EFFECTS OF DIFFERENT IRRIGATION LEVELS AND MYCORRHIZAL INOCULANT ON FRENCH MARIGOLD (*Tagetes patula* L.) GROWTH AND MORPHOLOGY

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Abstract

This research aimed to determine the effects of different irrigation levels, regarding drought stress, on the growth and morphology of French marigold (Tagetes patula L.). The effect of mycorrhizal inoculant on drought stress mitigation was also studied. Apart *from growth and aesthetic characteristics, the following morphological traits were observed: plant height, number of branches, green and dry above ground mass, flower mass, flower and stem diameter and number of flowers. Plants were grown in the Faculty of Agrobiotechnical Sciences Osijek greenhouse, and the treatments started when the plants reached the appropriate vegetative growth, a three-leaf stage. The research was set as a randomized block arrangement with five replications and three irrigation treatments (a1 = 100%, a2 = 70%, a3 = 50% of the substrate water retention capacity, WRC). The mycorrhiza treatments (b1 = without mycorrhiza, b2 = with mycorrhiza) contained MycoApply® preparation added during the transplantation (2.5 mL of mycorrhiza per plant) and along with irrigation treatments (10 mL of mycorrhizal preparation was dissolved with 3.5 L of water). Research results indicate that drought stress had a negative effect on observed marigold traits. Still, the mycorrhizal inoculant had a positive effect on some observed traits, regarding marigold tolerance to drought.*

Keywords: *French marigold, irrigation, water stress, mycorrhiza*

1. INTRODUCTION

Floriculture is a specialized branch of applied botany that includes the cultivation, production and study of flowering plants. Patel et al. (2024) emphasize that floriculture has a significant role in the global horticultural industry by focusing on cultivating and trading ornamental plants and flowers. Important agroecological factors impacting floriculture production include temperature, light and water. Temperature determines the appropriate time for planting and sowing. According to Thakur and Kumar (2020), temperature regulates many of the physical and chemical processes within the plant, which control its growth and development. Light is an essential abiotic factor in flower growth. Another significant irreplaceable resource in agriculture, i.e., floricultural production, is water and its sufficiency has become one of the most critical abiotic factors. Park (2019) emphasizes that water deficit is one of the most significant challenges affecting plant growth and development. Numerous scientific studies refer to the abiotic factors directly affecting agricultural production (Yasmin and Chakraborty, 2023, Kochar et al., 2020, Marino., 2021).

Rabiya (2024) stated that efficient water management strategies are important in long-term floriculture success. The author emphasizes that water waste could be reduced by installing drip irrigation systems, collecting rainwater, reusing irrigation runoff and choosing drought-tolerant plant species, which confirms the importance of this research.

Sushma and Prasad (2022) emphasize that Tagetes spp. is a member of the Asteraceae family, which contains a large number of species, including the two most popularly cultivated, French marigold (*Tagetes patula* L.) and African marigold (*Tagetes erecta* L.).

Tagetes patula L. is an economically important flower crop cultivated for cut and loose flowers, bedding and pot plants, essential oils, and carotenoid pigments for nutraceutical and pharmaceutical purposes (Gupta et al., 2022). Since flowering species require specific agroecological conditions, their tolerance to certain abiotic stress factors can be enhanced by applying mycorrhizal preparations. Lenoir et al. (2016) stated that mycorrhizal fungi are known to improve plant growth and health by increasing tolerance to abiotic and biotic stresses. Mycorrhiza is a vital biological symbiosis that supports ecosystem stability and function through a symbiotic relationship between fungi and plant roots. Mycorrhiza can enhance the plant's capacity to absorb water and nutrient uptake, resistance to high temperatures and diseases and tolerance to salt and soil pollution. Ahammed and Hajiboland (2024) stated that arbuscular mycorrhizal fungi have potential applications in crop production, forestry management, bioremediation, and ecological restoration, serving as biofertilizers and bioprotectors, while also enhancing plant root access to nutrients, particularly phosphorus (P) and nitrogen (N).

The objective of this research is to evaluate the effects of various water stress levels and mycorrhizal treatments on plant growth, aesthetic qualities and morphological traits, including plant height, branch number, green and dry above ground mass, flower biomass, flower mass, flower and stem diameter and number of flowers. Additionally, the study aims to identify potential correlations among the observed traits to better understand the interactions between plant responses and applied treatments.

2. MATERIALS AND METHODS

The research was conducted in 2023 in Osijek-Tenja, at a greenhouse at the experimental site of the Faculty of Agrobiotechnical Sciences Osijek. The mentioned experimental site is located northeast of Osijek at GPS coordinates, approximately: 45.5530°N (northern latitude), 18.6950°E (eastern longitude). The greenhouse is equipped with an automatic side-lifting system, preventing excessive air temperature increases and ensuring stable microclimate conditions.

The plants were grown from commercial seeds, sown on April 11, with germination occurring within 3 to 4 days. The seeds were sown in a standard plastic flower pot filled with a commercial substrate, Terra Brill Substrate 4. The selected substrate was chosen due to its high-water retention capacity, compact and stable structure and pH range of 5.5 to 6.5. The substrate comprises essential nutrients, white peat (80%) and black peat (20%).

The seedlings were transplanted into standard plastic flower-growing pots upon reaching the four-leaf development stage. A total of 30 plants were used in the study. During transplantation, mycorrhizal preparation was added to the root zone of half of the plants used in the study, in the amount of 2.5 mL per plant (half of a teaspoon). The mycorrhizal preparation, MycoApply® All Purpose Soluble, which consists of 4 species of endomycorrhizal (*Glomus intraradices*, *Glomus mosseae*, *Glomus aggregatum*, *Glomus etunicatum*) and 5 species of ectomycorrhizal fungi (*Pisolithus tinctorius*, *Rhizopogon villosulus*, *Rhizopogon luteolus*, *Rhizopogon fulvigleba*, *Rhizopogon amylopogon*), was used. The preparation is concentrated, water-soluble and consists of particles smaller than 300 microns, a powdery structure, which means that it can be applied by dissolving in irrigation water.

During the initial growth and development, plants were irrigated with stale water from the local water supply system. Irrigation was performed manually using consistent water volumes, with timing and frequency guided by visual estimation of substrate moisture status. Following successful root establishment and sufficient vegetative development, irrigation and mycorrhizal treatments were initiated.

The research was conducted using a Randomized Block Design (RBD) with five replications to assess the effects of water stress and mycorrhizal inoculant application on observed marigold traits. Three irrigation treatments were applied, based on the substrate's water retention capacity (WRC), along with two mycorrhiza treatments. The irrigation treatments were as follows: a1 = 100% WRC (70 mL of water), a2 = 70% WRC (49 mL of water), a3 = 50% WRC (35 mL of water). Water retention capacity (WRC) refers to the amount of water the substrate can hold after excess water has drained. In this study, WRC was determined using the gravimetric method, while real-time substrate water content was measured with the SM150 sensor (AT Delta-T Devices Ltd, Cambridge, UK), and calibrated according to the substrate type (Fig. 1). The SM150 sensor was used to measure the volumetric water content (VWC) of the substrate before applying the abovementioned treatments. This device operates based on Frequency Domain Reflectometry (FDR) and determines soil moisture by measuring the dielectric permittivity of the substrate. The sensor provides accurate and rapid readings of water content within a range of 0–60% VWC, with an accuracy of ±3% in mineral soils (after appropriate calibration). It is factory-calibrated for common soil types and greenhouse substrates, but custom calibration can be performed for specific media. Measurements were taken at a depth of approximately 3 cm. The device is waterproof (IP68 rated), suitable for repeated field use, and compatible with handheld readers or data loggers. Its application enables continuous monitoring of soil moisture under drought conditions and supports the assessment of plant responses to water stress.

Fig. 1. The SM150 sensor (Delta-T Devices Ltd., Cambridge, UK)

Source: Lulić, I (2023)

The mycorrhizal treatments were: b1 = without mycorrhiza, b2 = with mycorrhiza.

Mycorrhizal preparation was mixed with water at a ratio of one tablespoon (10 mL) per 3.5 liters of water and applied through irrigation, based on the designated treatments. Temperature and humidity levels were monitored using a Thermo-Hygro-Logger (KLIMALOGG PRO), which recorded data at 15-minute intervals (Fig. 2).

Fig. 2. Thermometer Thermo-Hygro-Logger

Source: Lulić, I (2023)

At the end of the study, the following parameters were measured: number of branches per plant, green above ground mass, plant height, flower mass, flower diameter, number of flowers per plant, number of buds per plant, stem diameter and dry above ground mass. Statistical analysis of the data was conducted using SAS Software 9.4 (ANOVA). The significance of the investigated characteristics was determined through the LSD test ($p<0.01$, $p<0.05$). The strength of the correlation was assessed using the Roemer–Orphal correlation coefficient (r).

3. RESULTS

The average temperature during the vegetation period was 25.78 °C, while the average relative air humidity (RH) was 59.06%. The temperature values were within the optimal requirements of *Tagetes patula* L., which positively influenced growth and development. Van Iersel (2006) stated that, in his research, marigold plants grew faster at 30 °C than at 20 °C, which is consistent with the average temperatures measured in our study. The effects of irrigation treatments without mycorrhiza application on observed *Tagetes patula* L. traits are represented in Table 1.

Morphological traits	Treatments				
	a1b1	a2b1	a3b1	LSD $_{(0,05)}$	F-value
Number of branches/plant (n)	14	13	12	1.2184	6.35*
Green above ground mass (g)	12.89	12.18	10.25	2.5354	2.43
Plant height (cm)	25.38	24.60	23.40	2.8697	1.02
Flower mass (g)	1.49	1.45	1.26	0.6761	0.29
Flower diameter (cm)	32.80	29.60	24.26	10.226	1.50
Number of flowers/plant (n)	2	1	1	0.6244	2.66
Number of buds/plant (n)	2	2	2	1.3645	0.14
Stem diameter (cm)	3.64	3.44	3.28	0.521	1.01
Dry above ground mass (g)	3.08	2.87	2.60	0.5437	1.75

a1 = 100% Water retention capacity, WRC, a2 = 70% WRC, a3 = 50% WRC; b1 = without mycorrhiza; $p<0.05$ = *

Table 1. The effects of irrigation treatments on the *Tagetes patula* L. morphological traits

A statistically significant difference ($p<0.05$) in the number of branches per plant was observed among the different irrigation treatments. The highest number of branches was recorded in treatment a1b1, which contained the highest amount of irrigation water. In contrast, the lowest number of branches was observed in the treatment with the lowest water amount, the a3b1 treatment. These results follow Babaei et al. (2021) research. The authors stated that decreasing water availability had a negative effect on *Tagetes minuta* L. growth. Asrar and Elhindi (2011) also asserted that *Tagetes erecta* L. plants grown under different levels of drought stress had reduced plant height, flower diameter as well as fresh and dry weight, which is in correlation with our research. Zulfiqar et al. (2020) conducted research to determine the impact of drought stress on two marigold cultivars, by applying three irrigation treatments (T0 = 100% of field capacity, FC; T1 = 60% FC; T2 = 40% FC). They stated that drought stress significantly affected anatomical features and root vascular areas in both cultivars. The aforementioned research confirms the negative effect of drought stress on marigold growth and quality. The effects of irrigation treatments with mycorrhiza on *Tagetes patula* L. are represented in Table 2.

Morphological traits	**Treatments**					
	a1b2	a2b2	a3b2	$LSD_{(0,05)}$	$LSD_{(0,01)}$	F-value
Number of branches/plant (n)	15	14	11	1.4008	1.8412	8.12**
Green above ground mass (g)	12.16	11.77	10.06	4.2763	5.0234	0.65
Plant height (cm)	25.84	26.20	21.00	4.1704	4.8277	4.60*
Flower mass (g)	1.63	1.46	1.14	1.0637	1.3664	0.53
Flower diameter (cm)	34.33	27.24	22.28	16.973	20.538	1.21
Number of flowers/plant (n)	2	1	1	1.0966	1.2508	1.37
Number of buds/plant (n)	2	2	3	2.4131	2.6827	0.59
Stem diameter (cm)	2.90	3.70	3.36	0.7988	0.9662	2.40
Dry above ground mass (g)	2.80	2.83	2.60	0.9705	1.0710	0.17

a1 = 100% Water retention capacity, WRC, a2 = 70% WRC, a3 = 50% WRC; b2 = with mycorrhiza; $p<0.05$ = *; $p<0.01$ **

Table 2. The effect of irrigation treatments (a) with mycorrhiza (b2) on the *Tagetes patula* L. morphological traits

A highly significant effect ($p<0.01$) of irrigation and mycorrhiza treatment on the number of branches per marigold plant was observed. The highest number of branches was recorded in the a1b2 treatment, while a significantly lower number of branches was observed in the treatment with the lowest irrigation water amount, a3b2. Irrigation and mycorrhiza treatments had a statistically significant effect ($p<0.05$) on the plant height. Notably, the greatest plant height was recorded in the a2b2 treatment. That is contrary to Jankowska and Andrzejak (2017) research. The authors stated that mycorrhizal inoculation had no effect on plant height on *Tagetes patula* L. and *Salvia splendens* Sellow ex J.A. Schultes. Onwards, similarly to our result in a2b2 treatment, regarding plant height, Asrar and Elhindi (2011) research resulted in higher plant height throughout mild, moderate and severe drought stress with mycorrhiza, unlike in the same drought stress levels without mycorrhiza. The highest number of buds was recorded in the a3b2 treatment, although there was no statistical significance. That suggests that marigold exhibits strong resistance to water stress concerning this variable.

Irrigation and mycorrhiza treatments did not have a statistically significant effect on stem diameter. However, it is noteworthy that the highest values were observed in treatments indicating mild (a2b2) and severe water stress (a3b2), which is in correlation with Wu and Zou (2017) assertion that mycorrhizal inoculation can modulate morphological adaptation to enhance drought tolerance of the plant. Similar results were shown in Abdel-Salam et al. (2018) research on *Rosa x damascena* Mill. plants. Authors stated that mycorrhizal fungi colonization enhances growth, flower number and adaptation of plants under high levels of drought stress.

A correlation analysis was performed to examine the relationships between the observed traits (Table 3). Statistically significant values are marked in red.

	NB	GAM	PH	FM	FD	NF	NB	SD	DAM
Number of branches/plant (n)	1.000								
Green above ground mass (g)	0.358	1.000							
Plant height (cm)	0.435	-0.118	1.000						
Flower mass (g)	0.027	0.149	-0.186	1.000					
Flower diameter (cm)	0.012	0.150	-0.246	0.892	1.000				
Number of flowers/plant (n)	0.124	0.288	-0.127	0.336	0.534	1.000			
Number of buds/plant (n)	-0.122	0.303	-0.327	-0.292	-0.115	-0.142	1.000		
Stem diameter (cm)	-0.147	0.371	-0.215	-0.053	-0.085	-0.337	0.357	1.000	
Dry above ground mass (g)	0.251	0.560	-0.233	0.082	0.187	0.270	0.374	0.159	1.000

NB-number of branches/plant (n); GAM-green above ground mass (g); PH-plant height (cm); FM-flower mass (g); FD-flower diameter (cm); NF-number of flowers/plant (n); NB-number of buds/plant (n); SD-stem diameter (cm); DAM-dry above ground mass (g)

Table 3. Correlations among the observed morphological traits ($p < 0.05$)

A weak positive correlation was observed between stem diameter and green above ground biomass (r = 0.371), as well as between dry above ground biomass and number of buds (r = 0.374). A moderate positive correlation was found between plant height and number of branches (r = 0.435). A strong positive correlation was observed between flower diameter and number of flowers (r = 0.534), and between dry and green above ground biomass (r = 0.560). A very strong positive correlation was identified between flower mass and flower diameter (r = 0.892).

CONCLUSIONS

The research results indicate a negative effect of drought stress on the growth and quality of *Tagetes patula* L. in terms of morphological traits. The highest values of the observed morphological traits were measured in the control irrigation treatment, i.e., with the highest irrigation rates (a1 = 100% WRC, 70 mL of water). The positive effects of mycorrhiza on the morphological parameters of *Tagetes patula* L. were observed in the a3b2 treatment (50% WRC with applied mycorrhiza) in terms of the number of buds and stem diameter, which emphasizes that *Tagetes patula* L. enhances tolerance to drought stress when a mycorrhizal inoculant is applied along with irrigation water.

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