0^{t}	2.3^{q}	0.0^{t}	5.3^{mn}	0.5^{s}	5.2^{mn}	6.5^{kl}	0.0^{t}	0.0^{t}	
		SH	4.6^{mno}	3.0^{pq}	6.0^{lm}	0.0^{t}	6.45^{kl}	9.0^{ef}	0.0^{t}	0.0^{t}	2.2^{q}	0.0^{t}	
		S	8.7^{efg}	7.8^{ghi}	3.5^{op}	0.0^{t}	8.0^{f-i}	8.2^{f-i}	8.0^{f-i}	0.0^{t}	0.0^{t}	0.0^{t}	
Winter	W	H	1.8^{r}	2.6^{q}	3.0^{pq}	2.5^{q}	5.5^{m}	6.5^{l}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	3.4^{a}
		SH	2.5^{q}	9.5^{de}	0.5^{s}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
		S	2.5^{q}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
	R	H	2.5^{q}	2.0^{r}	0.0^{t}	0.0^{t}	4.5^{no}	3.8^{op}	2.5^{q}	0.0^{t}	0.0^{t}	0.0^{t}	
		SH	8.5^{fgh}	8.5^{fgh}	5.0^{mn}	0.0^{t}	10.0^{d}	5.0^{mn}	11.5^{c}	7.9^{ghi}	5.1^{mn}	0.0^{t}	
		S	9.5^{de}	14.6^{b}	9.3^{e}	1.0^{s}	20.5^{a}	10.5^{c}	9.0^{ef}	11.6^{c}	5.0^{mn}	0.0^{t}	

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.
The abbreviations W and R denote white and red cultivars, respectively.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Shoot length

A statistically significant difference, as determined by Duncan's multiple range test at a 5% probability level, was observed in average branch lengths based on cutting timing, with spring cuttings yielding the longest shoots (Table 5). Hard wood cuttings of the red variety had the longest branch length, whereas soft wood cuttings of the same variety had the shortest. The shortest branch length was not significantly different from that of semihard wood cuttings in the white variety (Fig. 6-left). Significant differences in branch length were noted between the control group and PGR-treated groups. The control group exhibited the longest branch length (46.0 cm), while the shortest branch length (0.0 cm) was recorded for 1000 mg L^{-1} 2,4-D (Fig. 6-right). The longest branch length (46.0 cm) was achieved in soft wood cuttings of the red variety taken in winter under the control treatment (Table 5).

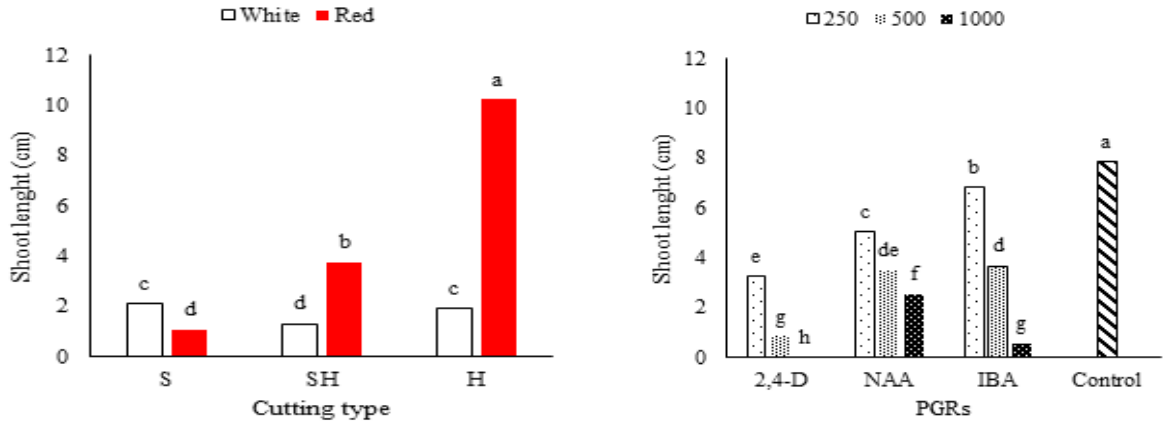

Fig. 6. Interaction effects of cultivar and cutting type (left); and PGRs and concentrations (Right) on shoot lenght of bougainvillea.
*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

Table 5. Effects of cultivar, cutting type, PGR type, and PGR concentration across two seasons on the longest shoot length of bougainvillea cuttings.

Time	Cultivar	Cutting	PGRs (mg L^{-1})										Season
			Control	IBA			NAA			2,4-D			
			0	250	500	1000	250	500	1000	250	500	1000	
Spring	W	H	3.5^{no}	3.2^{op}	4.5^{lmn}	4.0^{mno}	2.0^{pqr}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	2.0^{b}
		SH	4.6^{lm}	4.2^{mno}	1.5^{qrs}	0.0^{t}	1.0^{rs}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
		S	5.5^{kl}	5.0^{lm}	2.0^{pqr}	0.0^{t}	5.7^{kl}	5.0^{lm}	6.0^{kl}	6.5^{jk}	0.0^{t}	0.0^{t}	
	R	H	2.7^{p}	0.0^{t}	0.8^{rst}	0.0^{t}	1.0^{rs}	0.5^{st}	0.5^{st}	2.0^{pqr}	0.0^{t}	0.0^{t}	
		SH	3.8^{no}	1.5^{qrs}	3.5^{no}	0.0^{t}	2.0^{pqr}	1.0^{rs}	0.0^{t}	1.3^{qrs}	2.2^{pq}	0.0^{t}	
		S	4.0^{mno}	7.4^{hij}	2.0^{pqr}	0.0^{t}	7.5^{hij}	6.5^{jk}	6.2^{jkl}	0.0^{t}	0.0^{t}	0.0^{t}	
Winter	W	H	4.0^{mno}	7.8^{hi}	3.0^{op}	1.5^{qrs}	1.5^{qrs}	7.5^{hij}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	4.7^{a}
		SH	3.0^{op}	9.0^{gh}	3.0^{op}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
		S	2.5^{p}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	0.0^{t}	
	R	H	2.5^{p}	10.1^{g}	0.0^{t}	0.0^{t}	0.5^{st}	0.5^{st}	0.5^{st}	0.0^{t}	0.0^{t}	0.0^{t}	
		SH	12.0^{f}	8.2^{hi}	8.2^{hi}	0.0^{t}	12.0^{f}	4.0^{mno}	2.0^{pqr}	12.0^{f}	1.0^{rs}	0.0^{t}	
		S	46.0^{a}	25.5^{c}	15.6^{de}	1.0^{rs}	27.2^{b}	17.0^{d}	15.0^{e}	17.0^{d}	7.0^{ijk}	0.0^{t}	

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.
The abbreviations W and R denote white and red cultivars, respectively.
The abbreviations S, SH, and H denote softwood, semi-hardwood, and hardwood stem cuttings, respectively.

DISCUSSION

This study revealed no significant difference in the average rooting percentage, number of roots, or root length between cuttings taken in winter and those taken in spring. However, winter cuttings exhibited significantly longer root lengths compared to spring cuttings. Additionally, the timing of cuttings significantly affected branch length, with the longest shoot lengths observed in spring cuttings. These findings highlight the complex interplay of factors influencing rhizogenesis in woody plants, including species, cutting type, environmental conditions, and plant physiological state (Zhao *et al*., 2022). Below, we discuss these results in detail, their implications, and potential influencing factors.

Seasonal effects on rooting success: Seasonality plays a critical role in the rooting success of cuttings. During dormancy (late fall to early spring), hardwood cuttings benefit from high carbohydrate reserves stored in stems and roots, providing energy for root initiation (Zheng *et al*., 2020). However, endogenous auxin levels are low, and growth inhibitors are high, preventing premature growth (Hartmann *et al.*, 2002). This may explain why winter cuttings, despite their longer root lengths, did not show significant differences in rooting percentage compared to spring cuttings. The lack of significant differences in rooting parameters could also be attributed to the genetic variability of the plant material, environmental conditions during the experiment, or the specific hormonal treatments applied. For instance, variations in endogenous hormone levels among cuttings may have influenced rooting responses, masking potential differences (Druege *et al*., 2016). Future studies should explore these factors in greater detail to better understand their impact on rooting success.

In contrast, during active growth (spring and summer), softwood cuttings have high endogenous auxin levels, promoting rapid cell division and growth, but lower carbohydrate reserves, as they are used for shoot growth (OuYang *et al*., 2015). Softwood cuttings can root quickly but are susceptible to desiccation and fungal diseases, requiring high humidity and careful management (Saradha and Samydurai, 2015). Semihardwood cuttings, taken from

midsummer to early fall, strike a balance with moderate auxin and carbohydrate levels, offering moderate rooting speed and success. These seasonal variations underscore the importance of selecting the appropriate cutting type and timing based on the species and propagation goals.

Advantages of hardwood cuttings: Hardwood cuttings, taken from mature, fully lignified stems, offer several advantages over semihardwood or softwood cuttings, particularly for deciduous trees and shrubs. One key benefit is the greater carbohydrate reserves found in hardwood cuttings. During dormancy, stems accumulate high levels of stored carbohydrates, providing the energy needed to support early callus formation and subsequent root growth (Ashok and Ravivarman, 2021). Additionally, hardwood stems have tougher bark and thicker tissues, making them more robust and better able to tolerate stress. This reduces the likelihood of desiccation, fungal entry, or mechanical injury, allowing the plant tissue to focus more energy on producing roots instead of repairing damage (Mumtaz *et al*., 2022).

Hardwood cuttings also face reduced disease pressure. Taken during dormancy, they are less likely to carry active fungal or insect pests compared to softwood material, which can reduce disease and pest problems and thereby aid healthy root development (Kaushik and Shukla, 2020). Furthermore, hardwood cuttings benefit from reduced water loss through transpiration. Lacking leaves or having very few, small, dormant buds, hardwood cuttings significantly reduce water loss compared to softwood and semihardwood cuttings, which lose water much more rapidly (Dalbro, 1975). This makes hardwood cuttings more tolerant of drier conditions and less reliant on constant, high humidity, reducing the risk of fungal diseases that thrive in overly humid environments (Sytar *et al*., 2019). Despite being slower to root than softwood or semihardwood cuttings, hardwood cuttings often exhibit stronger, more vigorous growth and greater long-term health, particularly for woody species (Mumtaz *et al*., 2022). These attributes make hardwood cuttings a favorable choice for establelishing strong root systems in many woody plants.

Role of PGRs: The type and concentration of PGRs significantly influenced rooting success in this study. NAA-treated cuttings exhibited the highest rooting percentage, consistent with findings by Tripathi *et al*. (2022), who reported that auxins such as NAA are effective at promoting root initiation and development. NAA is often more effective than 2,4-D and IBA in stimulating root initiation, especially in hard-to-root plant species, as it promotes the formation of more root primordia, leading to better rooting success (Geneve and Heuser, 1982; Yan *et al*., 2014). Compared to 2,4-D, NAA promotes direct root formation with less callus production, leading to more efficient adventitious rooting (De Klerk *et al*., 1999). Additionally, NAA has a longer-lasting effect on promoting rooting than IBA, which degrades more quickly under some conditions (Dalbro, 1975). Conversely, cuttings treated with 2,4-D presented the lowest rooting percentage, corroborating the results of Sabagh *et al*. (2021), who noted the inhibitory effects of 2,4-D on root formation. The interaction between cultivar and cutting type further emphasized that woody cuttings of the red cultivar presented the highest rooting percentage, which is in line with the observations of Rademacher (2015) regarding the superior rooting potential of woody cuttings.

Practical implications for growers and horticulturists: The findings of this study have significant practical implications for growers and horticulturists. Hardwood cuttings, with their higher success rates and lower susceptibility to environmental stress, offer a cost-effective and reliable propagation method. This is particularly beneficial for large-scale production, where consistency and efficiency are critical. Additionally, the use of hardwood cuttings reduces the need for specialized equipment or controlled environments, making it accessible to small-scale growers and nurseries with limited resources (Dhillon, 2017). By adopting this method, growers can improve propagation outcomes and reduce losses, ultimately enhancing productivity and profitableility. Furthermore, the use of NAA as a PGR can significantly improve rooting success,

especially for hard-to-root species. Growers should consider the specific needs of the plants being propagated and choose the most appropriate auxin to achieve optimal rooting results. The advantages of NAA, such as faster root initiation, stronger root systems, and reduced callus formation, make it a valuable tool in the propagation of a wide variety of plant species (Yan *et al*., 2014).

CONCLUSION

The results indicated that the timing (winter vs. spring) had no significant effect on the rooting percentage. However, a significant difference was observed in branching, with the shortest average branch length (2.6 cm) occurring in early winter and the longest (4.2 cm) occurring in early spring. Significant differences were also found between the tested varieties, with the red variety outperforming the white variety in all the measured factors. While no significant difference was noted between the soft and semiwoody cuttings, the woody cuttings presented significantly better results. Among the PGRs, those treated with 250 mg L^{-1} IBA or NAA presented the highest rooting percentages. Increasing concentrations of these auxins resulted in decreased rooting, with the lowest rooting percentage observed at 1000 mg L^{-1} 2,4-D. This study demonstrated that the type and concentration of auxins significantly affect the rooting and branching of bougainvillea cuttings, with optimal results achieved using 250 mg L^{-1} IBA and NAA. These findings suggest that careful selection of the auxin type and concentration, as well as consideration of the cutting type and timing, are crucial for the successful propagation of bougainvillea. The findings of this study highlight the critical role of PGRs, particularly NAA, in enhancing rooting success and overall plant growth. The results also emphasize the importance of the cutting type and cultivar in determining rooting and growth outcomes. While seasonal timing did not significantly impact the rooting percentage, number of roots, or root length, it did influence branch length, with spring cuttings showing the longest shoot length. These insights can inform horticultural practices and propagation strategies, particularly for woody plants, by identifying optimal conditions for root initiation and plant development. Future research could further explore the interactions between different PGRs, cutting types, and environmental conditions to refine propagation techniques and improve plant growth outcomes.

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Conflict of interest: The manuscript was prepared and reviewed with the participation of the authors, who declare that there are no conflict of interest that puts at risk the validity of the presented results.

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دینامیک ریشه در قلمه‌های *Bougainvillea glabra* Choisy: بررسی تأثیر نوع اکسین، غلظت و نوع قلمه در طول زمان در دو واریته

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چکیده

این بررسی تأثیر سه نوع اکسین (اسید ایندول بوتیریک (IBA)، اسید نفتالین‌استیک (NAA) و اسید دی‌کلروفنوکسی‌استیک (2,4-D)) را بر ریشه‌زایی قلمه‌های نرم، نیمه‌چوبی و چوبی گل‌کاغذی (*Bougainvillea glabra* Choisy) در اوایل زمستان و بهار تحت شرایط گلخانه‌ای بررسی کرد. تنظیم‌کننده‌های رشد گیاهی با غلظت‌های ۲۵۰، ۵۰۰ و ۱۰۰۰ میلی‌گرم در لیتر تهیه شدند. قلمه‌های ساقه به مدت ۲۴ ساعت در این محلول‌ها غوطه‌ور شدند و سپس به محیط رشد منتقل شدند. آزمایش به‌صورت فاکتوریل در قالب طرح کاملاً تصادفی با ۱۰ تکرار طراحی شد. نتایج نشان داد که زمان (زمستان در مقابل بهار) تأثیر معنی‌داری بر درصد ریشه‌زایی ندارد. با این حال، تفاوت‌های معنی‌داری در شاخه‌دهی مشاهده شد. کوتاه‌ترین میانگین طول شاخه (۲ سانتی‌متر) در اوایل زمستان و بلندترین طول (۷/۴ سانتی‌متر) در اوایل بهار ثبت شد. تفاوت‌های معنی‌داری نیز بین واریته‌ها یافت شد، با عملکرد بهتر واریته قرمز نسبت به واریته سفید. قلمه‌های چوبی نتایج بهتری نسبت به قلمه‌های نرم و نیمه‌چوبی داشتند. از میان تنظیم‌کننده‌های رشد گیاهی، قلمه‌هایی که با ۲۵۰ میلی‌گرم در لیتر IBA یا NAA تیمار شده بودند، بالاترین درصد ریشه‌زایی را نشان دادند. افزایش غلظت این اکسین‌ها منجر به کاهش ریشه‌زایی شد، کمترین درصد ریشه‌زایی در ۱۰۰۰ میلی‌گرم در لیتر 2,4-D مشاهده شد. این مطالعه نشان داد که نوع و غلظت اکسین‌ها تأثیر معنی‌داری بر ریشه‌زایی و شاخه‌دهی قلمه‌های گل‌کاغذی دارد، با نتایج بهینه در ۲۵۰ میلی‌گرم در لیتر IBA و NAA. این یافته‌ها نشان می‌دهد که انتخاب دقیق نوع و غلظت اکسین، همراه با توجه به نوع قلمه و زمان‌بندی، برای تکثیر موفقیت‌آمیز گل‌کاغذی بسیار مهم است.

کلید واژه‌ها: اسید استیک، تولید گلخانه‌ای، تکثیر، تنوع فصلی، قلمه نر.

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تحلیل اقتصادی و گیاه‌شناسی گیاهان زینتی ایران مرکزی

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چکیده

در چند دهه اخیر گیاهان غیربومی زیادی به فلور فلات مرکزی به ویژه در فضاهای سبز معرفی شده‌اند. پژوهش حاضر با هدف شناسایی و شناخت اعضای مختلف زینتی ٦١ خانواده گیاهی متنوع در فلات مرکزی ایران انجام شد. بسیاری از گیاهان دارویی به دلیل تحمل به شوری و سازگاری با اقلیم‌های خشک و نیمه خشک در فضاهای سبز دست کاشت در مناطق کویری مورد استفاده قرار گرفته‌اند. ایجاد فضای سبز شهری با گیاهان معرفی شده در این تحقیق باعث حفظ و احیای تنوع زیستی می‌شود و فواید اکولوژیکی و اقتصادی بسیاری را به همراه دارد. دغدغه این تحقیق شناسایی گیاهان و بررسی اهمیت اقتصادی این گونه‌ها بود. از طریق کار میدانی و مشاهدات دقیق، گیاهان شناسایی شده به خانواده‌های مربوطه طبقه‌بندی شدند و بینش‌های ارزشمندی را در مورد تنوع زیستی غنی ایران مرکزی ارائه کردند. اهمیت اقتصادی این گیاهان با توجه به کاربردهای آن‌ها در پزشکی، محوطه‌سازی، مصارف سنتی و کاربردهای تجاری بالقوه به طور کامل مورد بررسی قرار گرفت. از این ۱۵۸ گونه مورد مطالعه، از نظر مصارف دارویی و خوراکی، ۱۱٦ گونه دارای مصارف خوراکی، ۱٤۹ گونه دارای مصارف دارویی و ۱۱۰ گونه دارای مصارف خوراکی و دارویی می‌باشند. در این مطالعه، مدلی برای ارزیابی ارزش‌های زیبایی‌شناختی و خدمات اقتصادی (تامین غذا؛ داروهای طبیعی، داروسازی؛ چوب، تولید الیاف) برای ۱۵۸ گونه زینتی مورد مطالعه طراحی شد. استفاده از مدل ارزیابی خدمات اکوسیستمی ارائه شده در این مطالعه برای مقایسه ارزش های زیبایی شناختی و مزایای اقتصادی گونه‌های زینتی، راهنمای انتخاب گیاهان در فضای سبز را ارائه می‌دهد. این مدل ارزش زیبایی شناختی و مزایای اقتصادی را در چهار سطح پایین (<۱/۵)، متوسط (< ۲/۵ ، > ۱/۵)، بالا (<۳/۵، > ۲/۵) و بسیار بالا (> ۳/۵) رتبه‌بندی می‌کند. شعار اصلی انتخاب گیاهان گلدار زینتی، برجسته کردن کاربرد گیاهان زینتی در صنایع مختلف است نه صرفاً زیباسازی باغ‌ها و مناظر.

کلید واژه‌ها: گیاهان اقتصادی، فضای سبز، گیاهان دارویی و صنعتی، مقاومت در برابر خشکی، تحمل به نمک.

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افزایش رشد کالوس در گیاه زینتی شیپوری (*Zantedeschia* Sun Club) با کاربرد فیپکسید، زغال فعال و اسید اسکوربیک در کشت درون شیشه‌ای

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چکیده

گل شیپوری گلدانی (*Zantedeschia* spp) یک گیاه زینتی محبوب در بازار جهانی گل و گیاهان زینتی است. مهمترین مشکل شیپوری در شرایط درون شیشه‌ای برای تولید به روش اندام‌زایی غیرمستقیم، تولید کالوس، ماندگاری و رشد کم کالوس به دلیل ترکیبات فنلی و آلکالوئیدی موجود است. بر همین اساس، آزمایشی برای بررسی اثر فیپکسید در مقایسه با زغال فعال و اسید آسکوربیک در محیط کشت برای بهبود کیفیت کالوس شیپوری گلدانی (*Zantedeschia* "Sun Club") انجام شد. فیپکسید یک از ترکیبات شیمیایی مورد استفاده در صنعت داروسازی بود که اخیراً برای شرایط درون شیشه‌ای در محیط کشت برای بهبود کیفیت مراحل مختلف رشد گیاه استفاده شد. این آزمایش به صورت فاکتوریل در قالب طرح کاملا تصادفی شامل فیپکسید در غلظت‌های ۰، ۱۵ و ۳۰ میکرومول بر لیتر، زغال فعال (۰ و ۱ گرم بر لیتر) و اسید اسکوربیک در غلظت‌های ۰ و ۲ گرم بر لیتر، شامل ۱۲ تیمار، ۳ تکرار، ۱۲ نمونه برای هر تیمار و در مجموعه با ۱۹۲ نمونه کالوس اجرا شد. در این تحقیق قطر کالوس، وزن تر کالوس، شاخص رشد کالوس و درصد کالوس‌های سالم ارزیابی شد. نتایج نشان داد که تیمار فیپکسید به ویژه در غلظت ۱۵ میکرومول بر لیتر با زغال فعال بیشترین تأثیر را بر صفات مورد بررسی مانند قطر کالوس (۹۸/۱۵ میلی متر)، درصد کالوس سالم (۹۰ درصد) در مقایسه با نمونه‌های شاهد (به ترتیب با میانگین ۳٦/۷ میلی‌متر و ٥٤ درصد) داشت. در مجموع فیپکسید با اثرگذاری بیشتر در این آزمایش، می‌تواند به عنوان یک ماده موثر و جایگزین در محیط رشد کالوس برای افزایش کیفیت و عملکرد در شرایط درون شیشه‌ای استفاده شود.

کلید واژه‌ها: رشد کالوس، ژئوفیت، گیاه زینتی، گیاه گلدانی، کشت بافت گیاهی.

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ارتباط بین کود زیستی ورمی‌کمپوست و بسترهای رشد با عملکرد و اجزای عملکرد همیشه‌بهار

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چکیده

کشت گل همیشه‌بهار (*Calendula officinalis* L) به‌دلیل فواید دارویی و ارزش زینتی آن اهمیت فزاینده‌ای پیدا کرده است. استفاده گسترده از مواد شیمیایی کشاورزی بر کیفیت خاک، عملکرد محصول و محیط زیست تأثیر منفی گذاشته است. به‌عنوان یک جایگزین مناسب، اصلاح‌کننده‌های آلی و کودهای زیستی برای بهبود سلامت خاک و گیاه پیشنهاد شده است. ورمی‌کمپوست رویکردی پایدار برای تامین تغذیه گیاه و افزایش تولید و در نتیجه ارتقای سلامت و حاصلخیزی خاک ارائه می‌دهد. این آزمایش در شرایط مزرعه‌ای به‌منظور بررسی اثر ورمی‌کمپوست (صفر، ۱، ۲ و ۳ کیلوگرم بر متر مربع) و بسترهای رشد (خاک مزرعه، خاک مزرعه همراه با شن، و خاک مزرعه همراه با شن و ورمی‌کمپوست) بر برخی پارامترهای گل همیشه‌بهار انجام شد. تعداد شاخه، تعداد گل، قطر گل، وزن خشک بوته و وزن خشک گل اندازه‌گیری شدند. نتایج نشان داد که سطوح بالاتر ورمی‌کمپوست باعث افزایش تمام پارامترهای اندازه‌گیری‌شده گردید. مقایسه بین بسترهای مختلف و سطوح مختلف ورمی‌کمپوست نشان داد که بستر کشت شامل خاک مزرعه به‌همراه شن و کود دامی نسبت به سایر تیمارهای آزمایش به جز ورمی‌کمپوست ۳ کیلوگرم بر متر مربع، عملکرد و اجزای آن را بیشتر القا کرد. این نتایج نشان‌دهنده پتانسیل کود زیستی و آلی در بهبود القای گل همیشه‌بهار با امکان اجتناب از کوددهی شیمیایی است.

کلید واژه‌ها: کود زیستی، بسترهای کشت، همیشه‌بهار، کودهای آلی، گیاهان زینتی و دارویی.

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افزایش پاسخ رشد و فعالیت آنزیم آنتی‌اکسیدانی سینره (*Pericallis* × *hybrida* L)

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چکیده

سینره (*Pericallis hybrida* L.) با موانع رشدی و کلروز مواجه شده است. اسید هیومیک، سولفات آهن و کودهای کلات آهن برای کشت سینره در خاک قلیایی مورد بررسی قرار گرفتند. این پژوهش به صورت آزمایشی فاکتوریل در قالب طرح کاملاً تصادفی با سه تکرار و در هر تکرار ٤ نمونه انجام شد. محیط‌های گیاهی با استفاده از اسید هیومیک (۰، ۰/۵ و ۱ گرم بر کیلوگرم خاک) و کودهای آهن (۰، ۵ و ۱۰ میلی‌گرم بر کیلوگرم سولفات آهن و ۵ و ۱۰ میلی‌گرم بر کیلوگرم کلات آهن) غنی‌سازی شدند. گیاهانی که با خاک حاوی ۱ گرم در کیلوگرم اسید هیومیک همراه با ۱۰ میلی گرم در کیلوگرم کلات آهن تیمار شدند، در ارتفاع بوته (٨٦٪)، قطر ساقه (۱۰۰٪)، وزن تر ریشه (۱۷۰٪)، دوره گلدهی (۱٦٦٪)، تعداد گل (۱۸۲٪)، تعداد گل آذین (۲۵۲٪)، قطر گل (۵۹٪) و میزان کلروفیل کل (۳۰۰٪) بهبود یافتند. کاربرد همزمان ۱ گرم بر کیلوگرم اسید هیومیک و ۱۰ میلی‌گرم بر کیلوگرم کلات آهن، میزان عناصر معدنی پتاسیم، نیتروژن و فسفر را به‌ترتیب ۱۷۹، ۱۹۳ و ٦٧٥ درصد افزایش داد. ترکیبات ۰/۵ گرم بر کیلوگرم اسید هیومیک و ۱۰ میلی گرم بر کیلوگرم کلات آهن باعث افزایش آنتوسیانین (۱۳۱ ٪) سطح برگ (۱٤۰٪) قندهای کل محلول (۳۲۲٪) و نشاسته (٦٤۲ ٪) شد. کوددهی با ۰/۵ گرم بر کیلوگرم اسید هیومیک در ترکیب با ۵ میلی گرم بر کیلوگرم کلات آهن منجر به بیشترین فعالیت آنزیم های آنتی‌اکسیدانی POD، SOD و CAT (با ۳۲٤، ۲۳۸ و ٦٦٧ درصد) و کاهش نشت یونی (٦۰٪) شد. اسید هیومیک (۱ گرم بر کیلوگرم) به‌عنوان یک کود زیستی در ترکیب با کلات آهن (۱۰ میلی‌گرم بر کیلوگرم) برای استفاده در تولید گیاهان در خاک هایی با شرایط pH بالا و استرس زا پیشنهاد می‌شود.

کلید واژه‌ها: فعالیت آنزیم‌های آنتی‌اکسیدانی، سینره، pH بالای خاک، کلروز برگ، *Pericallis* × *hybrida*.

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اثر نیترات، آمونیوم و گروه مسوس بر تکثیر درون شیشه گیاه ژربرا (*Gerbera jamesonii*)

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چکیده

گیاه ژربرا با نام علمی *Gerbera jamesonii* یکی از گل‌های شاخه بریده تجاری در جهان محسوب می‌شود. به‌دلیل هتروزیگوسیتی بالا، ریزازدیادی ارقام مختلف ژربرا یک روش مناسب برای تکثیر آن می‌باشد. بهینه‌سازی محیط کشت برای تهیه یک پروتکل ریزازدیادی اهیمت داشته و نیاز به تنظیم مواد غذایی پرمصرف و ریز مصرف در داخل محیط کشت می‌باشد. در این تحقیق اثرات متقابل سه گروه از مواد معدنی پرمصرف شامل NH_4NO_3، KNO_3 و گروه مسوس ($MgSO_4 . 7H_2O$, $CaCl_2 . 2H_2O$ and KH_2PO_4) و نقش آن‌ها بر روی رشد درون شیشه‌ای ژربرا مطالعه شده است. یک آزمایش فاکتوریل (۲ × ۲ × ۳ × ۳) شامل دو سطح 0.5X و 1X از NH_4NO_3، KNO_3 در سه سطح 0.5X، 1X و 1.5X و گروه مسوس در سه سطح 0.5X، 1X و 1.5X بر روی دو رقم مختلف ژربرا طراحی شد و اثرات ساده و چندگانه بر روی پارامترهای مختلف رشد ژربرا درون شیشه بررسی گردید. نتایج نشان داد که اثر متقابل سه طرفه نیترات آمونیوم، نیترات پتاسیم و گروه مسوس بر روی تمام فاکتورهای رشد از لحاظ آماری معنی‌دار بود و جالب اینکه اثر متقابل دوطرفه نیترات پتاسیم و نیترات آمونیوم بدون گروه مسوس بر روی تعداد شاخساره اثری نداشت. استفاده از غلظت 0.5X نیترات پتاسیم و نیترات آمونیوم و 1.5X از گروه مسوس منجر به تولید بیشترین تعداد شاخساره، کمترین تعداد ریشه و کمترین طول ریشه شد. این مطالعه نتایج جدیدی در مورد عناصر پرمصرف و چگونگی بهینه سازی مقادیر آن‌ها برای افزایش راندمان ریزازدیادی و کیفیت گیاهان تولید شده ارائه می‌دهد.

کلید واژه‌ها: آمونیوم، ژربرا، مسوس، ریزازدیادی، نیترات.

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