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

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Abstract

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

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

INTRODUCTION

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

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

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

MATERIALS AND METHODS

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

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

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

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

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

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

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

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

RESULTS AND DISCUSSION

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

According to the comparison of means presented in table 3, the highest carotenoid content was achieved through the application of estradiol. Brassinosteroids have been shown to enhance carotenoid levels, thereby inhibiting chlorophyll degradation and increasing chlorophyll a and b in various plant species such as marigolds, wild pears, mustard, and peas (Ahmadi Lashaki *et al.,* 2018; Zahedi *et al.,* 2017; Fariduddin *et al.,* 2009; Ali *et al.,* 2007). Conversely, the lowest carotenoid synthesis rate was observed in plants treated with glutamine and estradiol. Drought stress has been found to elevate carotenoids, dry matter, proline, and gas exchange through stomata in wild pear plants (Zarafshar *et al.,* 2014). Furthermore, research has demonstrated that the use of bio-elicitors on thyme can boost essential oil yield, chlorophyll a content, and carotenoid levels (Miri *et al.,* 2015). Carotenoids, as crucial light-absorbing pigments in thylakoids, serve as light receptors and protectors of photosystems, combating reactive oxygen species (ROS). By dissipating excess energy from photosystems I and II as heat or chemical reactions, carotenoids safeguard chloroplast membranes, thereby enhancing plant resilience to stress factors (Juan *et al.,* 2005). These pigments indirectly diminish ROS production and regulate free oxygen activity. Studies have indicated that the highest carotenoid content in marigold petals was associated with estradiol treatment, while the lowest was linked to the control group (Sedaghathoor and Raof Haghparvar, 2022). Leaf carotenoid content has been shown to increase with the foliar application of glutamine and amino acids, with reported benefits on plant growth and biochemical properties. Amino acids play a vital role in synthesizing various plant compounds, including proteins, enzymes, phenolics, and flavonoids, thereby influencing plant processes positively (Aghaye Noroozlo *et al.,* 2019; Haghighi *et al.,* 2022).

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

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

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

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

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

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

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

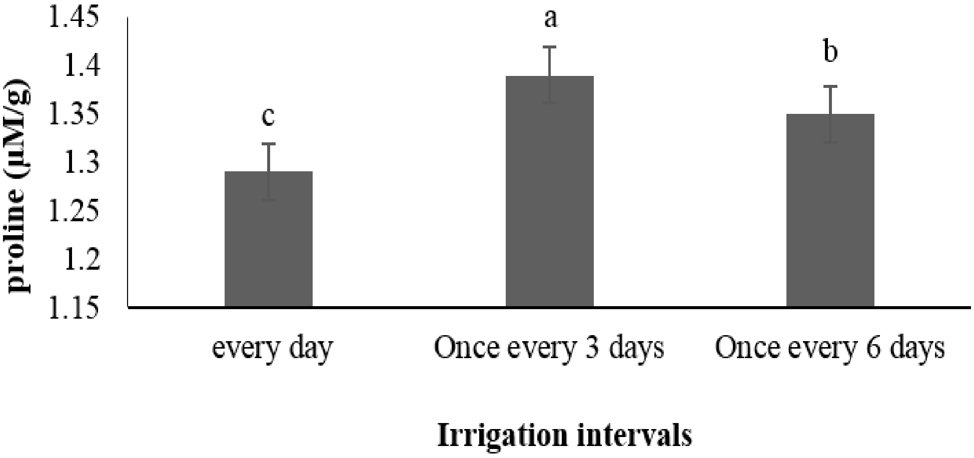

Fig. 1. The simple effect of irrigation on proline content.

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

Antioxidant capacity was one of trial traits that did not affected significantly under experimental factors and their interaction. Based on the results, the maximum phenolics synthesis was associated with the treatment involving glutamine and estradiol with a six-day irrigation interval, while the lowest was observed in the control group (Table 4). As indicated in table 3, the application of elicitors resulted in a notable increase in phenolics content, with the highest level recorded at 9.37 mg/g for the glutamine treatment and the lowest at 5.74 mg/g for the control. This enhancement in phenolics and flavonoids in marigolds due to elicitor application aligns with previous findings demonstrating increased phenolic compounds in various plants (Schwambach *et al.,* 2008) under drought conditions. Notably, research by Khajehhosseini *et al.* (2020) emphasized the significant impact of irrigation and foliar application of amino acids on total phenolics. Additionally, studies by Rezaie Alulu *et al.* (2020) highlighted that heightened water deficit stress led to increased phenolics, total flavonoids, and antioxidant activity in carla plants, corroborating our observations. The rise in phenolics serves as a defensive antioxidant mechanism in plants facing water deficit stress, aiding in the stabilization of cell membranes and the prevention of lipid peroxidation by scavenging reactive oxygen species. Phenolic compounds possess potent antioxidant properties, capable of scavenging free radicals and inhibiting the breakdown of hydroperoxides into free radicals (Razali *et al.,* 2008).

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

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

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

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

CONCLUSION

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

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Literature Cited

Abaspour Esfaden, M., Kallaterjari, S. and Fatehi, F. 2019. The effect of salicylic acid and L-arginine on morpho-physiological properties and leaf nutrients of *Catharanthus roseus* under drought stress. Journal of Horticultural Science, 33(3): 417-432. https://doi.org/10.22067/jhorts4.v33i3.73631

Aghaye Noroozlo, Y., Souri, M.K. and Delshad, M. 2019. Stimulation effects of foliar applied glycine and glutamine amino acids on lettuce growth. Agriculture, 4: 164–172. https://doi.org/10.1515/opag-2019-00166

Ahmadi Lashaki, M., Sedaghathoor, S., Kalatehjari, S. and Hashemabadi, D. 2018. The Physiological and growth response of *Petunia hybrida*, *Tagetes erecta*, and *Calendula officinalis* to plant and human steroids. AIMS Agriculture and Food, 3(2): 85-96. https://doi.org/10.3934/agrfood.2018.2.85

Ali, B., Hayat, S. and Ahmad, A. 2007. 28-Homobrassinolide ameliorates the saline stress in chickpea (*Cicer arietinum* L). Environmental and Experimental Botany, 59: 217–223.

Amin, A.A., Gharib, F.A.E, El-Awadia, M. and Rashad, E.S.M. 2011. Physiological response of onion plants to foliar application of putrescine and glutamine. Scientia Horticulturae, 129: 353-360. https://doi.org/10.1016/j.scienta.2011.03.052

Bates, I.S., Waldern, R.P. and Tear, I.D. 1973. Rapid determination of free praline for water stress studies. Plant and Soil, 39: 205-207. https://doi.org/10.1007/BF00018060

Erdal, S. and Dumlupinar, R. 2011. Mammalian sex hormones stimulate antioxidant system and enhance growth of chickpea plants. Acta Physiologiae Plantarum, 33(3): 1011-1017. https://doi.org/10.1007/s11738-010-0634-3

Fariduddin, Q., Khanam, S., Hasan, S.A., Ali, B., Hayat, S. and Ahmad, A. 2009. Effect of 28-homobrassinolide on drought stress induced changes in photosynthesis and antioxidant system of *Brassica juncea* L. Acta Physiologiae Plantarum, 31: 889-897. https://doi.org/10.1007/s11738-009-0302-7

Faten, S., Abd El-Aal, F.S., Shaheen, A.M., Ahmed, A.A. and Mahmoud, A.R. 2010. Effect of foliar application of urea and amino acids mixtures as antioxidants on growth, yield and characteristics of squash. Journal of Agricultural and Biological Science, 6: 583-588.

Gawronska, H. 2008. Biostimulators in modern agriculture (General aspects). Plant Press Warsaw University of Life Sciences (WULS). pp 89.

Haghighi, M., Barzegar Sadeghabad, A. and Abolghasemi R. 2022. Effect of exogenous amino acids application on the biochemical, antioxidant, and nutritional value of some leafy cabbage cultivars. Nature (Scientific Reports), 12: 17720. https://doi.org/10.1038/s41598-022-21273-6

Harborne, J.B. and Williams, C.A. 2000. Advances in flavonoid research since 1992. Phytochemistry, 55: 481-504. https://doi.org/10.1016/s0031-9422(00)00235-1

Hasanzade, S., Habibi, F., Amiri, M.E. and Naeini, M.R. 2017. Study of aril and leaf mineral compositionof pomegranate cv. Naderi with spraying fertilizer containing amino acid under drought stress conditions. Iranian Journal of Horticultural Science, 48(1): 229-236. https://doi.org/10.22059/ijhs.2017.138293.891

Janeczko, A. and Skoczowski, A., 2005. Mammalian sex hormones in plants. Folia Histochemica et Cytobiologica, 43(2): 71-79.

Juan, M., Rivero, R.M., Romero, L. and Ruiz, J.M. 2005. Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. Environmental and Experimental Botany, 54(3): 193-201.

Khajehhosseini, S., Fanoodi, F., Tabatabaee, S.A., Yazdani Biouki, R. and Masoud Sinaki, J. 2020. Evaluation of usage and time of glycine amino acid application on growth and vegetative organs yield and antioxidant activity of Hyssop (*Hyssopus officinalis* L.) under different irrigation conditions. Environmental Stresses in Crop Sciences, 13(2): 533-546. https://doi.org/10.22077/escs.2019.1909.1460

Khalid Hussein, Z. and Qader Khursheed, M. 2014. Effect of foliar application of ascorbic acid on growth, yield components and some chemical constituents of wheat under water stress conditions. Jordan Journal of Agricultural Sciences, 10: 1-15. https://doi.org/10.12816/0029871

Mahmood, T., Anwar, F., Abbas, M., Boyce, M. C. and Saari, N. 2012. Compositional variation in sugars and organic acids at different maturity stages in selected small fruits from Pakistan. International Journal of Molecular Sciences, 13: 1380-1392. https://doi.org/10.3390/ijms13021380

Mazumdar, B.C. and Majumder, K. 2003. Methods on physcochemical analysis of fruits. University College of Agriculture. Calcutta, 136-150.

McDonald, S., Prenzler, P.D., Autolovich, M. and Robards, K. 2001. Phenolic content and antioxidant activity of olive extracts. Food Chemistry, 73: 73-84. https://doi.org/10.1016/S0308-8146(00)00288-0

Miri, S.M., Ahmadi, S. and Moradi, P. 2015. Influence of salicylic acid and citric acid on the growth, biochemical characteristics and essential oil content of thyme (*Thymus vulgaris* L.). Journal of Medicinal Plants and By-products, 2: 141-146.

Miri Nargesi, M., Sedaghathoor, S. and Hashemabadi, D. 2022. Effect of foliar application of amino acid, humic acid and fulvic acid on the oil content and quality of olive. Saudi Journal of Biological Sciences, 29(5): 3473–3481. https://doi.org/10.1016/j.sjbs.2022.02.034

Noktehsanj, M., Jahedi, A., Mahdian, R. and Azari Anpar, M. 2018. An assessment of the effect of olive leaf extract as a useful compound on the physicochemical, microbial and sensory features of pulped tomato juice. Innovation in Food Science and Technology, 10(4): 119-136.

Olennikov, D. N. and Kashchenko, N. I. 2022. Marigold metabolites: diversity and separation methods of *Calendula* genus phytochemicals from 1891 to 2022. Molecule, 27(23): 8626. https://doi.org/10.3390/molecules27238626

Porcel, R. and Ruiz-Lozano, J.M. 2004. *Arbuscular mycorrhizal* influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. Journal of Experimental Botany, 55: 1743-1750. https://doi.org/10.1093/jxb/erh188

Pourcel, L., Routaboul, J.M., Cheynier, V., Lepiniec, L. and Debeaujon, I. 2006. Flavonoid oxidation in plants: From biochemical properties to physiological functions. Trends in Plant Science, 12 (1): 29-36. https://doi.org/10.1016/j.tplants.2006.11.006

Rabeie, V. and Jozghasemi, S. 2013. Applied laboratory methods in agricultural and horticultural science. Jahad-e Daneshghahi Press: Urmia, Iran.

Radkowski, A. 2018. Influence of foliar fertilization with amino acid preparations on morphological traits and seed yield of timothy. Plant, Soil and Environment, 64(5): 209-213. https://doi.org/10.17221/112/2018-PSE

Razali, N., Razab, R., Mat Junit, S. and Abdul Aziz, A. 2008. Radical scavenging and reducing properties of extracts of cashew shoots (*Anacardium occidentale*). Food Chemistry, 111: 38-44. https://doi.org/10.1016/j.foodchem.2008.03.024

Rezaie Alulu, A., Kheiry, A., Sanikhani, M. and Arghavani, M. 2020. The effect of salicylic acid, glycine betaine and gamma amino butyric acid foliar spray on carla antioxidant activity under water deficit stress. Journal of Plant Ecophysiology, 12(40): 140-151.

Roosens, N.H., Al Bitar, F., Loenders, K., Angenon, G. and Jacobs, M. 2002. Overexpression of ornithine-δ-aminotransferase increases proline biosynthesis and confers osmotolerance in transgenic plants. Molecular Breeding, 9: 73-80. https://doi.org/10.1023/A:1026791932238

Sawahel Wagdy, A. and Hassan, A. 2002. Generation of transgenic wheat plants producing high levels of the osmoprotectant proline. Biotechnology Letters, 24: 721-725. https://doi.org/10.1023/A:1015294319114

Schwambach, J., Ruedell, C.M., Almeida, M.R., Penchel, R.M., Araujo, E.F. and Neto, A.G. 2008. Adventitious rooting of (*Eucalyptus glubus ×maidennii*) mini-cutting derived from mini-stumps grown in sand bed and intermittent flooding trays: A comparative study. New Forests, 36(3): 261-271. http://dx.doi.org/10.1007%2Fs11056-008-9099-2

Sedaghathoor, S. and Raof Haghparvar, F. 2021. Physiological and antioxidant properties of marigold (*Calendula officinalis* L.) as influenced by methyl jasmonate and estradiol. Journal of Ecophysiology and Phytochemistry of Medicinal and Aromatic Plants, 9(1): 1-12.

Sedaghathoor, S. and Zakibakhsh-Mohammadi, P. 2019. Effect of time of application and amounts of mammalian sex hormone progesterone and gibberellic acid on the growth of *Zinnia elegans*. Revista Chapingo Serie Horticultura, 25 (1): 61-73. https://doi.org/10.5154/r.rchsh.2018.08.017

Seleiman, M.F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu,T., Ab-dul-Wajid, H.H. and Battaglia, M. L. 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants, 10(3): 259. https://doi.org/10.3390/plants10020259

Seyoum, A., Asres, K. and El-Fiky, F.K. 2006. Structure radical scavenging activity relationships of flavonoid. Phytochemistry, 67: 2058-2070. https://doi.org/10.1016/j.phytochem.2006.07.002

Slinkard, K. and Singleton, V.L. 1977. Total phenolics analyses: Automation and comparison with manual methods. American Journal of Enology and Viticulture, 28: 49-55. https://doi.org/10.5344/ajev.1977.28.1.49

Starck, Z. 2005. Growing assistant: Application of growth regulators and biostimulators in modern plant cultivation. Rolnik Dzierawca, 2: 74 - 76.

Sun, B., Ricardo-da-Silva, J.M. and Spranger, I. 1998. Critical factors of vanillin assay for catechins and proanthocyanidins. Journal of Agricultural and Food Chemistry, 46: 4267-4274. https://doi.org/10.1021/jf980366j

Thomas, J., Mandal, A. and Raj Kumar, R. 2009. Role of biologically active amino acid formulations on quality and crop productivity of tea (*Camellia* sp.). International Journal of Agricultural Research, 4: 228 - 36. https://doi.org/10.3923/ijar.2009.228.236

Torab Ahmadi, S., Abedy, B. and Saberali, S.F. 2019. Effect of foliar spray with a fertilizer containing amino acids and seaweed extract on quality and yield components of Ahmad Aghaei pistachio. Pomology Research, 4(2): 95-106.

Wang, M., Tsao, R., Zhang, S., Dong, Z., Yang, R., Gong, J. and Pei, Y. 2006. Antioxidant activity, mutagenicity/anti-mutagenicity, and clastogenicity/anti clastogenicity of lutein from marigold flowers. Food and Chemical Toxicology, 44: 1522–1529. https://doi.org/10.1016/j.fct.2006.04.005

Yari, A., Sedaghathoor, S., Padasht Dahkaei, M.N. and Ansari, M.H. 2024. The effect of foliar application of abiotic elicitors on the physiological traits of marigolds under water deficit conditions. Biologia, 79(1): 59-72. https://doi.org/10.1007/s11756-023-01558-5

Zahedi, M., Hosseini, M.S. and Karimi, M. 2017. The effects of drought stress and brassinosteroid solution spray on some morphological, physiological and biochemical characteristics of wild pear (*Pyrus biossieriana* Buhse). Plant Process and Function, 8(29): 181-192.

Zarafshar, M., Akbarinia, M., Askari, H., Hosseini, S.M., Rahaie, M., Struve, D. and Striker, G.G. 2014. Morphological, physiological and biochemical responses to soil water deficit in seedlings of three populations of wild pear tree (*Pyrus boisseriana*). Biotechnology, Agronomy and Society and Environment, 18(3): 353-366.

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