

RESPONSE OF TARRAGON (*ARTEMISIA DRACUNCULUS* L.) TO THE APPLICATION OF ZINC SULFATE AND GLYCINE BETAINE UNDER WATER STRESS CONDITIONS

Soheil KHANCHI^{1,2}, Asad ROKHZADI^{1,2},
Sirwan MOHAMMDIAZAR³, Elena Maria DRĂGHICI⁴

¹Department of Agronomy and Plant Breeding, Sa.C.,
Islamic Azad University, Sanandaj, Iran

²Research Center for Agriculture, Animal Husbandry, and Medicinal Plants, Sa.C.,
Islamic Azad University, Sanandaj, Iran

³Department of Chemistry, Sa.C., Islamic Azad University, Sanandaj, Iran

⁴University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author emails: asadrokh@iau.ac.ir, draghiciem@yahoo.com

Abstract

An experiment was conducted to investigate the effects of zinc sulfate and glycine betaine on tarragon (*Artemisia dracunculus* L.) under different irrigation regimes, using a central composite design (CCD) in greenhouse conditions. The experimental factors included irrigation at 30, 65, and 100% of field capacity (FC), zinc sulfate at 0, 3, and 6 g/L, and glycine betaine (GB) at 0, 75, and 150 mM. The imposition of drought stress led to a decrease in plant height and shoot dry weight (DW), while the highest rate of essential oil (EO) content was obtained under mild stress conditions. The highest plant height was obtained with moderate amounts of zinc sulfate, while the maximum DW was obtained with the application of 6 g/L of zinc sulfate. GB application had no significant effect on plant height and DW, but applying intermediate GB level maximized the EO content. In general increasing stress intensity reduced plant growth traits, but by imposing a moderate drought stress and applying 75 mM of GB, the EO content can be maximized.

Key words: tarragon, drought stress, zinc sulfate, glycine betaine, essential oil.

INTRODUCTION

Tarragon (*Artemisia dracunculus* L.) is a perennial herb species from Asteraceae family with numerous edible, spice, and medicinal uses (Kordali et al., 2005; Karimi et al., 2015; Koul and Taak, 2017). This plant is widespread in most temperate regions of Asia, Eastern and Central Europe, and Western North America (Obolskiy et al., 2011). It is a medicinal and culinary plant with antioxidant, anti-inflammatory, and hypoglycemic properties. Used traditionally in Asia for various ailments, it is now studied for its pharmacological benefits and is gaining importance in food, cosmetics, and biotechnology (Ekiert et al., 2021)

Hassanzadeh et al. (2016) stated that *Artemisia dracunculus* L., an aromatic plant from the Asteraceae family, is rich in essential oils containing methyl ethers, ocimene, and other compounds. Due to its pharmacological,

antibacterial, and antifungal properties, it is extensively used in food preservation as a natural substitute for synthetic additives.

Mumivand et al. (2021) studied *Artemisia dracunculus* under drought, finding reduced water content and chlorophyll but increased antioxidant activity. HPLC analysis showed enhanced phenolic acids and flavonoids, with Hamadan, Varamin, and Estahbanat accessions proving drought-tolerant, aiding tarragon breeding and cultivation.

Zinc (Zn) is essential for plant and human growth, with dietary zinc deficiencies being a global issue. A study conducted by Majid et al. (2023) over two years evaluated the effects of foliar application of zinc sulfate (0.44 g Zn/L) at different phenological stages on wheat agronomic traits and grain zinc content. Foliar application during the branching and grain filling stages significantly improved grain yield and plant height, while early application at stem elongation had no effect on zinc

concentration. The most effective strategy for alleviating zinc deficiency was application during the branching and grain filling stages.

Salinity stress affects crop growth and yield, prompting research on mitigation strategies. This study examined the effects of NaCl salinity (0, 50, 100 mM) and foliar sprays (ZnO, nano-ZnO, Fe-chelate, magnetized-Fe, graphene-oxide) on tarragon. Graphene spray improved K^+/Na^+ balance, while Zn sprays enhanced essential oil content. Salinity increased antioxidant activity and phenolics, with GC/MS identifying estragole (81-91.8%) as the dominant oil component. Despite salinity's adverse effects, Fe and Zn foliar treatments helped mitigate stress (Hassanpouraghdam et al., 2021).

Kordali et al. (2005) studied the effects of NaCl salinity (0, 50, 100 mM) and foliar sprays (ZnO, nano-ZnO, Fe-chelate, magnetized-Fe, graphene-oxide) on tarragon. The results indicated that graphene improved the K^+/Na^+ ratio, while Zn sprays enhanced essential oil content. Salinity induced oxidative stress, but foliar applications of Fe and Zn partially mitigated these effects. GC/MS analysis revealed estragole as the dominant compound, with increased concentrations under ZnO treatment at 50 and 100 mM NaCl.

Zinc (Zn) is essential for plants as a micronutrient and is involved in crucial physiological processes like protein and carbohydrate metabolism, auxin hormone regulation, and enzyme formation (Hafeez et al., 2013). Due to its numerous physiological roles in plants, Zn can protect plants from damage under stress conditions (Umair Hassan et al., 2020).

Hassanpouraghdam et al. (2022) investigated the impact of NaCl salinity (0, 50, 100 mM) and foliar sprays (ZnO, Fe-chelate, graphene-oxide) on tarragon. The study found that graphene enhanced the K^+/Na^+ ratio, while salinity elevated oxidative stress markers. Zinc treatments increased catalase activity and essential oil content, with estragole being the predominant compound. Both Fe and Zn sprays helped reduce salinity-induced stress, improving the overall physiological response of tarragon.

Glycine betaine (GB) serves as a compatible osmolyte in plant cells, aiding in cell protection

from environmental stressors like drought by facilitating osmotic regulation within the cell (Hernandez-Leon and Valenzuela-Soto, 2023). It has been shown that external application of GB to plants can improve the effects of drought stress in plants (Ashraf and Foolad, 2007; Obolskiy et al., 2011; Mohammad, 2023).

The study conducted by Oliveira et al. (2023) evaluated the response of tarragon to different concentrations of nutrient solution in hydroponic cultivation. The standard solution (100%) provided the best overall results. A 15% reduction maximizes fresh mass but significantly decreases dry mass, stem number, and height. For fresh consumption, a 25% reduction minimally affects production (1.3%). Water deficit stress is one of the most important abiotic stresses damaging to plants. Today, due to global warming and climate change, this stress has shown more detrimental effects on plants than in the past (Liu et al., 2022).

This study aimed to model the response of tarragon plant to the application of zinc sulfate and glycine betaine under different levels of drought stress using the response surface methodology (RSM).

MATERIALS AND METHODS

The experiment was conducted in 2023 at the Yashil Inji Azarbayjan research extension greenhouse in Urmia, Iran (37° 44' N, 45° 10' E, 1338 m above sea level). The experimental layout was based on a central composite design (CCD) with three experimental factors. The experimental factors included water deficit stress (30% FC, 65% FC, and 100% FC), zinc sulfate foliar application (0, 3, and 6 g/L), and glycine betaine (GB) foliar application (0, 75, and 150 mM).

The 4-leaf tarragon seedlings were transplanted into pots and subjected to drought stress treatments after reaching the 8-leaf stage. Foliar spraying with zinc sulfate and glycine betaine was carried out in three stages with 20-day intervals. At the flowering stage, plant height, shoot dry weight (DW), and essential oil (EO) content of tarragon plants were measured.

The collected data were subjected to analysis of variance and the effect of experimental factors on the measured responses of tarragon was

modeled using the response surface methodology (RSM). Statistical operations and graphing were performed using Design Expert 12 software.

RESULTS AND DISCUSSIONS

The fitted models for plant height, DW, and EO content were statistically significant (Tables 1-3). The effect of water stress on the measured parameters was significant. Zinc sulfate application had a significant quadratic effect on plant height and a significant linear effect on DW, but had no significant effect on EO content. Foliar application of GB had no significant effect on plant height and DW, but its quadratic effect on EO content was significant (Tables 1-3).

The response surface plot of plant height versus water stress and zinc sulfate indicates that plant height decreased linearly by reducing irrigation from 100 to 30% FC (Figure 1). The response of plant height to zinc sulfate was quadratic, so that under severe stress conditions (30% FC), the application of a moderate amount of zinc sulfate (3 g/L) resulted in the maximum plant height, and under normal irrigation conditions (100% FC), the plant height was maximized with the application of about 4 g/L of zinc sulfate (Figure 1). The plot of the interaction effect of water stress and zinc sulfate on DW shows that the highest amount of DW was obtained by applying the maximum rate of zinc sulfate at an irrigation level close to 100% FC (Figure 2).

Water deficit stress decreases plant growth by decreasing cell division and development, lowering stomatal conductance, and ultimately reducing the photosynthesis rate (Farooq et al., 2012). In accordance with our results, Thakur and Thakur (2018) declared that drought stress caused a significant decrease in plant height and biomass of various medicinal plants.

The positive impact of Zn on the height and DW of tarragon plants could be due to its influence on the meristematic regions of the plant, where it triggers the biosynthesis of the hormone indole-acetic acid (IAA) and promotes plant growth (Castillo-González et al., 2018).

The response plot of DW to water stress and GB indicates that although the application of

GB did not have a statistically significant effect on DW, it did lead to an increasing trend in DW (Figure 3). It has been reported that GB can affect plant growth and development, especially under stress conditions, through interactions with nucleic acids and proteins (Hernandez-Leon and Valenzuela-Soto, 2023).

The EO content reacted as a parabolic graph versus GB and water stress (Figure 4). In other words, the highest amount of EO was obtained with the use of medium levels of GB (75 mM) and under medium stress conditions (about 80% FC).

In accordance with the present result, Feiz et al. (2019) found that using 100 mM of GB and moderate levels of water stress led to the highest amount of EC in marigold plants.

The response surface plot of water stress and zinc sulfate interaction shows that despite the non-significant effect of zinc sulfate on EO content, an increasing trend in EO content was observed with zinc sulfate application. (Figure 5). The improving effect of zinc sulfate on EO content could be attributed to the role of Zn in photosynthesis and carbohydrate metabolism, as carbohydrates are known to serve as an energy source for the production of terpenoids and EO constituents (Said-Al Ahl and Mahmoud, 2010).

Table 1. The ANOVA results and regression coefficients of the fitted models for plant height

Source	df	Plant height	
		Regression coefficient	p-value
Model	9		0.0038
Intercept		53.9	
Linear			
x ₁ (Water stress)	1	4.625	0.0040
x ₂ (Zinc sulfate)	1	1.525	0.2486
x ₃ (GB)	1	-1.375	0.2952
Quadratic			
x ₁ ²	1	-3.625	0.1577
x ₂ ²	1	-5.925	0.0317
x ₃ ²	1	-0.375	0.8776
Interaction			
x ₁ x ₂	1	4.6875	0.0071
x ₁ x ₃	1	2.9375	0.0610
x ₂ x ₃	1	0.3125	0.8268
Lack of fit	5		0.5428
Pure error	5		
R ²		0.852	
CV (%)		8.04	

Table 2. The ANOVA results and regression coefficients of the fitted models for shoot dry weight (DW)

Source	df	Shoot DW	
		Regression coefficient	p-value
Model	9		0.0040
Intercept		1.15	
Linear			
x ₁ (Water stress)	1	0.2450	0.0003
x ₂ (Zinc sulfate)	1	0.1280	0.0164
x ₃ (GB)	1	0.0370	0.4248
Quadratic			
x ₁ ²	1	-0.1991	0.0408
x ₂ ²	1	-0.0591	0.5017
x ₃ ²	1	0.0009	0.9917
Interaction			
x ₁ x ₂	1	0.0525	0.3158
x ₁ x ₃	1	0.0275	0.5923
x ₂ x ₃	1	0.0675	0.2044
Lack of fit	5		
Pure error	5		
R ²		0.851	
CV (%)		13.76	

Table 3. The ANOVA results and regression coefficients of the fitted models for essential oil (EO) content

Source	df	EO content	
		Regression coefficient	p-value
Model	9		0.0098
Intercept		1.51	
Linear			
x ₁ (Water stress)	1	0.2743	0.0042
x ₂ (Zinc sulfate)	1	0.0543	0.4822
x ₃ (GB)	1	-0.0257	0.7367
Quadratic			
x ₁ ²	1	-0.3221	0.0465
x ₂ ²	1	0.0636	0.6632
x ₃ ²	1	-0.3649	0.0277
Interaction			
x ₁ x ₂	1	0.0679	0.4335
x ₁ x ₃	1	-0.0036	0.9666
x ₂ x ₃	1	-0.0036	0.9666
Lack of fit	5		
Pure error	5		
R ²		0.817	
CV (%)		19.69	

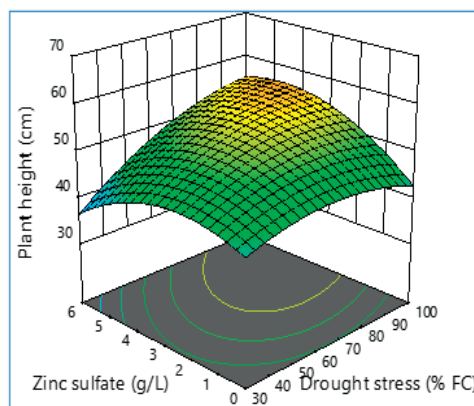


Figure 1. Response surface plot for the effect of drought stress and zinc sulfate on plant

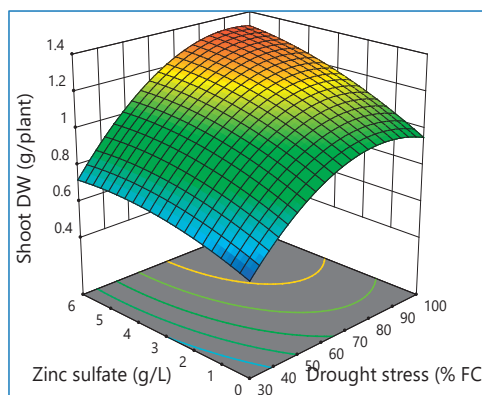


Figure 2. Response surface plot for the effect of drought stress and zinc sulfate on shoot dry weigh (DW)

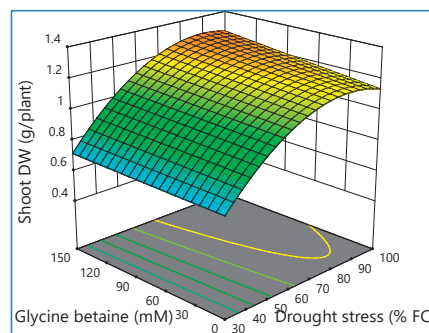


Figure 3. Response surface plot for the effect of drought stress and glycine betaine on shoot dry weigh (DW)

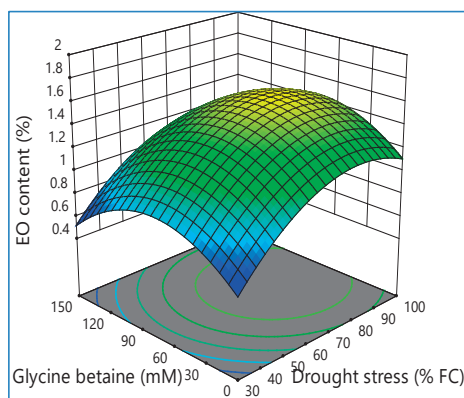


Figure 4. Response surface plot for the effect of drought stress and glycine betaine on EO content

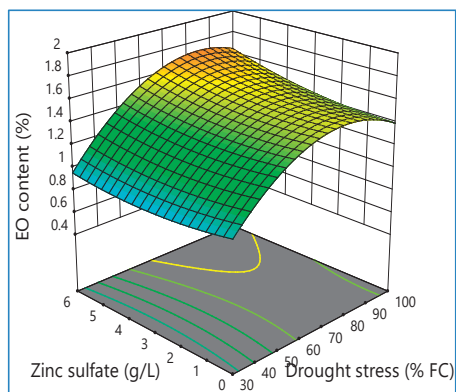


Figure 5. Response surface plot for the effect of drought stress and zinc sulfate on EO content

CONCLUSIONS

In this experiment, the effects of drought stress and the application of zinc sulfate and glycine betaine (GB) on tarragon growth and its essential oil (EO) content were modeled using the RSM technique. The fitted models for the studied traits were significant and had the necessary efficiency in predicting the responses. The effect of drought stress on the plant characteristics was significant. Increasing the intensity of drought stress caused a decrease in plant height and shoot dry weight (DW), while the EO content increased with the imposition of mild drought stress. The application of zinc sulfate increased the plant height and DW. The effect of GB on plant height and DW was insignificant, while its effect on the EO content was significant. In

general, the results showed that despite the negative effects of severe drought stress on plant growth traits, the EO content of tarragon can be optimized by applying 75 mM GB and 6 g/L zinc sulfate, under a mild drought stress conditions.

REFERENCES

- Ashraf, M. F. M. R., & Foolad, M. R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany*, 59(2), 206-216.
- Castillo-González, J., Ojeda-Barrios, D., Hernández-Rodríguez, A., González-Franco, A. C., Robles-Hernández, L., & López-Ochoa, G. R., 2018. Zinc metalloenzymes in plants. *Interciencia*, 43(4), 242-248.
- de Oliveira R. C.; Ferraz-Almeida R.; Luz J. M. Q, 2023. Performance on nutritive solution utilization by Tarragon (*Artemisia dracunculus* L.) cultivated in hydroponic, *Scientia Plena*, vol 19, no. 8, pp 1-7.
- Ekiert Halina, Joanna Świątkowska, Ewa Knut, Paweł Klin, Agnieszka Rzepiela, Michał Tomczyk, Agnieszka Szopa, 2021. *Artemisia dracunculus* (Tarragon): A Review of Its Traditional Uses, Phytochemistry and Pharmacology, *Front Pharmacol*, Vol. 12, <https://www.frontiersin.org/journals/pharmacology/articles/10.3389/>
- Farooq, M., Hussain, M., Wahid, A., & Siddique, K. H. M., 2012. Drought stress in plants: an overview. In: Aroca R (ed). *Plant responses to drought stress: From morphological to molecular features*, 1-33.
- Feiz, F. S., Hakimi, L., Mousavi, A., & Ghanbari Jahromi, M., 2019. The effects of glycine betaine and L-arginine on biochemical properties of pot marigold (*Calendula officinalis* L.) under water stress. *Iranian Journal of Plant Physiology*, 9(3), 2795-2805.
- Hafeez, B. M. K. Y., Khanif, Y. M., & Saleem, M., 2013. Role of zinc in plant nutrition-a review. *American Journal of Experimental Agriculture*, 3(2): 374-391.
- Hassanpouraghdam M. B., 2021. Foliar application of graphene oxide, Fe, and Zn on *Artemisia dracunculus* L. under salinity, *Plant Physiology and Biochemistry*, vol 80, <https://www.scielo.br/jl>.
- Hassanpouraghdam, M. B., Mehrabani, L. V., Kheirollahi, N., Soltanbeigi, A., & Khoshmaram, L., 2022. Foliar application of graphene oxide, Fe, and Zn on *Artemisia dracunculus* L. under salinity. *Scientia Agricola*, vol. 80, <https://acikerisim.afsu.edu.tr/xmlui/handle/20.500.12933/1028>
- Hassanzadeh M. K., Zahra Tayarani Najaran, Maryam Nasery, Seyed Ahmad Emami, 2016. Chapter 92 - Tarragon (*Artemisia dracunculus* L.) Oils, *Essential Oils in Food Preservation, Flavor and Safety*, Pages 813-817,
- Hernandez-Leon, S. G., & Valenzuela-Soto, E. M., 2023. Glycine betaine is a phytohormone-like plant growth

- and development regulator under stress conditions. *Journal of Plant Growth Regulation*, 42(8), 5029-5040.
- Karimi, A., Hadian, J., Farzaneh, M., & Khadivi-Khub, A., 2015. Phenotypic diversity and volatile composition of Iranian *Artemisia dracunculus*. *Industrial Crops and Products*, 65, 315-323.
- Kordali, S.; Kotan, R.; Mavi, A.; Cakir, A.; Ala, A.; Yildirim, A., 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. *Journal of Agricultural and Food Chemistry*, 53: 9452-9458. <https://doi.org/10.1021/jf0516538>.
- Koul B., Taak P., 2017, The *Artemisia* genus: a review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation. *Journal of Glycomics & Lipidomics*, Vol 7, issue 1, 1-7. 10.4172/2153-0637.1000142
- Liu, X., Liu, W., Tang, Q., Liu, B., Wada, Y., & Yang, H., 2022. Global agricultural water scarcity assessment incorporating blue and green water availability under future climate change. *Earth's Future*, 10(4), e2021EF002567.
- Majid Abdoli, Ezatollah Esfandiari, Seyed Bahman Mousavi & Behzad Sadeghzadeh, 2014. Effects of foliar application of zinc sulfate at different phenological stages on yield formation and grain zinc content of bread wheat (cv. Kohdasht), *Azarian Journal of Agriculture*, vol 1, issue 1, pp 1-17, <https://www.researchgate.net/publication/26990591>
- Mohammad, B.H.; Lamia, V.M. Data acquisition: Lamia, V.M.; Mohammad, B.H.; Nahideh, K.; Amir, S., 2023. Foliar application of graphene oxide, Fe, and Zn on *Artemisia dracunculus* L. under salinity, *Plant Physiology and Biochemistry*, Sci. agric. (Piracicaba, Braz.), vol. 80.
- Mumivand H., Ebrahimi A., Shayganfar A., & Hamid Hassaneian Khoshro, 2021. Screening of tarragon accessions based on physiological and phytochemical responses under water deficit, *Scientific Report*, vol. 8, issue 11: pp. 17839.
- Obolskiy, D., Pischel, I., Feistel, B., Glotov, N., & Heinrich, M., 2011. *Artemisia dracunculus* L. (tarragon): a critical review of its traditional use, chemical composition, pharmacology, and safety. *Journal of agricultural and food chemistry*, 59(21), 11367-11384.
- Said-Al Ahl, H. A. H., & Mahmoud, A. A., 2010. Effect of zinc and/or iron foliar application on growth and essential oil of sweet basil (*Ocimum basilicum* L.) under salt stress. *Ozean Journal of Applied Sciences*, 3(1), 97-111.
- Thakur, A., & Thakur, C. L., 2018. Evaluation of four medicinal herb species under conditions of water-deficit stress. *Indian Journal of Plant Physiology*, 23(3), 459-466.
- Umair Hassan, M., Aamer, M., Umer Chattha, M., Haiying, T., Shahzad, B., Barbanti, L. & Guoqin, H., 2020. The critical role of zinc in plants facing the drought stress. *Agriculture*, 10(9), 396.
- Yildirim, A., 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils, *Journal of Agricultural and Food Chemistry*, vol 53, 9452-9458. <https://doi.org/10.1021/jf0516538>