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Research Article

The effect of melatonin on quality and growth characteristics of ajowan (*Carum copticum* L.) to irrigation cut-off the development of different periods

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Abstract

The study was carried out to explore the influence of varying melatonin concentrations on the improvement of morphological, biochemical, and yield parameters of ajowan under conditions of irrigation cut-off at various stages of plant growth. The experimental treatments included irrigation cut-off at various stages of the plant: Control (full irrigation), vegetative stage, flowering, and vegetative + flowering, as well as three levels of melatonin foliar spraying (0, 0.5, and 1 mM). The experiment was carried out in a split plot arrangement based on a completely randomized blocks design, in the research farm of the University of Jiroft in 2023. The results showed that drought stress in different stages of plant growth caused a decrease in plant height, stem diameter, number of umbels per plant, number of seeds per umbel, weight of 1000 seeds, percentage of oil, seed yield, biomass yield, oil yield. Melatonin foliar spraying enhanced evaluated parameters under drought circumstances. Melatonin at 1 mM concentration exhibited the most favorable influence on photosynthetic pigments, morphological traits, yield components, seed yield, and biological yield. Only plant height, proline content, percentage of oil, biological yield, and essential oil yield were affected by the interaction of irrigation cut-off and melatonin treatment. The maximum plant height, biological yield, and essential oil yield were achieved with 0.5 foliar spraying and 1 mM melatonin concentration under full irrigation. In addition, the maximum concentration of proline was found when irrigation was halted during vegetative + flowering without melatonin. The highest percentage of essential oil (4.3%), biomass yield (14.65 kg ha^{-1}) and seed yield (19.56 kg ha^{-1}) were obtained in the treatment of full irrigation and melatonin foliar. In general, foliar spraying with melatonin increased resilience to drought stress and enhanced performance in ajowan seedlings, and the treatment with a 1 mM concentration of melatonin was more beneficial.

Keywords: Drought stress, Oxidative damage, Oil yield, Photosynthetic pigments, Proline

Introduction

Because of the detrimental effects of chemical medications, the growing of medicinal plants is becoming more popular. Medicinal plants are also employed in the food, pharmaceutical, and health industries due to the availability of secondary metabolites with numerous therapeutic qualities (Jamshidi-Kia *et al.*, 2018). The concentration of these chemicals is used to assess the quality of therapeutic medicines. Internal (genes, enzymes) and environmental (drought, salinity, light, and temperature) variables impact the production and accumulation of these chemicals (Li *et al.*, 2020). Drought stress is one of the most important environmental stresses that occurs as a result of temperature changes, high light intensity, and low rainfall and affects the morphological, physiological, biochemical, and molecular characteristics of the plant, negatively affecting photosynthetic capacity (Seleiman *et al.*, 2021; Brodersen *et al.*, 2019). Also, the accumulation of reactive oxygen species causes oxidative damage in this type of stress, resulting in decreased photosynthesis, stomatal closure, and changes in enzyme activities, electrolyte leakage, lipid peroxidation, cell membrane disruption, protein and nucleic acid degradation (Maksup *et al.*, 2014).

Increasing the rate of leaf senescence, chlorophyll degradation, canopy size decrease, and reduced

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efficiency of water and nutrient absorption are also among the negative impacts that lead the plant to die in the face of drought stress. Plants, on the other hand, employ a variety of methods to mitigate the impacts of drought and protect against damage produced by an overabundance of reactive oxygen species. The most common method is a defense mechanism that includes both non-enzymatic (glutathione, ascorbate, polyphenols, and vitamins) and enzymatic (superoxide dismutase, ascorbate peroxidase, peroxidase, and catalase) activities that effectively remove reactive oxygen species (ROS) produced in plant cells as a result of stress (Naudts *et al.*, 2015; Sinha *et al*., 2015; Dawood and Sadak, 2014). This plant's internal defense system is only active to a certain extent, and in the case of severe stress, it causes severe physiological abnormalities such as reduced photosynthesis, stomatal conduction, leaf area, growth rate, and plant height; disruption of the membrane system (cell, mitochondria, and chloroplast), loss of membrane fluidity and ionic imbalance, destruction of D1 protein in the reaction center of photosystem II, disruption of electron transport, and disruption of the electron transport system. Because the plant's defense mechanism is incapable of sufficiently protecting the plant under extreme stress circumstances, employing growth regulators such as melatonin is an appropriate approach for increasing the plant's tolerance to stress (Tiwari *et al.,* 2020). Melatonin (N-acetyl-5-methoxytryptamine) has an indole-based structure and a low molecular weight. It is present in many living creatures and has a role in many physiological and biochemical processes, including stem and root development, flower and fruit creation, and leaf senescence delay (Li, 2012; and Kabiri, 2018). Melatonin is soluble in both water and fat, giving it both hydrophilic and hydrophobic characteristics. Because of its tiny size, it may migrate between cells and neutralize ROS that accumulate in cells under stressful situations, therefore safeguarding the cells. Melatonin protects the chloroplast structure, prevents chlorophyll degradation, and improves the phytochemical capabilities of photosystem II to lessen the deleterious effects of oxidative stress on proteins, lipids, and nucleic acids (Sharma *et al.,* 2020), affecting biosynthesis and stimulating enzymatic and non-enzymatic systems (Li *et al,* 2012); as a result, it improves plant tolerance in the face of various environmental stresses, such as drought, salinity, and heat stress, and increases plant recovery potential after stress occurrence, resulting in increased plant growth and performance (Mohammadi *et al.,* 2018). Melatonin (seed priming, foliar application, or soil application) boosts the plant's defense system and increases stress tolerance in stress situations by modulating transcription factors and raising the expression of genes involved in hormonal message transmission pathways (Antoniou *et al.,* 2017). Drought stress reduced fenugreek (*Trigonella foenum-graceum* L.) biomass and plant height, photosynthetic pigments, and catalase enzyme activity, while increasing hydrogen peroxide and malondialdehyde concentrations (Zamani *et al.*, 2020). Melatonin use significantly boosted ROS scavenging and decreased chlorophyll degradation (Zamani *et al.,* 2020). A foliar application of 100 M melatonin boosted the level of carotenoids and proline while decreasing hydrogen peroxide. Melatonin is thought to produce free radical removal and enhanced tolerance to dehydration in the medicinal plant Agastache, or Mexican flower (*Agastache foeniculum),* by stimulating enzymatic and non-enzymatic activities (Mohammadi *et al.*, 2018).

Carum copticum (L.), often known as ajowan, is an annual medicinal plant in the Umbelliferae family that grows in arid and semi-arid locations such as Egypt, Iran, and Afghanistan. Thymol, carvacrol, alpha and beta pinene, and terpinene are among the chemicals discovered in this plant. Antimicrobial, antiviral, carminative, diuretic, antihypertensive, antitussive, and antilipid activities are found in the seeds of this plant (Panda *et al.,* 2020). Given the possibility of drought stress and the efficacy of melatonin in modulating the negative effects of stress on the ecophysiological and yield characteristics of plants, the purpose of this study is to investigate the effect of melatonin on the growth, physiological, and biochemical characteristics of ajowan medicinal plants under water stress conditions at various stages of plant growth.

Materials and methods

This investigation was conducted at the Jiroft University research farm in Jiroft, Kerman (35˚28'N, 47˚57'E). Jiroft has a subtropical climate and is located in south Kerman province. Based on a 30-year long-term average, the average yearly rainfall in this location is 152 mm, the average annual temperature is 26°C, and the average annual precipitation is 152 mm. Table 1 shows the properties of research farm soil.

The experimental design was carried out as a split plot arrangement using entirely randomized blocks. The main plot contained irrigation interruption at various times of the plant, such as control (full irrigation), vegetative stage, flowering, and vegetative + flowering, while the subplot included melatonin foliar spraying at three levels: Control, 0.5, and 1 mM. The plants were grown using the drip irrigation method in the early winter of 2020. Each experimental plot included 6 planting lines that were 3 meters long and planted 10 seeds per square meter with a gap of 40 and 25 cm between the rows and between the plants, respectively. The remaining plants were harvested at the physiological ripening stage in order to quantify seed yield and biomass by eliminating the marginal rows. To determine the amount of chlorophyll and carotenoid pigments, 0.2 grams of fresh plant leaves were ground in a Chinese mortar with 15 ml of 80% acetone, and after filtering, the absorption was measured with a spectrophotometer (UV-Visible model T80+) at

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Table 1. Physical and chemical characteristics of the experiment site

Organic matter (%)	Electrical conductivity (dS.m-1)	pH	Available k (ppm)	Available P (ppm)	Total N (%)	Soil texture
0.25	1.1	7.6	235	10.2	0.009	S-L

wavelengths of 646.8, 663.20, and 470 nm (Lichtenthaler and Buschmann, 2001).

(1)

$$Chla = (12.25A_{663.2} - 2.79A_{646.8})$$

(2)

$$Chlb = (21.21A_{646.8} - 5.1A_{663.2})$$

(3)

$$Car = \left(\frac{(1000A_{470} - 1.8Chla - 85.02Chlb)}{198}\right)$$

Chla, Chlb, and carotenoids are the concentrations of chlorophyll a, chlorophyll b, and carotenoids (including carotenes and xanthophylls) in these equations.

Ethanol 95% was employed to evaluate phenolic chemicals. The sample was then centrifuged for 10 minutes at 5000 rpm. After that, it was combined with Folin reagent and sodium carbonate, and the optical absorption of the samples was measured with a spectrophotometer (UV-Visible model T80+) at a wavelength of 725 nm. The standard curve was created using various amounts of gallic acid (Soland and Laima, 1999). To quantify proline, 2 cc of the plant extract solution, 2 cc of ninhydrin acid, and 2 cc of acetic acid were put in a test tube in a boiling water bath at 100°C for one hour. Using a Cloninger machine and water distillation, the essential oil was extracted from the seeds. The essential oil was weighed using a digital scale with 0.0001 g resolution. Dastborhan *et al.* (2012) utilized Equation 4 to compute the proportion of essential oil.

(4)

weight of essential oil (gr) × 100/dry weight of primary material (gr) = essential oil %

(5)

essential oil % × seed yield (kg) = essential oil yield

Statistical analysis was performed using SAS ANOVA, followed by Duncan's test (at the 5% level) to determine whether the means were statistically different.

Results

Photosynthetic pigments: Irrigation cut-off and melatonin application treatments had an effect on the quantity of chlorophylls a, b, and carotenoids (Table 2). The largest concentrations of photosynthetic pigments, such as chlorophylls *a* and *b* and carotenoids, were found in the control and irrigation cut-off treatments during the vegetative stage, and the lowest in the vegetative + reproductive stage irrigation cut-off treatment. As 0.5 and 1 mM melatonin were administered, the quantity of chlorophyll increased by 4.4 and 4.1%, respectively, as compared to the control. Using 1 mM melatonin had the greatest positive effect on raising the quantity of chlorophyll b, which was not statistically different from the consumption of 0.5 mM. Although irrigation interruption reduced the quantity of chlorophylls a and b relative to the control, stoppage of irrigation during the vegetative and flowering phases increased the amount of carotenoids. When compared to the control and 0.5 mM melatonin, applying 1 mM melatonin resulted in a 10% and 4.4% increase in the quantity of carotenoids in leaves, respectively (Table 3).

Proline: The irrigation cut-off and melatonin application treatments, as well as their interaction, influenced the quantity of proline. The maximum level of proline was seen in seedlings when irrigation was interrupted between the vegetative and flowering stages, and there was no significant difference between no usage and use of 0.5 mM melatonin. There was no significant change in the quantity of proline in seedlings between the use and non-use of melatonin in the conditions of normal watering and interruption of irrigation solely during the vegetative stage.

Flavonoid and phenol: When irrigation was halted throughout the vegetative and flowering stages, and no melatonin was consumed, the largest level of flavonoid was recorded. The combination of irrigation cut-off and melatonin ingestion treatments did not affect the quantity of flavonoids. Stopping irrigation throughout the flowering phases, as well as during the flowering and vegetative stages, increased the quantity of phenol in ajowan seedlings.

Morphological characteristics: Different quantities of irrigation and melatonin had an effect on plant height and stem diameter (Table 4). Using 1 mM melatonin in full irrigation and interrupted irrigation throughout vegetative and flowering phases resulted in an increase in plant height compared to the control. However, employing 1 mM melatonin throughout the vegetative + flowering stages resulted in a reduction in plant height as compared to the control; however, this difference was not statistically significant. The highest beneficial effect of melatonin on raising plant height as one of the parameters affecting yield was reported in full irrigation circumstances with a 1 mM concentration of melatonin (1.9% increase compared to the control) (Table 5). The biggest adverse effect of irrigation cut-off on stem diameter was noticed during the vegetative and flowering periods, where it exhibited a 25% drop compared to full irrigation. Melatonin doses of 0.5 and 1 mM improved stem diameter by 4% and 9%, respectively, as compared to the absence of melatonin (Table 5).

Yield components, seed yield, and essential oil: There was no significant variation in the quantity of

Table 2. ANOVA of different irrigation and melatonin levels effects on biochemical traits of ajowan

S. O. V.	df	Mean squares					
		Chl a	Chl b	Carotenoids	Proline	Flavonoids	Phenol
Block	2	0.008	0.002	0.011	0.006	6.25	0.029
Irrigation (I)	3	4.555^{**}	5.458^{**}	3.481^{**}	5.290^{**}	335.5^{*}	7.707^{**}
First error	6	0.011	0.073	0.028	0.011	54.17	0.036
Melatonin (M)	2	0.101^{*}	0.422^{**}	0.428^{**}	0.173^{*}	119.61^{**}	0.159^{*}
I × M	6	0.025^{ns}	0.036^{ns}	0.013^{ns}	0.109^{*}	20.48	0.042^{ns}
Total error	16	0.414	0.541	0.341	0.498	56.99	0.696
CV (%)	-	8.6	3.6	7.10	7.4	4.0	6.2

ns, * and **: non-significant and significant in the probability levels of 5%, and 1%, respectively

Table 3. Means comparison of biochemical traits of ajowan affected by experimental treatments

Treatments	Chl a (mg.gFW^{-1})	Chl b (mg.gFW^{-1})	Carotenoids (mg.gFW^{-1})	Proline (µm.gFW^{-1})	Flavonoids (mg.gFW^{-1})	Phenol (mg.gFW^{-1})
			Irrigation			
Control	4.32^{b}	3.82^{a}	3.165^{d}	1.283^{d}	48.98^{c}	1.340^{c}
Vegetative stage	4.55^{a}	3.62^{a}	3.772^{c}	2.250^{c}	55.25^{b}	2.440^{b}
Flowering stage	4.02^{c}	3.28^{b}	4.360^{b}	2.698^{b}	57.78^{b}	3.245^{a}
Vegetative+ Flowering stage	2.94^{d}	2.08^{c}	4.538^{a}	3.056^{a}	63.72^{a}	3.334^{a}
Control	3.85^{b}	3.02^{b}	3.765^{c}	2.417^{a}	59.73a	2.720^{a}
0.5 mM	4.02^{a}	3.20^{ab}	3.968^{b}	2.362^{a}	56.12ab	2.545^{b}
1 mM	4.01^{a}	3.39^{a}	4.143^{a}	2.187^{b}	53.44^{b}	2.504^{b}
			Interactions			
Control×Control	-	-	-	1.133^{d}	-	-
Control× 0.5 mM	-	-	-	1.433^{d}	-	-
Control×1 mM	-	-	-	1.283^{d}	-	-
Vegetative stage× Control	-	-	-	2.300^{c}	-	-
Vegetative stage× 0.5 mM	-	-	-	2.200^{c}	-	-
Vegetative stage× 1 mM	-	-	-	2.250^{c}	-	-
Flowering stage× Control	-	-	-	2.967^{ab}	-	-
Flowering stage× 0.5 mM	-	-	-	2.780^{b}	-	-
Flowering stage× 1 mM	-	-	-	2.347^{c}	-	-
Vegetative+ Flowering stage× Control	-	-	-	3.267^{a}	-	-
Vegetative+ Flowering stage× 0.5 mM	-	-	-	3.033^{ab}	-	-
Vegetative+ Flowering stage× 1 mM	-	-	-	2.867^{b}	-	-

Means within each column followed by the same letter are not significantly different (LSD, 0.05 probability level).

Table 4. ANOVA of different irrigation and melatonin levels effects on yield and yield component of ajowan

S. O. V.	df	Mean squares				
		Plant height	Stem diameter	N. of umbel per plant	N. of umbellate per umbel	N. of Seed per umbel
Block	2	14.77	0.994	44.19	0.646	1.51
Irrigation (I)	3	1022.30^{**}	5.562^{**}	3824.25^{**}	8.340^{**}	21.71^{**}
First error	6	12.18	0.219	46.97	0.424	0.93
Melatonin (M)	2	11.86^{*}	1.505^{**}	948.86^{**}	10.021^{**}	2.84^{ns}
I×M	6	9.71^{*}	0.071^{ns}	76.75^{ns}	0.799^{ns}	1.43^{ns}
Total error	16	94.31	0.755	427.95	1.763	2.87
CV (%)	-	6.3	5.2	3.0	6.5	5.8

ns, * and **: non-significant and significant in the probability levels of 5%, and 1%, respectively

umbels per plant when irrigation was interrupted during the vegetative and flowering periods. In general, drought stress reduced the quantity of umbels per plant significantly when compared to the control. The treatment of interrupting irrigation during two vegetative and flowering phases resulted in the highest reduction (19% reduction compared to the control). The usage of 1 mM melatonin increased umbel production by 8 and 3%, respectively, as compared to the control and the concentration of 0.5 mM (Tables 4 and 5).

The number of umbellates per umbel, one of the major yield components, was affected by the treatment of irrigation cut-off and melatonin. Their interaction on the quantity of umbellates was not significant (Table 4).

Only the stoppage of irrigation during the vegetative and flowering periods resulted in a decrease in the

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Continu of table 4.

S. O. V.	df	Mean squares				
		1000 seed weight	Seed yield	Biological yield	Oil	Essential yield
Block	2	0.0002	9.33	10067.4	0.0091	0.17
Irrigation (I)	3	0.0050^{**}	28334.32^{**}	252777.5^{**}	1.946^{**}	124.12^{**}
First error	6	0.0001	75.63	5163.7	0.0119	0.18
Melatonin (M)	2	0.0037^{**}	683.08^{**}	21909.0^{**}	0.0698	0.48ns
I×M	6	0.0012	48.82	5621.99^{*}	0.119^{*}	2.35^{*}
Total error	16	0.0007	2521.39	26144.3	0.207	11.39
CV (%)	-	2.8	11.2	8.4	4.9	5.2

ns, * and **: non-significant and significant in the probability levels of 5%, and 1%, respectively

Table 5. Means comparison of morphological, yield components and yield traits of ajowan affected by experimental treatments

Treatments	Plant height (cm)	Stem diameter (cm)	N. of umbel per plant	N. of umbellate per umbel	N. of Seed per umbel	1000 seed weight (g)	Seed yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	Oil (%)	Essential yield (kg.ha^{-1})
					Irrigation					
Control (A)	91.33^{a}	9.14^{a}	245.33^{a}	12.556^{a}	19.11^{a}	0.347^{ab}	458.78^{a}	1416.1^{a}	4.13^{a}	18.98^{a}
Vegetative stage (B)	81.89^{b}	8.68^{b}	235.67^{b}	12.222^{a}	18.67^{a}	0.353^{a}	433.33^{b}	1657.6^{b}	3.62^{b}	15.69^{b}
Flowering stage (C)	74.11^{c}	7.34^{d}	228.78^{b}	12.000^{a}	17.28^{b}	0.341^{b}	425.00^{c}	1302.8^{c}	3.41^{b}	14.50^{c}
(B) + (C)	66.44^{d}	8.01^{c}	197.67^{c}	10.389^{b}	15.67^{c}	0.301^{c}	330.56^{d}	1036.7^{d}	3.02^{c}	10.00^{d}
control	77.75^{a}	7.94^{b}	217.67^{c}	10.792^{b}	-	0.316^{c}	404.50^{b}	1229.2^{b}	3.57^{a}	-
0.5 mM	78.00^{a}	8.29^{ab}	227.50^{b}	12.000^{a}	-	0.341^{b}	411.67^{b}	1298.0^{a}	3.46^{a}	-
1 mM	79.58^{a}	8.65^{a}	235.42^{a}	12.583^{a}	-	0.350^{a}	419.58^{a}	1307.6^{a}	3.60^{a}	-
Control×Control	89.67^{b}	-	-	-	-	-	-	1340.0c	4.13^{ab}	18.65
Control×0.5mM	89.67^{b}	-	-	-	-	-	-	1465.0^{a}	4.30^{a}	19.56^{a}
Control×1 mM	91.33^{a}	-	-	-	-	-	-	1443.3^{ab}	3.98^{ab}	18.72^{a}
(B)× (A)	81.00^{c}	-	-	-	-	-	-	1378.7^{bc}	3.96^{b}	17.05^{b}
(B)× 0.5 mM	81.00^{c}	-	-	-	-	-	-	1345.3^{c}	3.37^{cd}	15.20^{c}
(B)× 1 mM	83.67^{c}	-	-	-	-	-	-	1348.7^{c}	3.53^{c}	14.82^{cd}
(C)× (A)	74.00^{d}	-	-	-	-	-	-	1245^{d}	3.30^{cd}	13.70^{d}
(C)× 0.5 mM	73.33^{d}	-	-	-	-	-	-	1320^{c}	3.430^{c}	14.93^{cd}
(C)× 1 mM	75.00^{d}	-	-	-	-	-	-	1343^{c}	3.50^{c}	14.88^{cd}
(B) + (C) × (A)	66.33^{e}	-	-	-	-	-	-	953^{f}	2.9^{e}	9.33^{e}
(B) + (C) × 0.5 mM	68.00^{e}	-	-	-	-	-	-	1062^{e}	3.10^{de}	10.39^{e}
(B) + (C) × 1 mM	65.00^{e}	-	-	-	-	-	-	1095^{e}	3.06^{de}	10.27^{e}

Means within each column followed by the same letter are not significantly different (LSD, 0.05 probability level).

quantity of umbellates, with no significant difference detected between the other irrigation cut-off treatments and the control. There was no significant difference in the number of umbellate per umbel between 0.5 and 1 mM melatonin levels, and both treatments boosted umbellate production compared to the control (Table 5).

Only irrigation cut-off treatments impacted the quantity of seeds, whereas irrigation cut-off treatments and melatonin levels affected the 1,000-seed weight (Table 4). The control and irrigation cut-off at the vegetative stage treatments had the highest 1,000-seed weight and seeds per umbel (Table 5). When compared to control and 0.5 mM melatonin treatments, using 1 melatonin treatments. Melatonin use at various doses increased biomass yield compared to the control (Table 5).

The control treatments and irrigation cut-off in the vegetative stage produced the maximum percentage of essential oil and yield of essential oil. Melatonin foliar mM melatonin had the most positive effect on 1,000-seed weight.

Different irrigation levels affected the seed yield, biomass yield, and percentage of essential oil (Table 4). The highest adverse effect of irrigation cut-off on these parameters was detected throughout the vegetative, flowering, and flowering phases. In other words, while the irrigation cut-off produces a drop in yield and yield components, the biggest detrimental effect is noticed during the vegetative and flowering periods. The highest seed yield was seen in the 1 mM melatonin treatment, and there was no significant difference in seed yield between the absence of melatonin and the 0.5 mM spraying had no positive effect on the essential oil yield, and no significant difference was found between the use and non-use of melatonin (Table 5).

Discussion

Our findings show that irrigation cut-off treatments have a detrimental effect on the quantity of chlorophylls a and b, whereas treatment with 0.5 and 1 mM melatonin increased the plant's photosynthetic pigments. The treatment with no irrigation had a detrimental effect on the quantity of chlorophylls a and b, but treatments with 0.5 and 1 mM melatonin increased the plant's photosynthetic pigments. Many other studies have reported the adverse effects of drought stress on various plants, including a significant decrease in growth, photosynthetic pigments, indole acetic acid (IAA), element contents, yield and yield components, and an increase in proline content, phenol, flavonoid, lipid peroxidation in the form of malondialdehyde, and various antioxidant enzymes (Liu *et al.*, 2015; Ye, *et al.*, 2016; Li *et al.*, 2018; Naghizadeh *et al.*, 2019; Sadak, *et al.*, 2020 (A); Sadak *et al.*, 2020 (B); Li *et al.*, 2021). Biological stresses also contribute to the buildup of ROS (Ye *et al.*, 2016; Naghizadeh *et al.*, 2019; Bidabadi *et al.*, 2020; Li *et al.*, 2018; Guo, *et al.*, 2020 (B); Li *et al.*, 2021). Although a moderate level of ROS is required for the activation of the stress response pathway and the activation of the defense pathway, high levels of ROS damage cell membranes and increase membrane permeability, ultimately leading to organelle destruction and cell death (Najafi *et al.*, 2021). Cell shrinkage and the formation of ROS, peroxidation of membrane lipids, increased membrane permeability, and damage to chloroplast grana lamellae are all caused by a lack of water, which may be the result of a lowered photosynthesis rate. Drought stress causes a reduction in photosynthesis rate, stomatal conductance, transpiration rate, photochemical efficiency of photosystem II, and photosynthetic electron transfer rate in plants (Campos *et al.*, 2019).

According to Naghizadeh *et al*. (2019), under drought stress circumstances, melatonin as an antioxidant produced chemical detoxification, membrane stability, and membrane fluidity restoration. Melatonin was found to reduce the effects of drought stress by activating the antioxidant defense system. Melatonin, according to Li *et al*. (2018), lowers the formation of free radicals, H_2O_2, and other compounds that are detrimental to cell membranes by enhancing the activity of antioxidant enzymes. Melatonin preserves the plant's photosynthetic apparatus and the photosynthetic electron transport chain, as well as improving the plant's photosynthetic efficiency recovery in the face of drought stress (Liang *et al.*, 2018; Guo *et al.*, 2020 (a)). Melatonin has also been shown to boost the plant's root system, improving water and nutrient absorption, photosynthetic capability, and biomass production (Posmyk and Janas, 2009; Liu *et al.*, 2015; Li *et al.*, 2018). Ye *et al.* (2016) studied the effect of melatonin foliar application on the development of maize seedlings under drought stress and found that melatonin boosted drought tolerance of maize seedlings by decreasing drought-induced photosynthetic inhibition and oxidative damage. They went on to say that by enhancing the activity of antioxidant enzymes and non-enzymatic antioxidants in plants under drought stress, they were able to diminish the buildup of ROS and, as a result, the damage caused by drought to the photosynthetic system. Melatonin also helps to eliminate ROS in cells during drought stress by modulating the ascorbate-glutathione cycle (Tiwari *et al.*, 2020). The rate of electron transport and photosynthesis, as well as the pace of the ascorbate-glutathione cycle, all play key roles in plant drought tolerance (Guo *et al*., 2020 (b)). According to Liang *et al.* (2019), melatonin treatment preserves and reduces chlorophyll degradation by inhibiting chlorophyllase activity, pheophytinase (PPH), chlorophyll-decomposing peroxidase, and pheophorbide oxygenase, and thus improves photosynthesis, respiration, and stomatal conduction of the plant under stress conditions. Melatonin administration alleviated the deleterious effects of drought stress, and seedlings treated with melatonin exhibited better antioxidant levels, lower ROS content, higher photosynthetic rate, and photosystem II quantum performance (Liu *et al.*, 2015; Ye *et al.*, 2015; Fleta-Soriano *et al.*, 2017; Guo *et al.*, 2020 (B)). Melatonin foliar spray increased the expression of ascorbate- and glutathione producing genes under drought stress conditions in wheat (Cui *et al.*, 2017) and maize (Guo *et al.*, 2020 (B)). Melatonin lowers oxidative damage greatly under drought stress circumstances (Ye *et al*., 2016; Cui *et al.*, 2017; Li *et al.*, 2018; Guo *et al.*, 2020 (b)). Melatonin-treated seedlings under drought stress had thicker lamina, unaltered leaf structure, and reduced levels of ROS (Cui *et al.*, 2017). Melatonin also reduces the content of malondialdehyde, hydrogen peroxide, and superoxide anion, thereby maintaining and increasing stomatal conductance and, as a result, improving the transfer of water, carbon dioxide, and oxygen to maintain photosynthesis stability in the face of drought stress (Zhang *et al.*, 2013; Cui *et al.*, 2017). Guo *et al*. (2020b) investigated two contrasting maize genotypes in terms of drought tolerance under drought stress with melatonin treatment and reported that melatonin application improved photochemical efficiency of photosystem II, effective quantum efficiency of photosystem II and I, and the rate of electron transfer between photosystem II and I under drought stress in both genotypes, but not the quantum efficiency of energy loss and donor and acceptor damage of photosynthetic. Melatonin can also mitigate the negative effects of drought stress by increasing photosynthetic pigments, indole acetic acid, phenolics, TSS, free amino acid content, proline, and antioxidant enzyme systems. According to Liu *et al*. (2015), the administration of melatonin increases the plants' ability to withstand stress and lowers damage to photosystem II by boosting non-photochemical energy loss. Furthermore, the influence of melatonin on stomatal activity during drought stress has been reported to promote photosynthesis and water usage efficiency (Liu *et al.*, 2015). Melatonin pretreatment enhanced relative water content,

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photosynthetic gas exchange, and stomatal behavior, according to Li *et al*. (2021). They continued, melatonin inhibits chlorophyll breakdown and minimizes photosynthetic damage in the plant in two ways: by preserving water content and breaking down ABA during drought stress circumstances.

One of the osmotic regulating molecules is proline (Arnao *et al.*, 2019). Drought stress increased the quantity of proline in alfalfa (*Medicago sativa* L.) and soybean (*Glycine max* L.) seedlings treated with melatonin (Antoniou *et al*., 2017; Cao *et al*., 2019). The accumulation of osmotic regulating chemicals and thicker epidermal cells in melatonin-treated seedlings may be attributable to greater relative pressure of mesophyll cells and improved water holding capacity (Turk *et al.*, 2014; Antoniou *et al.*, 2017). In the cells of seedlings treated with melatonin, water pressure, water balance, epidermal cell growth, reduction of water loss, improvement of antioxidant capacity, protection of photosynthetic apparatus, reduction of osmotic potential, and increase of water retention capacity were observed (Wei *et al.*, 2015; Fleta-Soriano *et al.*, 2017).

Melatonin has also been shown to assist plants in retaining their chlorophyll content by enhancing their capacity to control osmosis, minimizing oxidative damage, and moderating drought stress (Ye *et al.*, 2016; Li *et al.*, 2018). Drought stress suppresses cell division, elongation, and differentiation via increasing cyclin-dependent kinase (CDK) gene expression (Magwanga *et al.*, 2018), and as a result, it has a detrimental effect on plant vegetative characteristics such as height, number of branches per plant, wet and dry weight of the plant, and leaf weight, resulting in decreased plant growth (Sadak *et al*., 2020 (a); Hossain *et al.*, 2020). Our findings also revealed that irrigation cut-off during the vegetative + flowering stages had the most adverse effect on the plant's morphological parameters, such as stem height and diameter, and that utilizing melatonin enhanced the plant's morphological traits. Melatonin reduces the negative effects of drought stress on plant vegetative and physiological characteristics (Sharma *et al.*, 2020), and it maintains plant growth and increases tolerance to drought stress by regulating reactive oxygen and nitrogen species (ROS and RNS) and increasing photosynthetic efficiency (Liu *et al.*, 2015; Debnath *et al.*, 2019; Li *et al.*, 2021). Drought stress caused a significant decrease in various growth parameters, photosynthetic pigments, yield and yield components, and a significant increase in the content of phenol, total soluble sugars, proline, and free amino acids, as well as some antioxidant enzymes (superoxide dismutase, catalase, peroxidase, and polyphenol oxidase) in two varieties of flax, according to Sadak *et al.* (2020 a)). They claimed that exogenous melatonin boosted many growth and performance metrics, as well as biochemical and physiological parameters of the plant, and decreased the negative impacts of drought stress.

Conclusion

Drought stress reduced the amount of chlorophyll *a*, *b*, carotenoids, plant height, stem diameter, yield components, biomass yield, seed yield, and oil yield in ajowan during the vegetative + flowering phase. The most significant adverse effect of the irrigation cut-off was detected in the stoppage of irrigation during vegetative + flowering and flowering. The use of 1 mM melatonin as a phytohormone enhanced the plant's morphological and functional properties while decreasing the detrimental impacts of drought stress. A 0.5 mM melatonin concentration had no effect on height, seed yield, or proline content. In other words, the data suggested that increased melatonin concentrations improved the plant's growth and functioning qualities.

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