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SNAPDRAGON (*ANTIRRHINUM MAJUS* L.) RESPONSE TO FOLIAR APPLICATION OF ARGININE AND NANO-IRON

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SUMMARY

The latest study focused on snapdragon (*Antirrhinum majus* L.) plants during the fall season of 2021–2022 in a greenhouse at the Agricultural Division, University of Kufa, Najaf, Iraq. The study aimed to determine the response of snapdragon plants to foliar application of arginine (0, 20, 40, and 60 mg L^{-1}) and nano-iron (0, 30, 60, and 90 mg L^{-1}). The experiment comprised a randomized complete block design with a factorial arrangement and three replications. The arginine (60 mg L^{-1}) foliar spray led to a significant enhancement in the flower and physiological traits, i.e., flower carrier length (19.62 cm), age of the flowering inflorescence (25.01 days), leaf content of carbohydrates (12.75 mg g^{-1}) and iron (48.93 mg kg^{-1}), and flower content for anthocyanin dye (21.40 mg 100 g^{-1}) compared with the lowest values of the control treatment viz., 13.88 cm, 16.70 days, 11.25 mg g^{-1}, 15.22 mg kg^{-1}, and 9.82 mg 100 g^{-1}, respectively. The nano-iron (90 mg L^{-1}) also significantly improved the flower and physiological traits, i.e., the flower carrier length (18.06 cm), age of the flowering inflorescence (22.75 days), leaf content for carbohydrates (12.16 mg g^{-1}); iron (38.08 mg kg^{-1}), and flower content for anthocyanin dye (16.72 mg 100 g^{-1}) compared with the control, i.e., 15.79 cm, 20.02 days, 11.71 mg g^{-1}, 29.61 mg kg^{-1}, and 13.05 mg 100 g^{-1}, respectively. The interaction of arginine (60 mg L^{-1}) and nano-iron (90 mg L^{-1}) provided a positive effect of their individual application for flower and physiological traits (22.55 cm, 27.93 days, 12.93 mg g^{-1}, 50.04 mg kg^{-1} , and 23.06 mg100 g^{-1}) compared with the control (12.84 cm, 15.43 days, 10.84 mg g^{-1}, 13.0 mg kg^{-1} , and5.13 mg 100 g^{-1}), respectively.

Keywords: Snapdragon (*Antirrhinum majus* L.), arginine, nano-iron

Key findings: The single and dual interaction foliar application of arginine (60 mg L^{-1}) and nano-iron (90 mg L^{-1}) significantly improved the flower and physiological traits of snapdragon (*A. majus* L.) plants, followed by the individual application of arginine (60 mg L^{-1}) and nano-iron (90 mg L^{-1}).

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INTRODUCTION

The snapdragon (*Antirrhinum majus* L.) plays an important annual garden plant with beautiful flowers, widely cultivated in tropical and temperate zones as a bedding, rockery, herbaceous border, and container plant (Huxley and Griffiths, 1992). Cultivation of.

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snapdragon flowers serves for edging, pots, or window boxes, while other species work well for hanging baskets (Alshaib 2005). The snapdragon flower contains a wide array of colors, a relatively long vase life, and a stunning spike-type morphology, which has recently expanded (Abdulhadi *et al*., 2022).

Polyamines have an important role in the regulation of cell cycle and division, morphogenesis in phytochrome, and plant hormone-mediated processes; they also regulate plant senescence and plant response to various adverse environmental conditions (Walters, 2000). Arginine is an important amino acid thought to be the major precursor of polyamines, which are formed via decarboxylation of arginine by arginine decarboxylase to create putrescine in grapes (Hayder and Al-Janabi, 2020). Arginine application significantly promoted growth and increased fresh and dry weights, certain endogenous plant growth regulators, chlorophylls a and b, and carotenoids in beans (Nassar *et al.*, 2003; Khalil *et al*. 2009) and wheat (Abd-El-Moniem and Abd-Allah, 2008; El-Bassiouny *et al*., 2008). Furthermore, Khalil *et al*. (2009) documented the beneficial function of arginine in relieving inhibition caused by various stress conditions in wheat.

Minerals have a direct and indirect impact on apple fruit yield and quality (Spinelli *et al*., 2009). In agricultural systems, the efficient use of fertilizers aims to increase crop yield. However, synchronizing fertilizer administration per crop requirement proved difficult. With the ability of plant leaves to absorb nutrients, foliar application of fertilizers has become a common way of giving nutrients to mulberry fruit trees (Al-Janabi and Al-Hasnawi, 2021a). Since elements like nitrogen, phosphorus, zinc, copper, boron, and potassium play a physiological and vital role in stimulating and promoting vigorous plant growth and development, low application rates, uniform fertilizer distribution, and quick responses to applied foliar fertilization became advantageous. Micronutrients, such as, Fe, Zn, Mn, Cu, and B are not only necessary but also crucial due to their micro-quantity requirements for plant growth and productivity (Das and Bansal, 2019).

Iraqi soils mostly have a high amount of calcium carbonate. It is used as a fertilizer for agricultural soils since it increases the pH of acidic soils. The increase in pH enhances soil fertility and makes plants better assimilate nitrogen, phosphorus, and potassium. When macro- and micronutrients are introduced to the soil, some portion of the micronutrients may be exposed to loss, fixation, and sedimentation, and the plant will not benefit from them. As a result, researchers began looking for alternatives to using it as a foliar spray (Abd-El-Migeed *et al*., 2004). Given the harmful effects of chemical and inorganic fertilizers on the environment, and the quality of agricultural products, their use has long been discouraged or banned, and researchers began looking for better alternatives suitable for the management of water and fertilizers. From that standpoint, the studies turned toward alterations in the fertilizer's structure for their utilization as new technologies to have a positive impact (Abbasi and Abbasi, 2012).

Foliar fertilizers also help prevent signs of toxicity that can occur after the application of the same microelements to the soil (Al-Janabi *et al*., 2021b, c). In agriculture and food systems, the use of nanotechnologies nowadays helped in the development, processing, and implication of nanoscale complexes, solving several problems in multidisciplinary sciences and industries (Scott and Chen, 2003). Materials that are smaller than 100 nm are typically known as nano-material, at least in a single dimension. Applications of this new technology have been established in agriculture, and nano-technologies have served to produce, process, store, pack and transport agricultural products (Wiesner *et al*., 2006).

The nano-fertilizer market, which can gradually feed plants in a regulated manner, became the most significant application of nanotechnology in crop production. These nano-fertilizers are more effective in reducing soil contamination and other environmental threats by optimizing the formulation of chemical fertilizers (Naderi *et al*., 2011). One of the benefits of using nano-fertilizers provided for easier application of smaller quantities as compared with common inorganic fertilizers used for crop nutrition (Subramanian *et al*., 2015). Foliar application is an alternate way of dealing with nitrogen deficiency (Cakmak, 2008). It is useful to resolve soil issues, such as, growth media and nitrogen fixation, particularly in soils with high pH (El-Naggar, 2009). Foliar treatment also has the potential to increase the quality of cut flowers like lily flowers (*L. philadelphicum*) (Sajid *et al*., 2009) and mandarin fruit trees (Khalid *et al*., 2012). Likewise, it also boosts nutrient absorption by roots, resulting in improved plant roots and stalk development (Mia, 2015). The latest study aimed to determine the effects of foliar application of arginine and nano-iron on the growth, flowering, and physiological parameters of snapdragon (*A. majus* L.) and to formulate the best concentration of arginine and nano-iron and their interaction, with a focus on influences to the growth and flowering indicators.

MATERIALS AND METHODS

Plant material and procedure

The experiment on snapdragon (*A. majus* L.) plants took place during the fall season of 2021–2022 in a greenhouse at the Agricultural Division, University of Kufa, Najaf, Iraq. The plants were placed in small plastic dishes with dimensions of 55 cm × 28 cm containing peat moss. After the seeds germinated and the plant reached a height of 4–5 cm with the appearance of real leaves, they were transferred into plastic pots with a diameter of 25 cm and a height of 24 cm. The plant per pot contained a medium consisting of soil (river mixture) and peat moss in a ratio of 3:1 and a weight of 5 kg. Soil and peat moss were obtained from the Graduate Studies Laboratory at the Department of Soil and Water Sciences, Faculty of Agriculture, University of Kufa, Iraq (Tables 1 and 2).

Table 1. Soil properties of the experimental location used for the study.

Properties	Values
Acidity degree of soil (pH)	7.5
EC	3.9 Ds.
N	30.5 mg L^{-1}
P	12.6 mg L^{-1}
K	14.5 mg L^{-1}
$CaCO_3$	12.5%
Organic matter	2.1%
Soil texture	Sandy
Clay	16.5%
Silt	8.0%
Sand	75.5%

Table 2. Analysis of peat moss used in the study.

EC	pH	N (mg L^{-1})	P_2O_5 (mg L^{-1})	K_2O (mg L^{-1})	Mg (mg L^{-1})	S (mg L^{-1})
2.63	7.82	140	160	180	100	120

The study aimed to determine the response of snapdragon plants to foliar application of arginine (0, 20, 40, and 60 mg L^{-1}) and nano-iron (0, 30, 60, and 90 mg L^{-1}). The research employed a randomized complete block design with a factorial arrangement and three replications (Al-Rawi and KalafAllah, 2000). Analysis of the data used the Genstat V12 statistical program. The first spray of arginine proceeded on 8 December 2021, the second on 29 December 2021, and the third on 19 January 2022. For nano-iron, the first spray took place on 12 December 2021, the second on 2 January 2022, and the third on 23 January 2022. All the sprays took place until fully wet, using a two-liter hand-held sprinkler when the drops of the solution fell on the leaves in the early morning to avoid sunlight and prevent evaporation.

Data recorded

Inflorescence stalk length (cm)

On 18 April 2022, the length of the inflorescence and flower stand underwent measurement using a tape measure for each experimental unit, from the soil surface to the lower base of the flowering inflorescence where the flower bud appears, followed by computing averages for each treatment.

Lifespan of flowering inflorescence (days)

The lifespan of the plant's flower underwent calculation by counting the number of days the first flower of the inflorescence began opening until the flowering inflorescence withered, for all experimental units. Then computation of averages for each treatment took place.

Total carbohydrates content in leaves (mg 100 g^{-1} fresh weight)

The total carbohydrate content estimation in the leaves took place by taking 10 mg dry weight of plant leaves and placing them in 5 ml of sulfuric acid (H_2SO_4) at a concentration of 80% and 1 ml of phenol at a concentration of 5% for 10 min until reaching an orange color to indicate the reaction response. The spectrophotometer measured the solution at a wavelength of 490 nm by following Al-Janabi *et al*. (2021b) for each treatment. All the analyses took place at the Graduate Studies Laboratory, College of Agriculture, University of Kufa, Iraq.

Iron content in leaves (mg kg^{-1} dry weight)

The Atomic Absorption Spectrophotometer (Shimadzu AA-6200) determined the element iron.

Anthocyanin content in florets (mg 100 g^{-1})

The anthocyanin pigment estimation in flower petals used the following method: One g of fresh flower petals was taken and extracted in a solvent consisting of ethyl alcohol and HCl (1.5N) at a ratio of 85:15. Then 10 ml of the mixture was crushed in a ceramic mortar and then filtering 10 ml from the mixture solution in a filter paper. Having a complete volume of 30 ml, reading the samples used a UV-Visible Spectrophotometer at a wavelength of 535 nm. It was calculated through the following equation:

Quantity of anthocyanins = (535 wavelengths on optical density) / (98.2 × sample weight) × volume of solution used × extraction × dilutions × 100.

RESULTS

Inflorescence stalk length

Results revealed that arginine acid (60 mg L^{-1}) spray provided a significant increase in the inflorescence stand length of snapdragon (*A. majus* L.) plants (19.62 cm) compared with the control treatment (13.88 cm) (Table 3). The nano-iron (90 mg L^{-1}) foliar application also had a significant effect in enhancing flowering stand length (18.06 cm) compared with the control (15.79 cm) in the plant. The combined application of arginine acid (60 mg L^{-1}) and nano-iron (90 mg L^{-1}) had a significant effect in enhancing the stand length of the flowering inflorescence of snapdragon plants (22.55 cm) compared with the control treatment (12.84 cm).

Lifespan of flowering inflorescence

In snapdragon, the arginine acid (60 mg L^{-1}) foliar application increased the lifespan of the inflorescence (25.01 days), giving a significant effect compared with the control treatment (16.70 days) (Table 4). The spray of nano-iron (90 mg L^{-1}) significantly enhanced the lifespan of the inflorescence, reaching 22.75 days compared with the control (20.02 days). The combined foliar application and interaction of arginine acid (60 mg L^{-1}) with nano-iron (90 mg L^{-1}) caused a significant effect in increasing the lifespan of the flowering inflorescence (27.93 days) compared with the control treatment (15.43 days).

Total carbohydrates content in leaves

According to the findings, topically applying arginine acid (60 mg L-1) raised the amount of carbohydrates in snapdragon leaves (12.75 mg 100 g^{-1} fresh weight), having a substantial impact compared with the control treatment (11.25 mg 100 g^{-1} fresh weight) (Table 5). The nano-iron (90 mg L-1) application also had a positive effect by enhancing the carbohydrate content of leaves (12.16 mg 100 g^{-1} fresh weight) compared with the control (11.71 mg 100 g^{-1} fresh weight). The interaction effects of the foliar application of arginine acid (60 mg L^{-1}) with nano-iron (90 mg L^{-1}) displayed a relevant effect with the improved carbohydrate content of leaves (12.93 mg 100 g^{-1} fresh weight) compared with the control treatment (10.84 mg 100 g^{-1} fresh weight).

Iron content in the leaves

Results exhibited that arginine acid (60 mg L^{-1}) foliar application has a significant effect on the iron content of leaves in snapdragon, reaching 48.93 mg kg^{-1} dry weight compared with the control treatment (15.22 mg kg^{-1} dry weight) (Table 6). The nano-iron (90 mg L^{-1}) application also significantly enhanced the iron content in leaves (38.08 mg kg^{-1} dry weight) compared with the control treatment (29.61 mg kg^{-1} dry weight). The interaction of arginine acid (60 mg L^{-1}) with nano-iron (90 mg L^{-1}) revealed a significant effect, boosting the iron content in leaves (50.04 mg kg^{-1} dry weight) compared with the control treatment (13.00 mg kg^{-1} dry weight).

Table 3. Effect of arginine and nano-iron foliar applications on the inflorescence stand length in snapdragon plants***.***

Treatments		Nano-iron (mg L^{-1})				Arginine (means)
		0	30	60	90	
Arginine (mg L^{-1})	0	12.84	13.46	14.35	15.24	13.88
	20	15.24	15.70	15.70	16.76	15.85
	40	16.92	17.16	17.51	17.70	17.32
	60	18.51	18.61	18.83	22.55	19.62
Nano-iron (means)		15.79	16.23	16.60	18.06	$LSD_{0.05}$ Arginine = 0.226 $LSD_{0.05}$ Nano-iron = 0.226 $LSD_{0.05}$ Arginine × Nano-iron = 0.453

Table 4. Effect of arginine and nano-iron foliar applications on the lifespan of inflorescence in snapdragon plants.

Treatments		Nano-iron (mg L^{-1})				Arginine (means)
		0	30	60	90	
Arginine (mg L^{-1})	0	15.43	15.87	17.33	18.17	16.70
	20	19.31	19.97	20.77	21.43	20.37
	40	21.67	22.37	23.17	23.47	22.67
	60	23.63	23.84	24.84	27.93	25.01
Nano-iron (means)		20.02	20.51	21.47	22.75	$LSD_{0.05}$ Arginine = 0.843 $LSD_{0.05}$ Nano iron = 0.843 $LSD_{0.05}$ Arginine × Nano-iron = 1.686

Table 5. Effect of arginine and nano-iron foliar applications on total carbohydrates content of the leaves in snapdragon plants.

Treatments		Nano-iron (mg L^{-1})				Arginine (means)
		0	30	60	90	
Arginine(mg L^{-1})	0	10.84	11.06	11.21	11.37	11.25
	20	11.50	11.67	11.70	11.84	11.68
	40	11.94	12.05	12.44	12.49	12.23
	60	12.57	12.72	12.79	12.93	12.75
Nano-iron (means)		11.71	11.88	12.03	12.16	$LSD_{0.05}$ Arginine = 0.173 $LSD_{0.05}$ Nano iron = 0.173 $LSD_{0.05}$ Arginine × Nano iron = 0.347

Table 6. Effect of arginine and nano-iron foliar applications on the iron content of leaves in snapdragon plants.

Treatments		Nano-iron (mg L^{-1})				Arginine (means)
		0	30	60	90	
Arginine (mg L^{-1})	0	13.00	14.80	16.48	17.00	15.22
	20	17.62	24.31	34.21	37.27	28.35
	40	39.63	47.21	47.85	48.02	45.68
	60	46.22	48.35	49.12	50.04	48.93
Nano-iron (means)		29.61	33.66	36.91	38.08	$LSD_{0.05}$ Arginine=0.342 $LSD_{0.05}$ Nano iron=0.342 $LSD_{0.05}$ Arginine × Nano-iron =0.685

Anthocyanin content in florets

The spraying of the snapdragon plants with arginine acid (60 mg L^{-1}) showed a significant effect, boosting the anthocyanin pigment flower petals (21.40 mg 100 g^{-1}) compared with the control (9.82 mg 100 g^{-1}) (Table 7). The nano-iron (90 mg L-1) foliar application also increased the anthocyanin content in the flowers (16.72 mg 100 g^{-1}) compared with the control treatment (13.05 mg 100 g^{-1}). The combined foliar application of arginine acid (60 mg L^{-1}) and nano-iron (90 mg L^{-1}) positively affected and enhanced the anthocyanin dye in flower petals (23.06 mg 100 g^{-1}) compared with the control (5.13 mg 100 g^{-1}).

Table 7. Effect of arginine and nano-iron foliar applications on the anthocyanin content of the leaves in snapdragon plants.

Treatments		Nano-iron (mg L^{-1})				Arginine (means)
		0	30	60	90	
Arginine (mg L^{-1})	0	5.13	10.36	11.44	12.36	9.82
	20	12.50	12.63	13.40	15.12	13.41
	40	15.46	15.92	15.67	16.33	15.84
	60	19.14	21.16	22.23	23.06	21.40
Nano-iron (means)		13.05	15.02	15.66	16.72	$LSD_{0.05}$ Arginine=0.381 $LSD_{0.05}$ Nano iron=0.381 $LSD_{0.05}$ Arginine × Nano-iron = 0.636

DISCUSSION

Arginine is an essential and unique amino acid in crop plants. It serves in nitrogen reserve and recycling, as well as, a precursor of the biosynthesis of polyamines and nitric oxide. The arginine metabolism consists of two pathways: putrescine and catalyzing the enzyme nitric oxide synthase, which produces citrulline and nitric oxide. Arginine (amino acid) improves the hormonal balance, helps stimulate buds, and regulates the flowering rate, as well as, regulates the transfer of mineral elements and their accumulation in flowers of pomegranate (Al-Janabi *et al*., 2021c), grapes (Hayder and AL-Janabi, 2020), wheat (El–Bassiouny *et al*., 2008; Khalil *et al*., 2009), foliar application of plant growth regulators in lily flowers (Sajid *et al*., 2009), and nano-scale engineering in agriculture and food systems (Scott and Chen, 2003).

Nano-iron complex as a leaf spray helps for long-term feeding of the plants through roots. Iron serves as one of the essential micronutrients for plant growth and development. It is required for a wide range of biochemical processes, from photosynthesis to respiration. Iron has a crucial role in the system of many enzymes involved in the respiration process, such as, catalase, cytochrome oxidase, and peroxidase, as well as, in the representation of amino acids. Iron plays a significant part in various plant processes, through its direct participation as a synthetic part of the plant material and activation of the enzymatic processes in *Antirrhinum majus* L. (Abdulhadi *et al*., 2022), pomegranate (Al-Janabi *et al*., 2021b, c), plum trees (Hassan *et al*., 2010), nano-fertilizers use for balanced crop plants nutrition (Subramanian *et al*., 2015), and the vital role of nanotechnology in the optimization of the formulation of inorganic fertilizers (Naderi *et al*., 2011) and foliar application of macro- and micro-elements in *Dahlia hybrida* (Kashif *et al*., 2014).

Arginine, with its physiological role, highly contributes to osmotic pressure and other processes carried out in crop plant tissues, where its increase leads to a decrease in the osmotic pressure, proceeding to decrease water conservation in the cell. It eventually improves the cell's ability to withdraw water and the nutrients dissolved in it, enhancing vegetative growth. This then leads to an increase in the chlorophyll content and total soluble carbohydrates in the plant leaves. The iron element presence can also be a reason, which gave a boost in nitrogen percentage in the leaves since nitrogen is included in the construction of the group of four porphyrins. These complexes enter into the composition of the chlorophyll pigment with the magnesium element present, leading to an increase in the total dissolved carbohydrates in the leaves.

Nitrogen is the major component of amino acids in proteins and fats, which act as structural compounds in chloroplasts (Mia, 2015). The enzymatic chaperones such as NADH2, NADPH2, and the chlorophyll molecule (Nassar *et al*., 2003) play an important role in the redox reactions that occurs in photosynthesis. The chlorophyll formation undergoes the participation of the iron element (Ferrous Iron) in the condensation of succinic acid and glycine to form (Y-aminolevulinic acid), which in turn condenses to form pyrrole groups and condenses again to form Protopor Phyrin 1X. Afterward, the magnesium element also participates to form chlorophyll catalyzed by the iron. The amino acids, especially arginine, have multiple effects, playing an important role in the formation of polyamines (AL-Hadrawi and AL-Janabi, 2020) which are polyamines that participate in the process of modifying enzyme activities in cell division and elongation, and intervening in the plant growth and development (Naderi *et al*., 2011), as well as, in different biological processes, such as, gene expression and protein synthesis.

Nutrients' positive effect and their role in enhancing the photosynthesis products, represented by carbohydrates, are transformed within metabolic pathways in the plant Chalcone synthase, later turning into the naringenin compound, which oxidizes into anthocyanin in the cell vacuoles. The nano-iron (90 mg L^{-1}) has a significant impact on the anthocyanin content in flowers of snapdragons (*Antirrhinum majus* L.) because iron deficiency shows the symptoms of crop plants lacking iron, causing a decrease in all the pigments, including chlorophyll a and b, carotene, and xanthophylls.

CONCLUSIONS

Arginine and nano-iron foliar application notably affected the growth traits in the snapdragon plants. The arginine acid (60 mg L^{-1}) application served as the best among the concentrations, displaying a significant effect on most of the snapdragon plants traits showing more advantage in most of the indicators of the study. The combined foliar application of arginine (60 mg L^{-1}) and nano-iron (90 mg L^{-1}) positively influenced the plants to exhibit the highest rate of vegetative, flowering, root, and chemical growth indicators.

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