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Research paper

Improvement of grain and oil yield-related traits of marigold (*Calendula officinalis* L.) in response to exogenous application of spermidine under water-deficit and weed infestation conditions

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Abstract
A two-year experiment was performed to evaluate the efficacy of spermidine on the field performance of marigolds under water deficit and weed infestation conditions in the 2020 and 2021 growing seasons. The experiment was arranged as the split split-plot design based on a randomized complete block design with three replications. Irrigation intervals (I_1, I_2, and I_3: irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively) were assigned to main plots, weed infestation (weed-free and weed infestation) was assigned to the subplots, and foliar applications of spermidine (1 mM) (4-6th leaf stages and pre-blooming stage) were allocated to sub-subplots. The percentage of ground cover, leaf water content, leaf area index, and oil and essential oil yield decreased but leaf temperature increased in the I_2 and I_3 and also in the weed infestation conditions. This reaction led to a decline in the grain yield of the marigold plants. Foliar application of spermidine increased the percentage of ground cover, leaf water content, leaf area index, and oil and essential oil yield and decreased the leaf temperature in weed-free conditions. In conclusion, spermidine positively affected the grain and oil yield of marigold plants under water-stress conditions, when the weeds were absent.

Keywords: essential oil; grain yield; polyamine; weed infestation

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Introduction

Drought is one of the most critical abiotic stresses that limit the growth and development of plants. (Shen *et al.* 2014). The physiological response of plants to drought varies by species, nutrients, soil type, and climate. The drought decreases the water potential, transpiration rate, and relative water content of plant leaves (Farooq *et al.* 2009), and increases the reactive oxygen species (ROS), which leads to the peroxidation of membrane lipids (Singh *et al.* 2014).

Weeds are one of the most important biological constraints to crop production. Weeds have the potential to cause yield loss twice as much as animal pests and pathogens (Gharde *et al.* 2018). They reduce crop yield by competing for water, nutrients, space, and light (Soltani *et al.* 2017). Weeds have more variable characteristics than crops because they have not been selected for some favorable traits such as lack of seed dormancy, uniform growth, and high yields, therefore, they are more adaptable to stress conditions than crop plants (Korres *et al.* 2016).

Polyamines such as putrescine, spermine, and spermidine are present in all living cells (Handa *et al.* 2018). Their indispensable roles in adaptive responses to various abiotic stresses are becoming commonly known (Handa *et al.* 2018). The exogenous application of polyamines has indicated their possible role in the adaptation of plants to several abiotic stresses (Alcazar *et al.* 2012; Yin *et al.* 2014). Research on polyamines has increased significantly in recent years, providing information on how they can improve plant development processes (Satish *et al.* 2016).

Marigold (*Calendula officinalis* L.) has a long history of use in medicine and ornamental horticulture (Joly *et al.*, 2013). In water-deficit stress conditions, it is important to optimize the marigold essential oil yield and quality (Taherkhani *et al.* 2011). Weed infestation is also one of the factors that reduce yield and yield components as well as the percentage and yield of oil in

This study focused on the effect of the exogenous application of spermidine on marigold plants under water-deficit and weed stresses with respect to some physiological characteristics.

Materials and Methods

Field experiment

To investigate the physiological changes of the marigold (*Calendula officinalis* L.) under different irrigation intervals and weed-free conditions in response to spermidine, a field experiment was conducted as a split-split-plot design based on the randomized complete block design with three replications during the growing seasons of 2020 and 2021 at the Research Station of the University of Tabriz, Iran (Longitude 46°17′E, Latitude 38°05′N, and Altitude 1360 m above sea level). Irrigation intervals (irrigation after 40, 80, and 120 mm evaporation from the class A pan) were assigned to main plots, weed infestation (weed free and weed infestation) was assigned to the subplots, and foliar applications of spermidine (4-6th leaf and pre-blooming stages) were allocated to sub-subplots. Soil samples were taken from the 0 to 30 cm depths and their physical and chemical properties were measured (Table 1).

Each experimental plot consisted of six rows of 4 m in length. The distances within the rows and between the rows were 25 cm and 50 cm, respectively. Seeds were treated with 2 g kg^{-1} Benomyl and sown in late April 2020 at a depth of about 1 cm. Immediately after sowing, the plots were irrigated regularly until the seedling establishment, and then the irrigation intervals were applied. In the weed-free plots, frequent hand weeding was

Table 1. The soil physical and chemical properties of the experimental field

Depth (cm)	Soil type	Sand (%)	Clay (%)	Silt (%)	EC (dS m^{-1})	pH	OC (%)	Fe (mg kg^{-1})	K (mg kg^{-1})	P (mg kg^{-1})	N (mg kg^{-1})
0-30	Sandy-loam	74	12	14	2.92	8	0.37	2.6	255	4.9	0.04

accomplished during plant development. Spraying up to well-wetting of the plants was carried out at the 4-6th leaf s and pre-blooming stages. All characteristics were measured during the grain-filling period.

Measurements

Percentage of ground cover: The percentage of ground cover (PGC) was determined by observing the canopy via a 100-piece wooden frame (50 cm × 50 cm) when the area contained more than 50 percent crop greenery.

Leaf area index: Four plants were selected at random from the center rows of each plot and all leaves were counted and removed from the branches. A leaf area meter (LI-COR, Model Li-3100C Area Meter, USA) was utilized to determine the leaf size. The formula defined by Watson (1947) was used to get the leaf area index (LAI):

$$LAI = \text{Leaf area} / \text{Ground area}$$

Leaf water content: In each plot, the leaves of five plants were harvested and weighed. The leaves were then dried for 24 hours at 75 degrees Celsius and reweighed. The leaf water content (LWC) was measured according to González and González-Vilar (2001):

$$LWC= ((FW-DW) / FW) \times 100$$

Where FW represents the leaves' fresh weight, and DW their dry weight.

Leaf temperature: Before irrigation, leaf temperature (upper, middle, and lower leaves) was directly monitored on a plant from each plot using an infrared thermometer (TES-1327).

Grain yield: The grain yield per unit area was calculated by harvesting the mature grains from 1 m^2 of the middle of each plot in both years.

Seed oil: The seeds of the plant were crushed and ground into a fine powder. Oil was extracted from each seed sample (30 g) as a percentage of seeds weight using a Soxhlet extractor at 70° C for 6 hours using ethanol as the extraction solvent. The solvent was evaporated under vacuum by a rotary evaporator at 30 °C, and finally, the pure oil was collected (Folch *et al.* 1957).

Essential oil: Fresh flower heads of five plants from each plot were detached at the flowering stage. Directly after harvest, flower heads were dried in a room at about 25–26 ºC for three days and weighed. Each sample was then mixed with 300 ml of distilled water and the essential oil content was measured through hydro-distillation in a Clevenger apparatus for three hours (Clevenger 1928).

Statistical analysis

Analysis of variance of the data and consequently the comparison of means by Duncan's multiple range test ($p \leq 0.05$) was performed using SAS software. Figures were created using Microsoft Excel 2016 software.

Results

The results of the combined analysis of variance over two years for grain yield, oil characteristics, and measured physiological traits of the marigold affected by irrigation, weeds, and spermidine are shown in Tables 2 and 3. There was a significant difference between years for LWC, leaf temperature, and percentage of essential oil. Also, the interaction of year × irrigation for PGC, percentage of essential oil, percentage of oil, the interaction of year × weed for PGC, leaf temperature, percentage of oil, the interaction of year × irrigation × weed for the leaf temperature, essential oil yield, percentage of oil, the interaction of year × spermidine for PGC, LAI, LWC, percentage of oil, the interaction of year × irrigation × spermidine for the percentage of oil, the interaction of year × weed × spermidine for the percentage of oil, and the interaction of year × irrigation × weed × spermidine for LAI, leaf temperature, grain yield, essential oil yield, and percentage of oil was significant. A significant irrigation × weed interaction for PGC, LAI, LWC, and grain yield, irrigation × spermidine interaction for all traits, and weed × spermidine interaction for LAI was also observed.

Percentage of ground cover

PGC of the marigold decreased with increasing water-deficit stress. Weed infestation also decreased the PGC of plants (Table 4). Spermidine application, especially in the pre-blooming stage, increased the PGC of marigold plants under all irrigation treatments (Figure 1).

Leaf area index

When no spermidine was used and under weed-free conditions, the LAI in the optimum watering conditions was significantly higher than the moderate and severe water-deficit stresses (Figure 2), however, weed infestation decreased the LAI of the plants and the amount of this decrease was higher in the severe water deficit-stress conditions. In the weed-free treatments, foliar application of spermidine in both stages increased LAI under moderate and

Table 2. Combined analysis of variance of the data for the percentage of ground cover, leaf area index, leaf water content, leaf temperature, and grain yield of marigold affected by irrigation, weeds, and spermidine.

Source of variation	df	Mean squares				
		Percentage of ground cover	Leaf area index	Leaf water content	Leaf temperature	Grain yield
Year (Y)	1	0.89	0.02	58.21*	2.65*	420.32
Replication (R/Y)	4	2.35	0.002	7.32	0.21	532.7
Irrigation (A)	2	310.05**	6.30**	657.08**	78.35**	77534.2**
Y × A	2	3.35*	0.001	12.21	0.77	54.1
Error 1	8	0.68	0.03	7.47	0.28	129.7
Weed infestation (B)	1	54.05	2.18**	324.11**	8.21	5635.8**
Y × B	1	2.32*	0.001	11.32	16.11**	276.7
A × B	2	21.10**	1.62**	15.47**	0.65	3541.6**
Y × A × B	2	0.55	0.008	7.47	19.44 **	86.8
Error 2	12	0.45	0.06	3.87	0.18	568.6
Spermidine (C)	2	112.10*	0.88	88.54	66.04**	21115.2**
Y × C	2	2.68*	0.12**	22.32**	0.38	463.2
C × A	4	6.84**	1.44**	8.47**	6.47*	3542.4**
Y × C ×A	4	1.31	0.012	3.08	0.50	54.2
B × C	2	1.65	2.065**	15.45	3.32	852.3
Y × B × C	2	1.48	0.045	3.06	0.47	524.5
A × B × C	4	2.87	1.56**	3.44	0.66	2125.7**
Y × A × B × C	10	1.32	0.041*	1.45	1.54**	725.1**
Error 3	48	0.66	0.016	2.26	0.32	158.6
CV (%)	-	0.55	4.42	2.65	2.49	13.75

*, **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 3. Combined analysis of variance of the data for the percentage of essential oil, essential oil yield, percentage of oil, and oil yield of marigold affected by irrigation, weeds, and spermidine.

Source of variation	df	Mean squares			
		Percentage of essential oil	Essential oil yield	Percentage of oil	Oil yield
Year (Y)	1	0.465*	1.87	0.006	34.25*\
Repeat (R/Y)	4	0.021	4.57	0.035	0.32
Irrigation (A)	2	130.35**	6058.47**	3.335**	127.08**
Y × A	2	0.74**	3.49	0.032*	0.41
Error 1	8	0.08	2.66	0.006	0.47
Weed (B)	1	0.083	568.54**	0.055	8.11**
Y × B	1	0.041	4.36	0.032*	1.32
A × B	2	0.065	4.38	0.010	1.47
Y × A × B	2	0.044	8.30 **	0.035**	1.04
Error 2	12	0.06	4.54	0.005	1.07
Spermidine (C)	2	23.41**	750.63**	4.810*	12.54**
Y × C	2	0.07	8.46	0.065**	0.32
C × A	4	3.17**	39.31**	2.84**	1.47**
Y × C ×A	4	0.05	6.47	0.031**	0.18
B × C	2	0.02	7.31	0.067	0.45
Y × B × C	2	0.077	10.01	0.048**	0.16
A × B × C	4	1.06 **	5.32	0.087	20.54 **
Y × A × B × C	10	0.03	17.34**	0.025**	0.25
Error 3	48	0.08	5.42	0.006	0.47
CV (%)	-	0.49	3.45	2.74	12.15

*, **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 4. Percentage of ground cover and leaf water content of marigold for the combination of irrigation intervals and weed infestation conditions.

Irrigation	Weed	Percentage of ground cover	Leaf water content (%)
I_1	Weed free	94.35 a	85.18 a
	Weed infested	64.47 b	77.30 b
I_2	Weed free	70.47 b	62.68 c
	Weed infested	58.33 c	56.08 d
I_3	Weed free	57.04 c	33.23 e
	Weed infested	41.67 d	21.44 f

I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Different letters in each column indicate significant difference at $p \leq 0.01$.

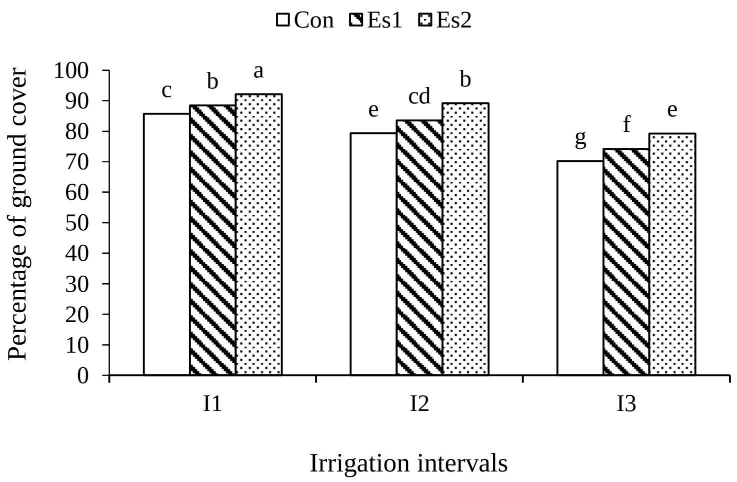

Figure 1. Percentage of ground cover of marigold for the combination of irrigation intervals and spermidine concentrations.

I_1, I_2, I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Con, Es1, and Es2: Control, application of spermidine at 4-6th leaf stage, and pre-blooming stage, respectively; Different letters indicate a significant difference at $p \leq 0.01$.

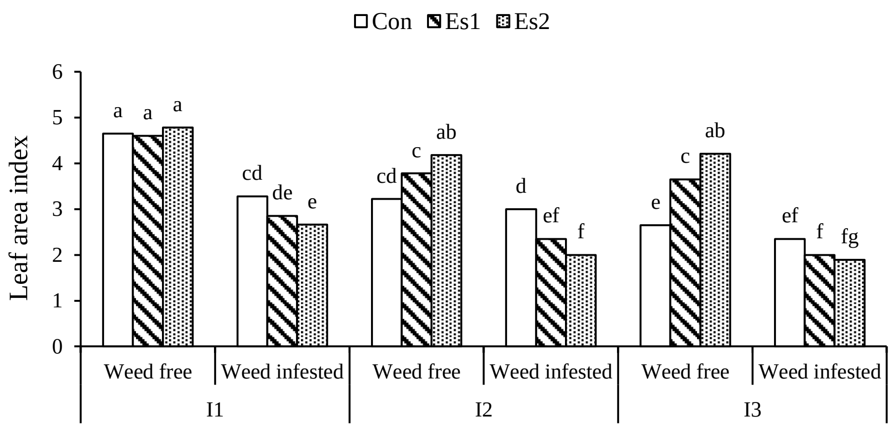

Figure 2. Leaf area index of marigold for the combination of irrigation intervals, weed infestation conditions, and spermidine concentrations.

I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Con, Es1, and Es2: Control, application of spermidine at 4-6th leaf stage, and pre-blooming stage, respectively; Different letters indicate a significant difference at $p \leq 0.01$.

severe water-deficit stresses. However, the use of spermidine at the pre-blooming stage in the weed-infested treatments significantly decreased the LAI of the marigold plants at both optimum and moderate water-deficit conditions (Figure 2).

Leaf water content

LWC also decreased with increasing water deficit and the lowest amount was obtained from the severe water-deficit stress conditions (Table 4). Plants grown under weed-infested conditions had lower leaf water content compared with the weed-free treatments (Table 4). Application of spermidine at the 4-6th leaf stage had no significant difference with the control under optimum and moderate water-deficit stress, but under severe stress increased LWC of the marigold plants (Figure 3). However, the use of spermidine at the pre-blooming stage caused a significant increase in LWC under all irrigation conditions.

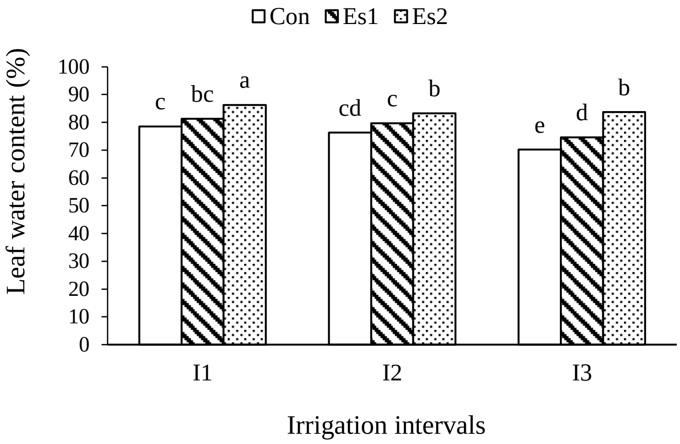

Figure 3. The leaf water content of marigold for the combination of irrigation intervals and spermidine concentrations. I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Con, Es1, and Es2: Control, application of spermidine at 4-6th leaf stage, and pre-blooming stage, respectively; Different letters indicate a significant difference at $p \leq 0.01$.

Leaf temperature

In both years, the leaf temperature of marigold plants increased with decreasing water availability (Table 5). In the second year, in the weed-free conditions, the marigold LT under moderate water deficit was significantly higher than that under normal irrigation, while in the first year, there was no significant difference between these conditions (Table 5). The highest LT was 27.67 and 30.44 ˚C in 2020 and 2021, respectively, which belonged to the plants under severe water stress with weed infestation (Table 5). Foliar application of spermidine at the 4-6th leaf stage did not alter the temperature of the leaf under normal irrigation and moderate stress conditions (Figure 4). However, in the severe water stress, LT was significantly decreased by spermidine application, particularly in the pre-blooming stage.

Table 5. The leaf temperature of the marigold for the combination of irrigation intervals and weed infestation conditions averaged over two years.

Irrigation	Weed	2020	2021
I_1	Weed free	19.35 de	17.18 e
	Weed infested	22.47 c	22.30 c
I_2	Weed free	20.28 d	20.68 d
	Weed infested	24.33 b	25.08 b
I_3	Weed free	24.04 b	25.23 b
	Weed infested	27.67 a	30.44 a

I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Different letters in each column indicate significant differences at $p \leq 0.01$.

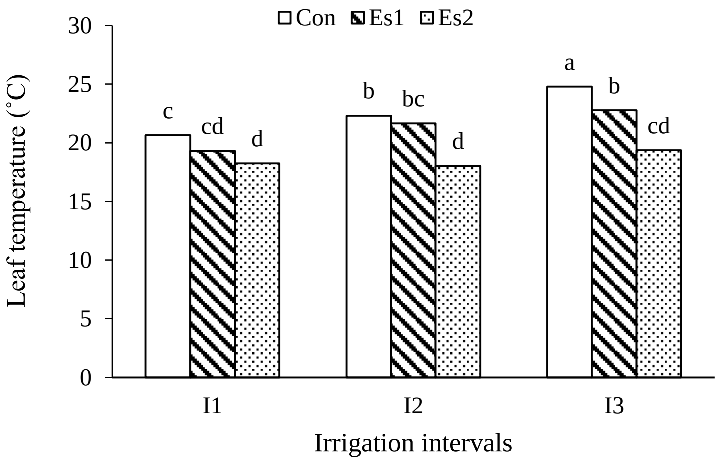

Figure 4. Leaf temperature of marigold for the combination of irrigation intervals and spermidine concentrations.
I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Con, Es1, and Es2: Control, application of spermidine at 4-6th leaf stage, and pre-blooming stage, respectively; Different letters indicate a significant difference at $p \leq 0.01$.

Grain yield

The results presented in Figure 5 showed that marigold's grain yield decreased with increasing water deficit, especially at severe stress conditions. Weed infestation also decreased the grain yield at all irrigation conditions. On the other hand, the use of spermidine caused an increase in the grain yield of the plants in weed-free conditions. In contrast, the foliar application of spermidine in the weed-infested plots reduced the grain yield of the marigold plants.

Percentage of essential oil

In the marigold plants grown in moderate water-deficit stress conditions, the percentage of essential oil increased significantly in the weed-infested plots, but it decreased by increasing the stress intensity at both weed-free and weed-infested conditions. The percentage of essential oil also decreased in plants infested by weeds. In the weed-free plots, foliar spraying of spermidine increased the percentage of essential oil under moderate and severe water-deficit stress conditions, however, in the weed-infected plots, the use of spermidine decreased the percentage of essential oil at the 4-6th leaf stage in all irrigation conditions (Figure 6). weed-free plots. However, in the first year of the experiment, weed infestation under normal irrigation conditions did not have a significant effect on the yield of essential oil (Table 6). Under normal irrigation and drought stress conditions, the use of spermidine in both the 4-6th leaf stage and the pre-blooming stage had a significant effect on the increase of essential oil yield in the marigold plants (Figure 7). In plants exposed to water-deficit stress, the application of spermidine in the pre-blooming stage had a more pronounced effect on

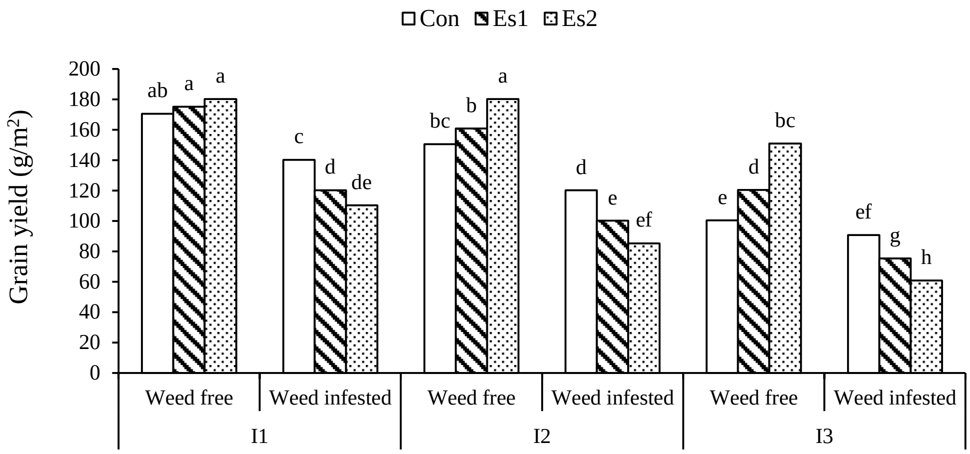

Figure 5. Grain yield of marigold for the combination of irrigation intervals, weed infestation conditions, and spermidine concentrations.

I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Con, Es1, and Es2: Control, application of spermidine at 4-6th leaf stage, and pre-blooming stage, respectively; Different letters indicate a significant difference at $p \leq 0.01$.

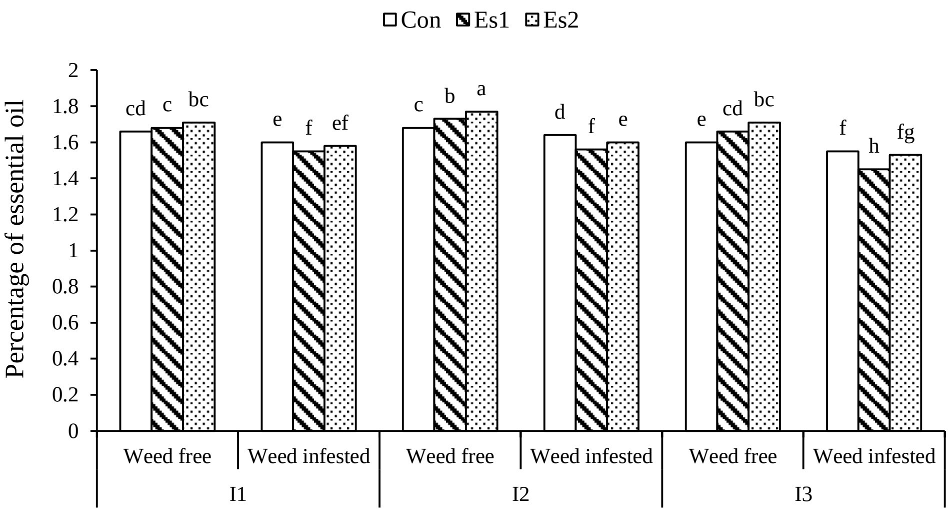

Figure 6. Percentage of the essential oil of the marigold seeds for the combination of irrigation intervals, weed infestation conditions, and spermidine concentrations.

I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Con, Es1, and Es2: Control, application of spermidine at 4-6th leaf stage, and pre-blooming stage, respectively; Different letters indicate a significant difference at $p \leq 0.01$

increasing the essential oil yield of the marigold (Figure 7).

Oil percentage

The oil percentage of the marigold plants grown under severe water-deficit stress was significantly reduced, but this reduction was not significant in moderate stress conditions. Foliar spraying of spermidine on the plants under water-deficit stress caused an increase in the oil percentage, which was higher in the pre-blooming stage (Figure 8).

Oil yield

Oil yield decreased in the marigold plants under drought stress. The reduction of oil yield in plants under drought stress was intensified by the weed infestation. In the weed-free conditions, application of spermidine at the 4-6th leaf stage and pre-blooming stage increased oil yield in the marigold seeds but this increase was not significant at the 4-6th leaf stage under severe water-deficit stress conditions. In the plants infested by weeds, the use of spermidine mostly caused a significant decrease in the oil yield. The lowest amount of oil yield in the marigold seeds was obtained in the plants under severe water stress and infested by weeds using spermidine at the 2-4th leaf stage (Figure 9).

Discussion

The drop in PGC observed due to water stress and weed infestation may be attributable to plant competition for water and nutrients. Limiting the availability of water in the soil during the vegetative stage affects the development of plant height and biomass (Araujo Rufino *et al.* 2018). Under dry conditions, reduced turgor pressure and a slow photosynthetic rate primarily restrict leaf development (Keyghobadi *et al.* 2020), thus PGC is reduced. Weed infestation also reduced the PGC of the marigold plants. Researchers believe that at higher weed densities, more leaves are lost due to crop shading, resulting in a reduction in the PGC in the field (Singh *et al.* 2022). Enhancing the PGC of the marigold with spermidine may be associated with this polyamine's growth and development-regulating properties. Spermidine is reported to increase the percentage of green cover of the plant by increasing the LAI and the number of leaves (Osman and Salim 2016). The application of spermidine also increases the fresh and dry weight of the plants under stress conditions (Tian *et al.* 2022). It seems that the role of polyamines in increasing plant growth has been related to their antioxidant effect, helping cation-anion balance, or acting as a source of nitrogen (Collado-Gonzalea *et al.* 2021).

Table 6. Essential oil yield of marigold for the combination of irrigation intervals and weed infestation conditions averaged over two years.

Irrigation	Weed	2020	2021
I_1	Weed free	4.68 a	3.78 a
	Weed infested	4.29 ab	3.01 b
I_2	Weed free	3.46 b	2.36 c
	Weed infested	2.72 c	1.82 d
I_3	Weed free	2.59 c	1.71 d
	Weed infested	1.33 d	1.16 e

I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Different letters in each column indicate significant difference at $p \leq 0.01$.

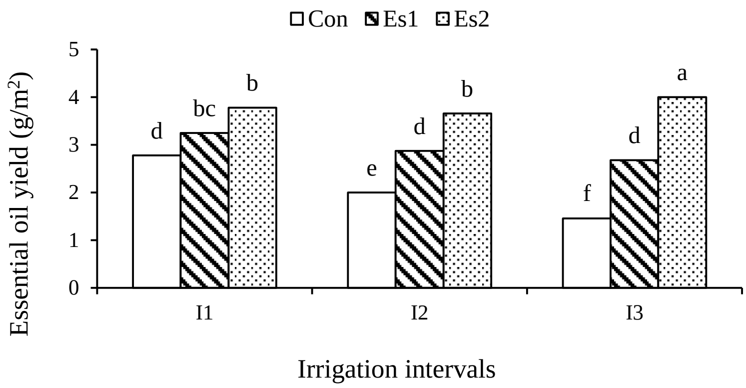

Figure 7. The essential oil yield of the marigold for the combination of irrigation intervals and spermidine concentrations.
I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Con, Es1, and Es2: Control, application of spermidine at 4-6th leaf stage, and pre-blooming stage, respectively; Different letters indicate a significant difference at $p \leq 0.01$.

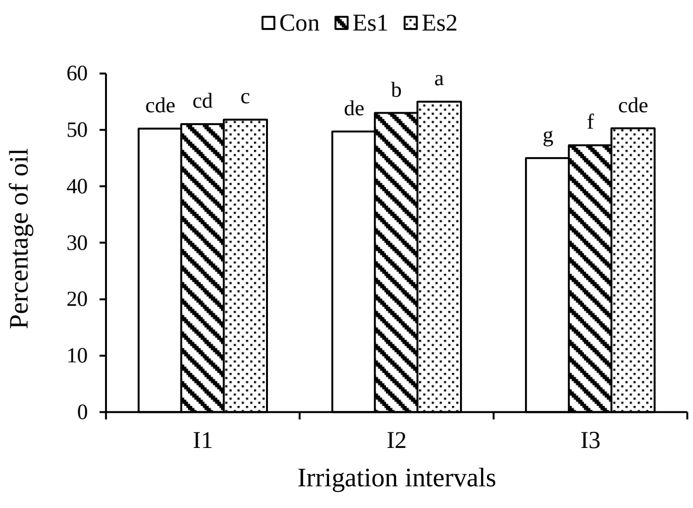

Figure 8. The oil percentage of the marigold seeds for the combination of irrigation intervals and spermidine concentrations.
I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Con, Es1, and Es2: Control, application of spermidine at 4-6th leaf stage, and pre-blooming stage, respectively; Different letters indicate a significant difference at $p \leq 0.01$.

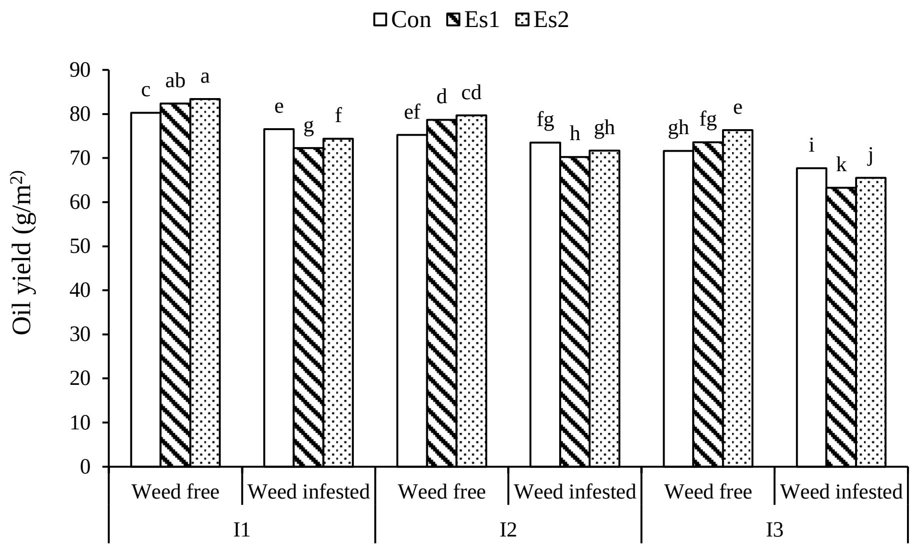

Figure 9. Oil yield of marigold for the combination of irrigation intervals, weed infestation conditions, and spermidine concentrations.
I_1, I_2, and I_3: Irrigation after 40, 80, and 120 mm evaporation from the class A pan, respectively; Con, Es1, and Es2: Control, application of spermidine at 4-6th leaf stage, and pre-blooming stage, respectively; Different letters indicate a significant difference at $p \leq 0.01$.

The LAI of marigolds decreased under limited irrigation conditions. Leaf area is often characterized by specific adaptations and is known to be sensitive to drought as well as weed infestation stress conditions. The reduction in leaf area is an adaptive response to a water deficit to avoid a higher transpiration rate and reduce radiation surfaces (Hayatu *et al*. 2014). Torabian *et al.* (2018b) in common bean and Rostami Ajirloo *et al.* (2019) in maize plants showed that LAI, leaf relative water content, shoot dry weight, and grain yield decreased when the drought stress increased. Increased leaf area depends on leaf turgor, temperature, and growth factors, which are affected by drought (Kapoor *et al.* 2020). Inadequate cell turgor pressure, reduced cell division, decreased stomatal conductance or closed stomatal, and reduced photosynthesis and plant growth, are considered secondary factors in reducing leaf area (Martínez *et al* 2004).

Spermidine application, especially at the pre-blooming stage, alleviated the adverse effect of water-deficit stress in weed-free conditions. Polyamines are implicated in numerous physiological processes, including cell growth and development and the response to various environmental stressors (Gill and Tuteja 2010; Chen *et al.* 2019). Polyamines provide the energy needed for cell growth by maintaining cell inflammation and increasing osmolality in the plant (Chen *et al.* 2019). Torabian *et al.* (2018b) reported that exogenous application of spermidine affected leaf characteristics and increased the growth of common beans under water deficit conditions. A decrease in LAI in the weed-infested plots by the spermidine application showed that the use of growth regulators will be more beneficial for weeds. Foliar application of spermidine in the weed-infested treatments strengthened the weed growth and increased their competitiveness with marigold plants, eventually reducing the LAI of the marigold.

Reduction in LWC due to drought stress and weed infestation in this study may depend on the plant vigor reduction (Nohong and Nompo 2015; Zhang *et al.* 2021). LWC of the spermidine-treated plants increased in the normal conditions and also the moderate and severe water-deficit stresses. Spermidine has a crucial role during environmental stress, particularly drought, and it can accumulate in reaction to a lack of water (Satish *et al.* 2018). An increase in LWC following the application of spermidine resulted from an increase in plant accessibility to water. Due to the partial closing of stomata (Mehri *et al.* 2018), reduction in transpiration, increase in root penetration, and suppression of shoot growth, water availability may be increased by conserving plant moisture (Yin *et al.* 2014). Torabian *et al.* (2018a) concluded that exogenous spermidine significantly improved leaf relative water content and better maintained the chlorophyll content, which demonstrates that spermidine application delayed leaf senescence in common beans under drought stress.

Leaf temperature can reflect the physiological activity of plants under stress conditions (Ahmadi-Lahijani and Emam 2016). Increasing the leaf temperature due to weed infestation and water-deficit stress is possibly related to a decrease in relative water content under these stressful conditions, resulting in a decrease in stomatal conductance and transpiration (Zhang *et al.* 2019). There is a significant negative association between leaf temperature and LWC, and hormonal applications under environmental stress, enhance LWC and decrease leaf temperature.

Increasing leaf temperature and decreasing LWC and PGC as a result of both water-deficit stress and weed infestation led to substantial decreases in grain yield per unit area. The enhanced grain yield of spermidine-sprayed plants, particularly in the pre-blooming stage, in the weed-free conditions, can be attributed to increases in the LAI and LWC of these plants. Radhakrishnan and Lee (2013) have demonstrated that polyamine sprays improve the reproductive characteristics of soybean pods and seeds under stress conditions. Under weed-infested conditions, the use of hormones makes weeds use hormones more effectively and increase their competitive power to cause more damage to the growth and yield of crops. Therefore, it is not recommended to use hormones under weed-infested conditions. Similar results were reported from the investigation about the effect of salicylic acid on corn under weed-free and infested conditions (Porheidar Ghafarbi *et al.* 2019).

When the plant is subjected to severe drought stress, most of the photosynthetic materials are used to produce osmotic regulator compounds such as proline, glycine betaine, and sugar compounds like sucrose, fructose, and fructan to reduce the cellular water potential (Blum 2017). Under these conditions, the percentage of essential oil and, accordingly, the yield of the essential oil decreases. Also, the decrease in essential oil percentage in drought stress can be due to the disruption of photosynthesis and production of carbohydrates, which suppress plant growth in drought conditions (Flexas and Medrano 2002). In this study, weed infestation also caused a decrease in the percentage and yield of the essential oil in marigold plants. Field weeds consume nutrients (Mohammad Doost-Chaman Abad 2010) and compete better with main crops, resulting in reduced essential oil yields. Weeds can cause depletion of nutrients from the soil, which reduces the amount of essential oil yield. The increase in the percentage and yield of essential oil with the use of polyamines under weed-free conditions can be related to the effect of these compounds on the mechanisms related to terpenoids and other hydrocarbons and the effect of these compounds in the production of isoprenoids (Gharib 2006). The foliar spraying of putrescine, spermidine, and spermine polyamines on chamomile and sweet marjoram medicinal plants under stress increased the vegetative performance and the amount of essential oil in these plants (Ali *et al.* 2007).

Oil is one of the main components in marigold seeds and its amount can be changed depending on the environmental conditions and genetic structure. There are conflicting reports about the effect of drought stress on oil percentage. Reducing the transfer of photosynthetic materials to seeds can be one of the most important reasons for the reduction of oil yield under drought-stress conditions (Nakagawa *et al.* 2018). Hussain *et al.* (2018) reported a reduction in the oil yield of sunflowers butthis reduction was lower than the reduction in grain yield. However, in severe drought during flowering and germination the reduction in oil yield was more than that of the grain yield. This is probably due to the reduced seed oil content of sunflowers. They also reported that low soil moisture combined with high temperatures during the flowering and seed-filling stages significantly reduced grain yield and oil quality. Research results indicate that spermidine appears to increase oil yield by improving the oil source-storage relationship, and increasing leaf area durability and seed filling time. Deotale *et al.* (2016) showed that 100 ppm putrescine applied to leaves significantly increased the soybean oil (*Glycine max* (L.) Merr.) content. On the other hand, spermidine foliar spraying has probably provided the necessary time for oil storage in the seeds by increasing the effective seed-filling period and increasing the yield of the oil produced per unit area (Li *et al.* 2016).

Conclusion

In the marigold plants under mild and severe water-deficit stresses and weed infestation conditions, LWC, LAI, PGC, grain yield, oil yield, and essential oil yield decreased, whereas leaf temperature increased. A foliar spray of spermidine (1 mM) reversed the drought stress damage. Therefore, the physiological repercussions of water-stress-mediated changes in the plant defense system, especially in conjunction with spermidine catabolism, are worthy of study.

Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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بهبود صفات مربوط به عملکرد دانه و روغن همیشه بهار (*Calendula officinalis* L.) در پاسخ به کاربرد خارجی اسپرمیدین تحت شرایط کم‌آبی و آلودگی علف‌های هرز

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چکیده

به منظور بررسی اثر اسپرمیدین بر عملکرد مزرعه‌ای همیشه‌بهار در شرایط کم‌آبی و آلودگی علف‌های هرز آزمایشی دو ساله در سال‌های ۲۰۲۰ و ۲۰۲۱ انجام شد. آزمایش به صورت طرح اسپلیت اسپلیت پلات بر پایه طرح بلوک کامل تصادفی با سه تکرار پیاده شد که در آن فواصل آبیاری (I_1، I_2 و I_3: به ترتیب آبیاری پس از ۴۰، ۸۰ و ۱۲۰ میلی متر تبخیر از تشتک کلاس A) به کرت‌های اصلی، آلودگی علف‌های هرز (عاری از علف‌های هرز و آلوده به علف‌های هرز) به کرت‌های فرعی و محلول‌پاشی اسپرمیدین (یک میلی مولار) (در مرحله ۴ تا ۶ برگی و پیش از غنچه‌دهی) به کرت‌های فرعی-فرعی اختصاص یافت. در سطوح I_2 و I_3 و نیز در شرایط آلودگی به علف‌های هرز درصد پوشش زمین، محتوای آب برگ، شاخص سطح برگ و عملکرد روغن و اسانس کاهش و دمای برگ افزایش یافت. این واکنش منجر به کاهش عملکرد دانه گیاهان همیشه‌بهار شد. کاربرد اسپرمیدین باعث افزایش درصد پوشش زمین، محتوای آب برگ، شاخص سطح برگ و عملکرد روغن و اسانس و موجب کاهش دمای برگ در شرایط عاری ازعلف‌های هرز شد. به طور کلی تیمار با اسپرمیدین بر عملکرد دانه و روغن گیاه همیشه بهار تحت شرایط تنش آبی در شرایط فقدان علف‌های هرز تأثیر مثبت داشت.

واژه‌های کلیدی: آلودگی علف‌هرز؛ .اسانس؛ پلی‌آمین؛ عملکرد دانه