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Influence of exogenous tryptophan application on production and carotenoids of *Calendula officinalis* under drip irrigation treatments

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Abstract*---Calendula officinalis* L. is primarily cultivated as cut flowers plant and also regarded as a medicinal plant with a variety of therapeutic uses. Irrigation management results in reduction in irrigation requirements, water saving and conservation. Tryptophan has a major role in improving plant stress tolerance to environmental stresses such as water deficiency. This study investigated the response of *C. officinalis* cultivated under drip irrigation system to the number of irrigation times week^{-1} and tryptophan foliar application in two successive seasons. Three irrigation treatments (once, twice and three times week^{-1}) were used as the main plots and four tryptophan treatments (0, 15, 30 and 45 mg l^{-1}) were applied as subplots with three replicates. Increasing the number of irrigation times from once to twice showed the highest values of investigated growth parameters, while three times a week decreased them to lower values than those of once a week. The opposite was true for flowers production in which

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increasing number of irrigation decreased the flower production. Increasing tryptophan concentration gradually up to 45 mg l^{-1} increased growth parameters. On the other hand, increasing tryptophan concentration decreased flowers production and proline under all used irrigation treatments. Irrigation of *C. officinalis* twice a week combined with application of tryptophan at 45 mg l^{-1} resulted in the highest values of photosynthetic pigments and carotenoids in petals and the lowest value of proline.

Keywords---calendula officinalis, irrigation, tryptophan, photosynthetic pigments, carotenoids.

Introduction

Calendula officinalis belongs family Asteraceae, is a well-known ornamental plant and therapeutic herb that was used for millennia. English marigold, pot marigold, bride of the sun, bull flower and butterwort are some of its common names (Ashwlayan *et al.*, 2018). It is widely grown in sunny areas and in a variety of soils. *C. officinalis* is a tall plant with sparsely branching erect stems, rectangular lance leaflets with tubular disc florets and thorns curving achene that is yellow or orange in color. Carotenoids, flavonoids, saponins, sterols, phenolic acids, lipids and other biological active constituents found in numerous organs of the plant such as leaves and flowers. It is believed to have medicinal properties and is widely used as an anti-inflammatory, diaphoretic, analgesic and antiseptic activities. It has some therapeutic properties and is used to treat gastrointestinal problems, gynaecological issues, oral disorders, eye diseases, skin injuries and certain burns (Chaparzadeh *et al.*, 2004 and Karthikeya *et al.,* 2022). The yellow pigment extracted from its flowers is used as food coloring additive. Flower decoction is utilized to treat cold, digestive system, kidneys and contains antioxidants that stimulate the immune system (Edwards *et al.*, 2015).

Amino acids are organic compounds that are synthesized by plants, but this process involves energy expenditure and takes time. Amino acids fulfill a wide variety of functions in plants, but their basic role is to serve as building blocks of proteins, which exert manifold functions in plant metabolism, and as metabolites and precursors involved in plant defense, vitamin, nucleotide and hormone biosynthesis, and as precursors of a huge variety of secondary metabolites (Mohamed *et al.*, 2018). The most biologically active plant-derived amino acids is tryptophan and it is a unique amino acid bearing an indole ring (Mustafa *et al.,* 2018). Tryptophan is known as -3-indolylalanine, it is one of the nine essential amino acids for humans. It may be applied to soil, used as a foliar spray and seed priming and its application increase plant yield and improve yield quality (Gondek and Hersztek, 2021).

Tryptophan (Trp) synthase β subunit 1 (TSB1) is essential for tryptophan synthesis in plants; thus, it affects protein biosynthesis, auxin accumulation and plant growth (Ursache *et al.,* 2014; Wang *et al.,* 2015). TSB1 sulfenylation by H_2O_2 functions in plant defense against bio-trophic pathogens (Yuan *et al.,* 2017). TSB1 acts as a key coordinator in the trade-off between plant growth and abiotic stress responses by regulating both Trp and ABA accumulation. Drought stressed reduced TSB1 expression and repressed growth, exhibit suppressed growth caused by repression of TSB1 expression and decreased synthesis of the auxin precursor tryptophan, which leads to less auxin accumulation. Decreasing TSB1 expression also results in increased accumulation of ABA and enhanced stress tolerance, because of the decreased interaction of TSB1 with b-glucosidase 1 (BG1), which frees this enzyme to hydrolyze glucose-conjugated ABA (ABA-GE) into ABA (Liu *et al.,* 2022). Water deficit or drought stress is considered as one of the most restrictive factors in plant growth and yield in many areas of the world with agricultural production (Hassan and Ali, 2014). Under drought stress, water potentials in the root zone become sufficiently negative, resulting in a reduction of water availability which affects the plant growth and development (Chai, *et al.*, 2016).

Drought stress generates changes at physiological and metabolic levels such as stomatal closure and reduction in photosynthesis rate and crops growth. Under these adverse conditions, the amount of oxidized reduction equivalents (NADP+) working as electron acceptors is reduced. Sequentially, large amounts of NADPH + H+ are accumulated, resulting in a high over-reduced state. As a consequence, it is necessary to have a high level of consumption of $NADPH^+$ H^+ in a plant, for instance, through the biosynthesis of highly reduced secondary compounds, such as phenols, terpenoids, alkaloids, cyanogenic glycosides and glucosinolates. (Caparros *et.al,* 2019). The issue of water is one of the hot issues in all agricultural societies in many countries of the world. In Egypt, water shortage is the issue that concerns those responsible for agriculture and its future, as fears of the negative effects of climatic changes in this area are increasing, in addition to the fact that the planned expansions in the new reclaimed land areas are located in low fertility or sandy lands with a low water holding capacity for the plant use. Therefore, the subject of irrigation water occupies an increasing interest in the scientific research field of water and agriculture. In the field of cultivating medicinal plants, the search for plant varieties with low water requirements, and natural and chemical additives that increase the plant resistance to water shortage are occupying increasing interest in this field. Thus, this study focuses on the effect of the number of irrigation per week with tryptophan application on growth, yield and carotenoids of calendula plant cultivated under drip irrigation system.

Material and Methods

The field experiment was carried out at SEKEM Company Farm, Belbes, Sharkia Governorate west of the Nile Delta, Egypt (30°35'15.65" N and 31°30'7.20" E) using a drip irrigation system during the two successive seasons of 2018/2019 and 2019/2020 to investigate the effect of irrigation intervals (number of irrigation times per week) and tryptophan foliar application on growth, production and some chemical constituents of *Calendula officinalis* plant. Seeds were obtained from SEKEM Company Farm and cultivated in trays on 8^{th} and 14^{th} of September 2018 and 2019, respectively. After the seedlings reached the suitable size and has a good root system (after 40 days), they were transplanted in the permanent field on 21^{st} and 24^{th} of October 2018 and 2019, respectively. The metrological data of the experimental farm region during the growing period are presented in Table (1).

Table 1
Monthly average of metrological data of the experimental area during 2018/2019 and 2019/2020 seasons

2018/2019 season					2019/2020 season				
Month	Air temperature °C			R.H. %	Month	Air temperature °C			R.H. %
	Max.	Min.	Aver.			Max.	Min.	Aver.	
Sept. 2018	37	23	29	57	Sept. 2019	37	20	28	58
Oct. 2018	34	17	26	54	Oct. 2019	35	19	26	59
Nov. 2018	31	15	21	59	Nov. 2019	32	13	22	54
Dec. 2018	23	10	17	58	Dec. 2019	24	9	16	56
Jan. 2019	25	7	14	46	Jan. 2020	21	6	13	59
Feb. 2019	31	10	16	54	Feb. 2020	26	9	15	61
Mar. 2019	29	10	18	55	Mar. 2020	30	10	18	55
Apr. 2019	35	13	21	48	Apr. 2020	34	14	21	50
May 2019	45	16	27	50	May 2020	43	17	26	45

R.H.: Relative humidity

The soil was prepared two weeks before transplantation and divided into rows (1 m in between). The physical and chemical analyses of the used soil were determined according to Jackson (1973) and are shown in Table (2), from which it could be observed that the soil is sandy loam soil in texture. During soil preparation, 20m^3 fed.$^{-1}$of compost, 75 kg fed.$^{-1}$ of elemental sulphur (99.9% S) and 400 kg fed.$^{-1}$of calcium superphosphate (15.5% P_2O_5) were added. All treatments received 300 kg fed^{-1} of ammonium sulphate (20.5% N) and 155 kg fed^{-1} of potassium sulphate (48.5%K_2O) in three doses during plant growth period.

Table 2
The physical and chemical properties of the experimental soil during 2018/2019 and 2019/2020 seasons

	Physical properties						
	Very coarse sand (2-1mm)	Coarse sand (1-0.5mm)	Medium sand (0.5-0.25mm)	Fine sand (0.25-0.1mm)	Very fine sand (0.1-0.05mm)	Silt +Clay (0.5> mm)	Texture
2018	15.00	50.90	3.21	19.50	8.80	2.59	Sandy
2019	11.84	56.12	2.51	17.87	9.67	1.99	Sandy

	Chemical properties									
	pH (2.5:1)	E.C. (dSm^{-1}) (1:1)	(meq / L) Cations				Anions			
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	So_4^{--}
2018	7.04	0.69	2.5	0.6	3.3	0.5	--	1.5	4.5	0.9
2019	7.97	1.03	3.8	0.8	6.2	0.6	--	2.6	5.6	3.2

The design of the factorial experiment was split plots with 12 treatments; the main plots consist of three irrigation treatments (once, twice and three times week^{-1}) and the sub plots contain four levels of tryptophan as foliar application (0, 15, 30 and 45 mg l^{-1}) with three replicates. Spraying of tryptophan treatments were performed twice; the 1stapplication was at first week of January and the second one at the first week of March in both seasons. Plant height, fresh and dry weight of herb (g plant^{-1}andton fed^{-1}) were recorded at the end of the experiment. Total yield of flowers (g plant^{-1} and ton fed^{-1}) were calculated using the weights that recorded from the nine cuts started from the middle of February till the beginning of May in both seasons. Photosynthetic pigments in leaves (chlorophyll a, chlorophyll b and total carotenoids (mg g^{-1} fresh leaves) were determined according to Moran (1982). Total carotenoids in petals (mgg^{-1}fresh petals) was determined according to Büyükçapar *et al.* (2007). Proline content in herb was determined according to Bates *et al.* (1973). The recorded data were analyzed as split plot design by analysis of variance (ANOVA) using the General Linear Models procedure of CoStat (Snedecor and Cochran, 1967). Least significant difference (LSD) test was applied at 0.05 probability level to compare the means of the treatments.

Results and Discussion

The mean values of plant heights, herb fresh and dry weights of *Calendula officinalis* plants as affected by irrigation interval/or tryptophan treatments are shown in Table (3). Statistical analyses indicated that irrigation intervals and tryptophan separately showed significant effects on plants height, fresh and dry weights of calendula plants in both seasons. In the same time, the interaction between irrigation intervals and tryptophan was insignificant on plant height and significant on herb fresh weights and dry weights in both seasons. Increasing irrigation times from once a week to twice a week significantly increased plant height, while increasing irrigation times to three times a week significantly decreased plant height comparing to the other two treatments. This may be attributed to the fact that the abundance of irrigation water causes the washing of nutrients, and the lack of aeration of the soil around the roots of the plant, which leads to poor plant growth. This result agreed with Neto *et al.* (2006) who suggested the constrained uptake of N (75% N less than control) observed in flooded studied plant was the result of the lower energy status of flooded roots and the energy costs of N uptake and assimilation, and García *et al.* (2008) who found that water excess treatment changed the soil chemical properties for plant growth by decreasing oxygen concentration in the soil and decreased level of nutrient uptake, so plant growth and root growth were decreased by water-logging.

Table 3
Effect of irrigation numbers week^{-1} and tryptophan on plant height (cm) and herb weight (g plant^{-1}) of *Calendula officinalis*

Treatment		Plant height (cm)		Herb fresh weight (g plant^{-1})		Herb dry weight (g plant^{-1})	
Irrigation numbers week^{-1}	Tryptophan (mg l^{-1})	1st season	2nd season	1st season	2nd season	1st season	2nd season
Once	0	44.0	46.7	845.7	903.3	162.6	173.7
	15	49.3	52.0	865.0	931.7	164.0	176.5
	30	54.0	58.0	924.0	1028.3	180.6	200.5
	45	58.7	63.7	953.3	1095.3	178.3	204.1
Twice	0	57.7	52.3	884.3	965.7	168.1	183.5
	15	63.3	59.0	947.3	1103.3	178.5	207.3
	30	67.3	65.7	985.0	1222.7	191.8	238.3
	45	72.7	69.0	1165.7	1518.0	233.1	303.1
Three times	0	44.7	47.0	789.3	749.0	146.1	138.7
	15	48.3	49.7	804.7	776.3	151.6	146.3
	30	50.7	53.0	826.3	841.7	158.9	162.0
	45	53.7	60.7	857.7	886.3	164.1	169.7
L.S.D. at 5%		ns	ns	38.60	120.24	18.24	24.82
Irrigation numbers week^{-1}							
Once		51.5	55.1	897.0	989.7	171.4	188.7
Twice		65.3	61.5	995.6	1202.4	192.9	233.1
Three times		49.3	52.6	819.5	813.3	155.2	154.2
L.S.D. at 5%		1.15	4.51	21.33	115.05	13.05	22.83
Tryptophan (mg l^{-1})							
0		48.8	48.7	839.8	872.7	158.9	165.3
15		53.7	53.6	872.3	937.1	164.7	176.7
30		57.3	58.9	911.8	1030.9	177.1	200.3
45		61.7	64.4	992.2	1166.6	191.8	225.6
L.S.D. at 5%		3.22	3.44	22.29	69.45	10.53	14.33

Application of tryptophan significantly increased plant height in both seasons. Increasing tryptophan concentration up to 45 mg l^{-1} gradually increased plant heights of calendula plants with significant values in both seasons. Calendula plant heights from 44 and 46.7 cm to 72.7 and 69 cm in the 1st and second season, respectively. From the combined treatments, it is clear that, increasing tryptophan concentration increased plant height under all used irrigation treatments. The shortest plant were recorded with 0 tryptophan treatment and irrigation once a week, while the highest ones were recorded with application of 45 mg l^{-1} tryptophan and irrigation twice a week in both seasons. The results in Table 3 also showed that the herb fresh and dry weights of calendula plants were affected by irrigation interval/or tryptophan treatments and indicated that irrigation intervals and tryptophan separately significantly affected the fresh and dry weighs of calendula plants in both seasons. In the same time, the interaction between irrigation intervals and tryptophan was significant on herb fresh weights and dry weights in both seasons.

Increasing irrigation numbers from once a week to twice times a week significantly resulted in the maximum fresh and dry weights, while increasing irrigation to three times a week significantly resulted in the lowest fresh and dry weights comparing to the other two treatments. Application of tryptophan significantly increased fresh and dry weighs in both seasons. Increasing tryptophan concentration up to 45 mg l^{-1} gradually increased fresh and dry weights of calendula plants with significant values in both seasons. The fresh weight of calendula plant ranged from 789.3 and 749.0 g to 1165.7 and 1518.0 g while, calendula plant dry weight ranged from 1518.0 and 146.1 g to 138.7 and 303.1 g in the 1st and second season, respectively. From the combined treatments, it is clear that increasing tryptophan concentration increased fresh and dry weights under all irrigation treatments. The lowest fresh and dry weights were recorded with 0 tryptophan treatment and irrigated three times a week, while the heaviest ones were recorded with application of 45 (mg l^{-1}) tryptophan and irrigated twice a week in both seasons.

The increase in plant height and fresh and dry weights by spraying tryptophan may be due to its role in plant growth, as it helps in the synthesis of auxins which work on the elongation of cells as well as improving the yield of plants. These results are in consistency to those of Mustafa *et.al* (2018) who found that plant growth regulators are biologically active signaling molecules that regulate a number of plant physiological processes. Auxin (indole-3-acetic acid) is an important plant growth regulator and is synthesized within plant tissues through tryptophan (TRP) dependent and -independent pathways. Foliar spray with TRP stimulates auxin synthesis within plants and improves growth and productivity of agricultural crops. Furthermore, TRP contains approximately 14% nitrogen in its composition, which is released upon its metabolism within a plant or in the rhizosphere and plays a role in enhancing crop productivity.

Means of comparisons in Table 4 shown the effect of irrigation interval/or tryptophan treatments on fresh and dry flower weights (g plant^{-1}) and total fresh and dry flower yield (ton ha^{-1}) of *Calendula officinalis* plants. Data indicated that irrigation treatments and tryptophan separately showed significant effects on fresh and dry flower weights (g plant^{-1}) and total fresh and dry flower yield (ton ha^{-1}) of Calendula plants in both seasons. In the same time, the interaction between irrigation treatments and tryptophan was significant in both seasons. Reducing number irrigation times from three times to once a week significantly increased flower fresh and dry weights (g plant^{-1}) and total flower fresh and dry yield (ton ha^{-1}), while increasing irrigation times to three times a week significantly decreased fresh and dry flower weights (g plant^{-1}) and total fresh and dry flower yield (ton ha^{-1}) comparing to the other two treatments.

Table 4
Effect of irrigation numbers week^{-1} and tryptophan on flowers total yield (g plant^{-1} and ton ha^{-1}) of *Calendula officinalis*

Treatment		Total fresh weight (g plant^{-1})		Total dry weight (g plant^{-1})		Total fresh yield (ton ha^{-1})		Total dry yield (ton ha^{-1})	
Irrigation numbers week^{-1}	Tryptophan (mg l^{-1})	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Once	0	624.5	749.8	85.8	102.2	20.8	25.0	2.9	3.4
	15	501.0	575.0	72.3	80.9	16.7	19.2	2.4	2.7
	30	407.3	481.0	57.4	69.0	13.6	16.0	1.9	2.3
	45	291.5	350.7	41.6	47.9	9.7	11.7	1.4	1.6
Twice	0	394.7	431.0	55.9	61.9	13.2	14.4	1.9	2.1
	15	331.8	404.8	47.3	58.2	11.1	13.5	1.6	1.9
	30	259.5	294.2	38.0	41.3	8.7	9.8	1.3	1.4
	45	209.5	265.2	31.4	39.5	7.0	8.8	1.0	1.3
Three times	0	283.9	328.1	40.7	48.3	9.5	10.9	1.4	1.6
	15	252.8	331.4	36.8	47.9	8.4	11.0	1.2	1.6
	30	213.1	244.6	31.6	35.4	7.1	8.2	1.1	1.2
	45	187.9	234.6	28.3	35.6	6.3	7.8	0.9	1.2
L.S.D. at 5%		17.42	22.92	2.46	4.44	0.58	0.77	0.10	0.14
Irrigation numbers week^{-1}									
Once		456.1	539.1	64.3	75.0	15.2	18.0	2.1	2.5
Twice		298.9	348.8	43.1	50.2	10.0	11.6	1.4	1.7
Three times		234.4	284.7	34.4	41.8	7.8	9.5	1.1	1.4
L.S.D. at 5%		18.49	20.06	2.70	3.38	0.61	0.68	0.12	0.09
Tryptophan (mg l^{-1})									
0		434.4	503.0	60.8	70.8	14.5	16.8	2.0	2.4
15		361.9	437.1	52.1	62.3	12.1	14.6	1.7	2.1
30		293.3	339.9	42.3	48.6	9.8	11.3	1.4	1.6
45		229.6	283.5	33.8	41.0	7.7	9.5	1.1	1.4
L.S.D. at 5%		10.06	13.24	1.42	2.56	0.33	0.45	0.06	0.08

Application of tryptophan had a negative effect on fresh and dry flower weights (g plant^{-1}) and total fresh and dry flower yield (ton ha^{-1}), whereas higher value of fresh and dry flower weights (g plant^{-1}) and total fresh and dry flower yield (ton ha^{-1}) were resulted from 0 tryptophan treatment in both seasons. Increasing tryptophan concentration up to 45 mg l^{-1} gradually decreased fresh and dry flower weights (g plant^{-1}) and total fresh and dry flower yield (ton ha^{-1}) in both seasons. Calendula total flower fresh weight (g plant^{-1}) ranged from 187.9 and 234.6 g to 624.5 and 749.8 g, as well as total flower dry weight (g plant^{-1}) ranged from 28.3 and 35.6 g to 85.8 and 102.2 g, The total flower fresh yield (ton ha^{-1}) ranged from 6.3 and 7.8 ton to 20.8 and 25.0 ton and as well as total flower dry yield (ton ha^{-1}) ranged from 0.9 and 1.2 ton to 2.9 and 3.4 ton in the 1st and second season, respectively. From the combined treatments, it is clear that increasing tryptophan concentration decreased total fresh and dry flower weights (g plant^{-1}) and total fresh and dry flower yield (ton ha^{-1}) under all used irrigation treatments. The highest total fresh and dry flower weights (g plant^{-1}) and total fresh and dry flower yield (ton ha^{-1}) were recorded with 0 tryptophan treatment and irrigated once a week, while the lowest ones were recorded with application of 45 mg l^{-1} tryptophan and irrigated three times a week in both seasons. This may be attributed to the stimulation influence of tryptophan on the growth of plants on the expense of flowering organs.

The influence of irrigation numbers/or tryptophan treatments on chlorophyll a, chlorophyll b, carotenoid in herb, carotenoid in petals and proline of *Calendula officinalis* plants are shown in Table (5). It could be conducted from data that irrigation numbers and tryptophan separately showed significant effects on chlorophyll a, chlorophyll b, carotenoid in leaves, carotenoid in petals and proline in both seasons. In the same time, the interaction between irrigation treatments and tryptophan was insignificant on chlorophyll b and carotenoid in petals but significant for chlorophyll a, carotenoid in herb and proline in both seasons. Increasing irrigation numbers from once a week to twice a week significantly increased chlorophyll a, chlorophyll b, carotenoid in leaves and carotenoid in petals, while increasing irrigation numbers to three times a week significantly decreased chlorophyll a, chlorophyll b, carotenoid in herb and carotenoid in petals comparing to the other two treatments. Data indicated that application of tryptophan significantly increased photosynthetic pigments and carotenoids of petals in both seasons.

Increasing tryptophan concentration up to 45 mg l^{-1} gradually increased chlorophyll a, chlorophyll b, carotenoid in herb and carotenoid in petals of calendula plants with significant values in both seasons. Chlorophyll a of calendula plant ranged from 1.13 mg to 2.13 mg, as well as chlorophyll b of calendula plant ranged from 0.47 mg to 0.78 mg. in the same time, carotenoid in calendula herb ranged from 0.60 mg to 1.01 mg, while carotenoid in petals ranged from 2.95 to 3.89 in the second season, respectively. From the combined treatments, it is clear that increasing tryptophan concentration increased chlorophyll a, chlorophyll b, carotenoid in leaves and carotenoid in petals under all used irrigation treatments. The lowest values were recorded with 0 tryptophan treatment and irrigated three times a week, while the highest ones were recorded with application of 45 mg l^{-1} tryptophan and irrigated twice a week in second season.

These results are in agreement with Naghizadeh *et al.* (2019), Sadak and Ramadan (2021) who reported that drought stress reduced photosynthetic pigments of studied plant. The reduction in photosynthetic pigments under water stress conditions might be correlated to the obliteration of chloroplast and photosynthetic apparatus, chlorophyll, photo-oxidation induced breakdown of chlorophyll biosynthesis precursors, hormonal imbalance and activation of chlorophyll degrading enzymes (Wang *et al.* 2013). Moreover, Sultana *et al.* (1999) showed that in water deficit plants, the decrease in carotenoids might be related to the destruction in b-carotene or formation of zeaxanthin in the xanthophyll cycle.

Table 5
Effect of irrigation numbers week^{-1} and tryptophan on photosynthetic pigments (mg g $^{-1}$ fresh leaves), carotenoids in petals (mg g $^{-1}$ fresh petals) and proline (mg g $^{-1}$ fresh leaves) of *Calendula officinalis*

Irrigation numbers week^{-1}	Tryptophan (mg l^{-1})	Chl. a	Chl. b	Carot. in herb	Carot. in petals	Proline
Once	0	1.20	0.54	0.64	3.12	6.43
	15	1.34	0.55	0.68	3.28	6.23
	30	1.71	0.58	0.70	3.35	5.84
	45	1.92	0.60	0.73	3.51	5.59
Twice	0	1.40	0.61	0.65	3.43	5.36
	15	1.42	0.62	0.80	3.41	5.10
	30	1.83	0.71	0.91	3.82	4.99
	45	2.13	0.78	1.01	3.89	4.79
Three times	0	1.13	0.47	0.60	2.95	7.26
	15	1.26	0.52	0.62	3.22	7.05
	30	1.31	0.57	0.64	3.30	6.19
	45	1.42	0.59	0.70	3.58	5.90
L.S.D. at 5%		0.19	ns	0.12	ns	0.33
Irrigation numbers week^{-1}						
Once		1.54	0.57	0.69	3.32	6.02
Twice		1.70	0.68	0.84	3.64	5.06
Three times		1.28	0.54	0.64	3.26	6.60
L.S.D. at 5%		0.15	0.11	0.07	0.08	0.19
Tryptophan (mg l^{-1})						
0		1.24	0.54	0.63	3.17	6.35
15		1.34	0.56	0.70	3.30	6.13
30		1.62	0.62	0.75	3.49	5.67
45		1.82	0.66	0.81	3.66	5.43
L.S.D. at 5%		0.11	0.04	0.07	0.16	0.19

Chl.: Chlorophyll, Carot.: Carotenoids

Data in Table (5) also represented that increasing irrigation numbers from once a week to twice a week significantly decreased proline while increasing irrigation to three times a week significantly increased proline comparing to the other two treatments. Application of tryptophan significantly decreased proline in both seasons. Increasing tryptophan concentration up to 45 mg l^{-1} gradually decreased proline in calendula plants with significant values in both seasons. Proline in Calendula plant ranged from 4.79 to 7.26 in the second season. From the combined treatments, it is observed that increasing tryptophan concentration decreased proline under all used irrigation treatments. The highest value was recorded with plants that received with 0 tryptophan and irrigated three times a week, while the lowest one was recorded with those received 45 mg l^{-1} tryptophan and irrigated twice a week in second season.

Osmotic stress induces cellular accumulation of compatible solutes, or osmolytes in all plants, regardless of them being tolerant or not to abiotic stress (Ashraf and Foolad, 2007). One ubiquitous osmolyte in plants is proline, which is synthesised under different stress conditions such as drought (Boscaiu *et al.,* 2013). In addition to its role in osmotic adjustment, proline shows many other functions, as 'osmoprotectant,' directly stabilizing proteins, membranes and other subcellular structures, scavenging free radicals, or balancing the cell redox status under stress conditions (Verbruggen and Hermans, 2008), as well as contributing to storage of carbon and nitrogen during stress, which will help cell and tissue recovery when stress eases or disappears (Cicevan *et al.,* 2016).

Conclusion

Increasing the number of irrigation times from once to twice showed the highest values of investigated growth parameters, while three times a week decreased them to lower values than those of once a week. The opposite was true for flowers production in which increasing number of irrigation decreased the flower production. Increasing tryptophan concentration gradually up to 45 mg l^{-1} increased growth parameters. On the other hand, increasing tryptophan concentration decreased flowers production and proline under all used irrigation treatments. Irrigation of *C. officinalis* twice a week combined with application of tryptophan at 45 mg l^{-1} resulted in the highest values of photosynthetic pigments and carotenoids in petals and the lowest value of proline. For growth and plant biomass production, the recommended combined treatment is irrigation twice a week and application of 45 mg l^{-1}. On the other hand for flowers production, the recommended combined treatment is irrigation once a week without tryptophan application.

References

Ashraf M and Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59(2):206-216.

Ashwlayan VD, Kumar A, Verma M, Garg VK and Gupta SK (2018). Therapeutic potential of *calendula officinalis*. Pharm Pharmacol Int. J, 6(2):149-155.

Bates LS, Waldren RP and Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39(1): 205-207.

Boscaiu M, Lull C, Llinares J, Vicente O and Boira H. (2013). Proline as a biochemical marker in relation to the ecology of two halophytic Juncus species. Journal of Plant Ecology, 6(2):177-186.

Büyükçapar HM, Yanar M and Yanar Y (2007). Pigmentation of rainbow trout (*Oncorhynchus mykiss*) with carotenoids from marigold flower (*Tagetes erecta*) and red pepper (*Capsicum annum*). Turk. J. Vet. Anim. Sci.,31(1):7-12.

Caparros GP, Romero JM, Llanderal A, Cermeño P, Lao MT and Segura ML (2019). Effects of Drought Stress on Biomass, Essential Oil Content, Nutritional Parameters, and Costs of Production in Six Lamiaceae Species. Water 11, 573: 1-12.

Cardinali A and Nason G (2013). Costationarity of locally stationary time series using costat. Journal of Statistical Software, 55(1): 1-22.

Chai Q, Gan Y, Zhao C, Xu HL, Waskom RM, Niu Y and Siddique KH (2016). Regulated deficit irrigation for crop production under drought stress. A review. Agron. Sustain. Dev., 36, (3): 1-21.

Chaparzadeh ND', Amico ML, Khavari-Nejad RA, Izzo R and Navari-Izzo F (2004). Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiol. Biochem. 42, 695–701

Cicevan R, Al Hassan M, Sestras AF, Prohens J, Vicente O, Sestras RE and Boscaiu M (2016). Screening for drought tolerance in cultivars of the ornamental genus *Tagetes* (Asteraceae) Peer J. (4): 1-20.

Edwards SE, da Costa Rocha I, Williamson EM, Heinrich M (2015). Phytopharmacy: An evidence-based guide to herbal medicinal products. John Wiley & Sons.

García I, Mendoza R and Pomar MC (2008). Deficit and excess of soil water impact on plant growth of Lotus tenuis by affecting nutrient uptake and arbuscular mycorrhizal symbiosis. Plant Soil 304:117–131.

Gondek K and Hersztek MM (2021). Effect of Soil-Applied L-tryptophan on the Amount of Biomass and Nitrogen and Sulfur Utilization by Maize. Agronomy, 11, 2582, 1-10.

Hassan FAS and Ali EF (2014). Impact of different water regimes based on class A pan on growth, yield and oil content of *Coriandrum sativum* L. plant. J. Saudi Soc. Agric. Sci., 13, 155–161.

Jackson ML (1973). Soil Chemical Analysis. Prentice-Hall Inc., Englewood Cliffs, New Jersey, USA, 498p.

Karthikeya P, Sanjay CJ, Doggallialli N, Krrenuka D and Harshitha N (2022). A Review of *Calendula officinalis*- Magic in Science. Journal of Clinical and Diagnostic Research. 16(2): 23-27.

Liu WC, Song RF, Zheng SQ, Li TT, Zhang BL, Gao X and Lu YT (2022). Coordination of plant growth and abiotic stress responses by tryptophan synthase b subunit 1 through modulation of tryptophan and ABA homeostasis in Arabidopsis. Mol. Plant. 15, 973- 990.

Mohamed MF, Thalooth AT, Essa REY and Gobarah ME (2018). The stimulatory effects of Tryptophan and yeast on yield and nutrient status of Wheat plants (*Triticum aestivum*) grown in newly reclaimed soil. Middle East J. Agric. Res., 7(1): 27-33.

Moran R (1982). Formulae for determination of chlorophyllous pigments extracted with n,n-dimethylformamide. Plant Physiol., 69: 1376-1381.

Mustafa A, Imran M, Ashraf M and Mahmood K (2018). Perspectives of using L tryptophan for improving productivity of agricultural crops: A review. Pedosphere, 28(1):16–34.

Naghizadeh M, Kabiri R, Hatami A, Oloumi H, Nasibi F and Tahmasei Z (2019). Exogenous application of melatonin mitigates the adverse effects of drought stress on morpho-physiological traits and secondary metabolites in Moldavian balm (*Dracocephalum moldavica*). Physiol Mol Biol Plants, 25:881-894.

Neto D, Carvalho LM, Cruz C and Martins-Loução MA (2006). How do mycorrhizas affect C and N relationships in flooded *Aster tripolium* plants? Plant Soil 279:51–63.

Sadak MSh and Ramadan AA (2021). Impact of melatonin and tryptophan on water stress tolerance in white lupine (*Lupinus termis* L.). Physiol. Mol. Biol. Plants ., 27(3): 469-481.

Snedecor GW and Cochran WG (1967). Statistical Methods. Iowa State University Press, Ames, Iowa, USA, 593p.

Sultana N, Ikeda T and Itoh R (1999). Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environ Exp Bot, 42:211-220.

Ursache R, Miyashima S, Chen QG, Vaten A, Nakajima K, Carlsbecker A, Zhao YD, Helariutta Y and Dettmer J (2014). Tryptophan-dependent auxin biosynthesis is required for HD-ZIP IIImediated xylem patterning. Dev. 141:1250–1260.

Verbruggen N and Hermans C (2008). Proline accumulation in plants: a review. Amino Acids, 35(4):753-759.

Wang B, Chu JF, Yu TY, Xu Q, Sun XH, Yuan J, Xiong GS, Wang GD, Wang YH and Li JY (2015). Tryptophan independent auxin biosynthesis contributes to early embryogenesis in Arabidopsis. Proc. Natl. Acad. Sci. U S A. 112: 4821-4826.

Wang P., Sun X, Chang C, Feng FJ, Liang D, Cheng LL and Ma FW (2013). Delay in leaf senescence of *Malus hupehensis* by long term melatonin application is associated with its regulation of metabolic status and protein degradation. J Pineal Res 55:424–434.

Yuan HM, Liu WC and Lu YT (2017). Catalase to coordinates SA-Mediated repression of both auxin accumulation and JA biosynthesis in plant defenses. Cell Host Microbe 2,143-155.