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Role of melatonin in improving growth, yield quantity and quality of *Moringa oleifera* L. plant under drought stress

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Abstract

Background: Melatonin, an indoleamine compound, has the ability to regulate a lot of physiological and biochemical processes under different environmental stresses such as drought stress.

Materials and methods: So, this investigation was done to study the physiological role of melatonin on *Moringa oleifera* plants grown in sandy soil at normal and drought stress.

Results: Subjecting *M. oleifera* plant to drought stress caused significant decreases in growth, photosynthetic pigment, indole acetic acid (IAA), element contents, yield, and yield attributes, meanwhile increased lipid peroxidation is expressed as malondialdehyde (MDA) and various antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX). On the other hand, foliar treatment with different concentrations of melatonin significantly increased growth parameters, yield quantity, and quality of *M. oleifera* plant at normal and drought-stressed conditions via improving photosynthetic pigments (IAA), phenolic and element contents, and antioxidant enzyme systems, whereas declined malondialdhyde (MDA) contents as compared with their corresponding untreated controls (M0). Foliar treatment with 100 mM melatonin showed the greatest growth criteria and yield components of *M. oleifera* plants at either normal irrigation or under drought stress. This concentration also improved amino acid constituents of the yielded *M. oleifera* plants compared with other concentrations under normal and stress conditions.

Conclusion: We can conclude that spraying *M. oleifera* with melatonin could alleviate the reduced effect of drought stress.

Keywords: Amino acids, Antioxidant enzymes, IAA, MDA, Melatonin, *Moringa oleifera*, Phenolics, Vegetative growth

Introduction

Moringa (*Moringa oleifera* L.) plant, family *Moringacea*, is commonly known as the miracle tree as it has a multitude of medicinal and nutritional values. Its leaves have a large amount of important minerals, vitamins, amino acids, and antioxidant compounds. *M. oleifera* plants are used as cardiac and circulatory stimulants, as well as it has antihypertensive, cholesterol-lowering, antioxidant, anti-diabetic, and hepatoprotective effects (Anjorin et al. 2010). Different parts of the tree are edible, such as leaves, roots, flowers, immature pods, and seeds, as they are a highly nutritive vegetable (Arabshahi and Devi 2007 and Ezzo et al. 2018). In developing nations, *M. oleifera* is used as an alternative to imported food supplements to treat and combat malnutrition, especially among infants and nursing mothers, by virtue of its chemical constituents (A. O. A. C. 1990).

Drought stress is a major problem that restricts crop growth and production because it adversely affects on different biochemical and physiological processes in plant cells. The extent of drought stress effects depends on the duration of time and intensity of stress, growth stage, and genetic tolerance capacity of plants (Dacosta and Huang 2007). The most important deleterious effects of drought stress are the reduction in relative water content, reduction in water potential of leaf, loss of

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turgor, and cell enlargement reduction, and then decreases of photosynthetic pigments, disturbance of different metabolic processes, and finally plant death (Boyer 1992). Also, drought stress caused oxidative damage via increasing accumulation of reactive oxygen species (ROS), which reduces photosynthesis and stomatal closure and alters the activities of enzymes. ROS formation is considered a threat to cell as it causes electron leakage, lipid peroxidation, and subsequent membrane damage, as well as damage to nucleic acids and proteins (Maksup et al. 2014). To decrease these damages, plants have evolved different pathways such as increasing antioxidant compounds either non-enzymatic antioxidant (such as glutathione, ascorbic acid carotenoids, and α-tocopherols) or enzymatic antioxidants (including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GPX)) (Abd Elhamid et al. 2014). Another antioxidant compound that improves plant tolerance in plant tissue is the different phenolic compounds. In plant tissues, many phenolic compounds are potential antioxidants such as flavonoids, tannins, and lignin precursors, and these compounds might act as ROS-scavenging compounds (Rice-Evans et al. 1997). These antioxidants act as a cooperative network, employing a series of redox reactions. Moreover, it has been shown that phenolic compounds can be involved in the ROS scavenging cascade in plant cells (Dawood and Sadak 2014). The global climate change in the last 50 years significantly reduced plant growth and yields due to the increases in drought and heat stresses (Hillel and Rosenzweig 2002). Thus, the enhancement of plant yields under different environmental stresses is an urgent task to meet the booming food demands of the ever-increasing population. Treatment of exogenous natural plant growth substances has been demonstrated to be an effective strategy to improve plant tolerance to drought (Peleg and Blumwald 2011).

Melatonin (N-acetyl-5-methoxytryptamine) is an indole-based structure and low molecular weight compound that exists in different living organisms. Melatonin plays an important role as a potential modulator on plant growth and development (Li et al. 2012). In plant cells, melatonin affects on various physiological processes and biochemical aspects such as shoot and root growth, flower formation, flower and fruit development, and delaying leaf senescence (Park et al. 2013). Melatonin helps in homeostasis of various ions (Sarropoulou et al. 2012). The lipophilic and hydrophilic nature of melatonin and the ease of passing through morpho-physiological barriers resulted in rapid transport of the molecule into plant cells (Tan et al. 2012a). Melatonin has an important role in improving plant tolerance against different abiotic or environmental stress such as drought, salinity, and heat (Kabiri et al. 2018). Melatonin acts as a growth promoter in plant cells and as an antioxidant compound under oxidative stress and those are the main functions of it (Arnao and Hernandez-Ruiz 2014). Melatonin could alleviate the harmful effects of oxidative damages on proteins, lipids, and nucleic acids via its role as ROS scavenger or by regulating non-enzymatic antioxidants or enzymatic antioxidant biosynthesis or activities (Li et al. 2015). The improved role of melatonin on decreasing oxidative damage induced by water deficit has been proven earlier in different plant species of cucumber (Zhang et al. 2014a), apple (Li et al. 2015), soybean (Wei et al. 2015), wheat (Sadak 2016a), **Moldavian balm** (Kabiri et al. 2018), and maize (Huang et al. 2019). Treatment of plants with melatonin enables plants to tolerate abiotic stress via enhancing the recovery potential (Tan et al. 2012b).

So the aim of this study was to investigate the physiological role of the foliar treatment of melatonin with different concentrations on growth, some biochemical aspects, and yield of *M. oleifera* plant grown under drought stress.

Materials and methods

Plant material and growth conditions

Moringa oleifera L. plants were transplanted in the Experimental Station of National Research Centre, Nubaria District, Beheira Governorate, Egypt. This experiment was done during two successive seasons of 2015/2016 and 2016/2017. The soil of both experimental sites was newly reclaimed sandy soil where mechanical and chemical analyses are presented in Table 1, according to Chapman and Pratt (1978).

The experimental design was split-plot into four replications. The main plots were devoted to the irrigation treatments, while melatonin treatments were randomly occupied by the subplots. Each plant was fertilized with 40 g calcium superphosphate (15.5% P_2O_5), 20 g potassium sulfate (48.0% K_2O), and 40 g urea (46.5% N) mixed with 500 g green manures (compost). Melatonin foliar treatment consisted of three levels of melatonin namely 0.0 control, 50, 100, and 150 mM considered as Mel0, Mel1, Mel2, and Mel3, respectively, at 45 and 60 days after *M. oleifera* cutting. Drought stress includes normal irrigation (D0) and skipping two irrigation times (D1). After the second foliar treatment by 2 weeks and 3 weeks, plant samples were taken after the second skipping irrigation by week. An estimation of some growth parameters such as plant height (cm), number of leaves/plant, fresh and dry weights of leaves and shoots (g), photosynthetic pigments of leaves, indole acetic acid (IAA) and phenolic contents, lipid peroxidation expressed as MDA, and some antioxidant enzyme activity (SOD, CAT, and POX), as well as the yield of *M. oleifera*, here referred to as foliage yield, the yield and its

Table 1 Mechanical and chemical analyses of the experimental soil sites

A. Mechanical analysis									
Sand					Silt 20 – 0 μ (%)		Clay < 2 μ (%)		Soil texture
Course 2000 – 200 μ (%)			Fine 200 – 20 μ (%)						
47.46			36.19		12.86		4.28		Sandy
B. Chemical analysis									
pH (1:2.5)	EC (dSm^{-1})	$CaCO_3$ (%)	OM (%)	Macroelement (ppm)			Microelement (ppm)		
				N	P	K	Zn	Fe	Mn
7.60	0.13	1.5	0.06	52	12.0	75	0.14	1.4	0.3

components (foliage yield) as plant height (cm), stem diameter (cm), and the nutritive value of the yield of nitrogen, phosphorus, potassium, calcium, and magnesium contents in addition to amino acid content of the yielded leaves, was performed..

Biochemical analysis

Photosynthetic pigments

Total chlorophyll a and b and carotenoid contents in fresh leaves of *M. oleifera* plant were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue was ground in a mortar and pestles using 80% acetone. The optical density (OD) of the solution was recorded at 662 and 645 nm (for chlorophyll a and b, respectively) and 470 nm (for carotenoids) using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in milligrams per gram FW.

Indole acetic acid content

A known weight of the fresh samples was taken and extracted with 85% cold methanol (v/v) for three times at 0 °C. The combined extracts were collected and made up to a known volume with cold methanol. Then take 1 ml of the methanolic extract and 4 ml of PDAB reagent (para-dimethylamino benzoic acid 1 g dissolve in 50 ml HCl and 50 ml of ethanol 95%) and left for 60 min in 30-400 °C. The developing color was spectophotometrically measured at wave length of 530 nm, as described by Larsen et al. (1962).

Total phenol content

The extract was extracted as IAA extraction, and then 0.5 ml of the extraction was added to 0.5 ml Folin and shaked and allowed to stand for 3 min. Then 1 ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60 min. The optical density was determined at wave length of 725 nm using spectrophotometer as described by Danil and George (1972).

Lipid peroxidation

The levels of lipid peroxidation were measured by determining the levels of malondialdehyde (MDA). Malondialdehyde is a product of lipid peroxidation and was assayed by thiobarbituric acid reactive substrate (TBARS) contents using the method of Hodges et al. (1999) with some modifications. Fresh leaf samples (0.2 g) were ground in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) at 4 °C. Following the centrifugation at 12,000×*g* for 5 min, an aliquot of 1 ml from the supernatant was added to 4 mL of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA. Samples were heated at 90 °C for 30 min. Thereafter, the reaction was stopped in ice bath. Centrifugation was performed at 10,000×*g* for 5 min, and absorbance of the supernatant was recorded at 532 nm on a spectrophotometer (Model Camspec M330 UV/Vis) and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The following formula was applied to calculate malondialdehyde content using its absorption coefficient (ε) and expressed as *n* mol malondialdehyde g − 1 fresh mass following the formula:

MDA (nmol g − 1 FM) = [(A532 − A600) × V × 1000/ε] × W, where ε is the specific extinction coefficient (= 155 mM cm − 1), V is the volume of the crushing medium, W is the fresh weight of the leaf, A600 is the absorbance at 600 nm wavelength, and A532 is the absorbance at 532 nm wavelength.

Assay of enzyme activities

Enzyme extractions were collected following the method described by Chen and Wang (2006). Leaf tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation at 8000 rpm and 4 °C for 15 min. The supernatant was used immediately to determine the activities of enzymes.

Superoxide dismutase Assay of superoxide dismutase was carried according to the method of Chen and Wang (2006). 0.5 ml of plant extract, 1 ml of 125 mM sodium carbonate, 0.4 ml of 25 μM NBT, and 0.2 ml of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride, and the

absorbance was read at 560 nm using a spectrophotometer at 1-min intervals. Units of SOD were expressed as amount of enzyme required for inhibiting the reduction of NBT by 50%.

Catalase activity Catalase (CAT) (EC 1.11.1.6) activity was determined spectrophotometrically by following the decrease in absorbance at 240 nm (Chen et al. 2000). The mixture (3 ml) contained 1.9 ml phosphate buffer (50 mM, pH 7.0), 100 μl enzyme extract, and 1 ml 0.3% H_2O_2. The reaction was initiated by adding an enzyme extract. One unit of CAT activity was defined as the 0.01 deduction in absorbance at 240 nm per minute.

Peroxidase activity Peroxidase (POX, EC 1.11.1.7) activity was assayed by the method of Kumar and Khan (1982). The reaction mixture used for estimating the peroxidase enzyme (POX) contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2, and 0.5 ml of the enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a reagent blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at the zero time.

Macro element contents Macro element contents of *M. oleifera* plants were determined according to Chapman and Pratt (1978). Nitrogen and phosphorus were determined using Spekol Spectrocololourimeter VEB Carl Zeiss. Ca and K contents were estimated by using a flame photometer. Mg contents were estimated using an atomic absorption spectrophotometer.

Amino acid constituents Based on the results of the first season (2015/2016), the most promising treatments were selected for the determination of amino acid compositions of the *M oleifera* plants and were carried out by using HPLC (Eppdrof, Germany).

Statistical analysis

Statistical analysis was performed using the MSTATC computer software package. Using analysis of variance procedure of split-plot design according to Snedecor and Cochran (1990), treatment means were compared using the Duncan (1955) test at 5% of probability and presented with standard errors. Combined analysis of the two growing seasons was carried out.

Results

Changes in growth criteria

Table 2 shows the effect of melatonin foliar treatment with different concentrations on the growth criteria of *M. oleifera* plants grown under drought stress. Data clearly show that subjecting *M. oleifera* plant to drought stress significantly decreased growth parameters such as plant height, number of branches/plant fresh, and dry weights of leaves and shoots. The percentages of decreases were 19.29%, 28.57%, 38.29%, 12.70%, 14.97%, and 24.88% of plant height, number of branches/plant fresh, and dry weights of leaves and shoots, respectively. On the other hand, treatment of *M. oleifera* plants with different concentrations of melatonin increased all the abovementioned growth parameters at either normal irrigation or skipping two irrigation times, as compared with their corresponding controls (Table 2). Data clearly show that 100 mM was the most effective treatment as it caused the greatest increase in growth criteria of *M. oleifera* plant at either normal or drought irrigated plants.

Changes in photosynthetic pigments

The effect of foliar application of melatonin with different concentrations (0, 50, 100, and 150 mM) on *M. oleifera* plant grown under drought stress is presented in Fig. 1. The obtained data show that subjecting *M. oleifera* plants to drought stress (by skipping two irrigation

Table 2 Effect of melatonin (0, 50, 100, and 150 mM) on growth characters of *M. oleifera* plants subjected to drought stress (normal irrigation and skipping two irrigation times). Data are means of two seasons

Drought	Melatonin	Plant height (cm)	Number of leaves/plant	Leaves fresh wt. (g)	Shoot fresh wt. (g)	Leaves dry wt. (g)	Shoot dry wt. (g)
D0	M 0	103.67	14.00	24.97	29.22	5.01	8.44
	M 1	107.00	17.67	26.19	42.02	5.91	13.81
	M 2	115.33	17.67	35.65	50.53	10.29	13.95
	M 3	113.00	16.33	32.91	36.66	9.48	13.11
D1	M 0	83.67	10.00	15.41	25.51	4.26	6.34
	M 1	91.33	11.33	17.79	36.25	4.88	7.55
	M 2	95.67	13.33	24.19	44.54	8.69	9.04
	M 3	92.67	12.33	18.95	32.17	7.31	8.5
LSD at 5%		5.65	1.35	1.85	6.75	1.02	0.86

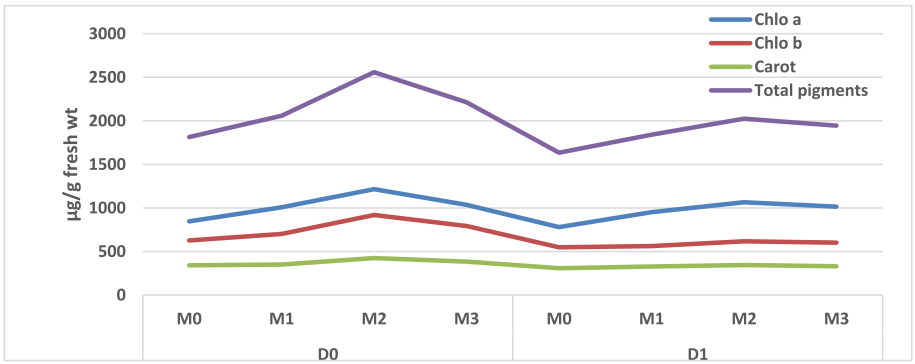

Fig. 1 Effect of melatonin (0, 50, 100, and 150 mM) on photosynthetic pigments (µg/g fresh wt.) of *M. oleifera* plants subjected to drought stress (normal irrigation and skipping two irrigation times)

times) caused significant decreases in photosynthetic pigment constituents (chlorophyll a, chlorophyll b, carotenoids, and total pigments) as compared with control plants. With respect to foliar treatment of melatonin, data showed that melatonin foliar treatment increased chlorophyll a, chlorophyll b, carotenoids, and consequently total pigments significantly and gradually till 100 mM then decreased but still greater than control plants as compared with untreated control plants. A total of 100 mM was the most effective treatment as it causes the highest increases of all photosynthetic pigment constituents either of normal irrigated plants or drought-stressed plants (Fig. 1).

Changes in endogenous indole acetic acid (IAA) and phenolic contents

The changes in endogenous indole acetic acid content of *M. oleifera* plant treated with different concentrations of melatonin and grown under drought stress are presented in Fig. 2. Subjecting *M. oleifera* plants to drought stress by skipping two irrigation times caused significant decreases in indole acetic acid contents, meanwhile significantly increased phenolic content relative to the control plant (Fig. 2). On the other hand, melatonin concentrations (M1, M2, and M3) under normal irrigation caused gradual increases in indole acetic acid and phenolic content relative to the control plant. Under drought stress, the effect of melatonin on indole acetic acid and phenolic contents of *M. oleifera* plants was similar to its effect on the plants normally irrigated as compared with their corresponding untreated controls. The increases were gradual and significant with increasing melatonin concentrations till 100 mM, and then at 150 mM foliar treatment, IAA and phenolic contents decreased but still higher than their corresponding controls. It is worthy to mention that 100 mM melatonin was the most effective treatment on the enhancement of the studied parameters (indole acetic acid and phenolic contents) at either normal conditions or stressed conditions.

Changes in lipid peroxidation

The changes in MDA which is an indicator of lipid peroxidation of plant cells are presented in Fig. 3. Data clearly show the increased lipid peroxidation of *M. oleifera* cells expressed as increased levels of malondialdehyde (MDA) contents when plants subjected to drought stress as compared with those irrigated normally. These

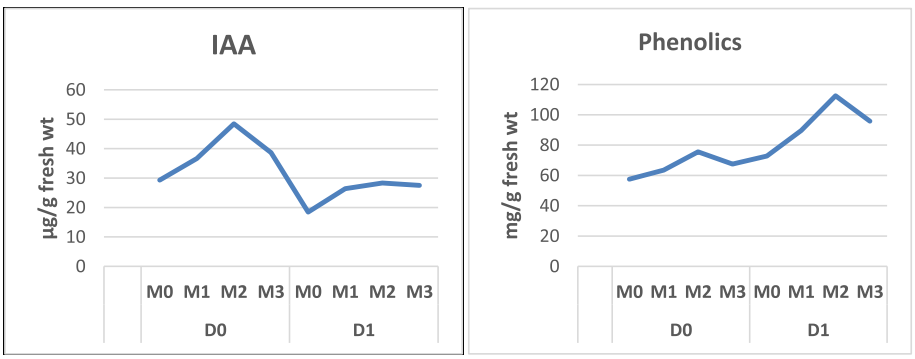

Fig. 2 Effect of melatonin (0, 50, 100, and 150 mM) on indole acetic acid (µg/g fresh wt.) and phenolic contents (mg/g fresh wt.) of *M. oleifera* plants subjected to drought stress (normal irrigation and skipping two irrigation times)

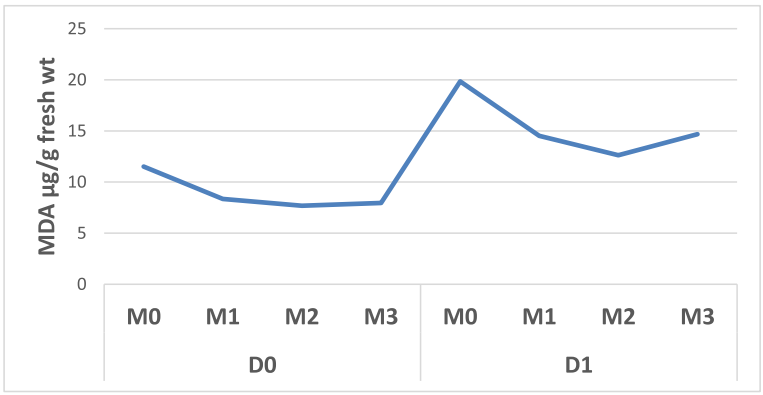

Fig. 3 Effect of melatonin (0, 50, 100, and 150 mM) on MDA (µmol/g fresh wt.) of *M. oleifera* plants subjected to drought stress (normal irrigation and skipping two irrigation times)

increases were significant increases. Meanwhile, foliar treatment of *M. oleifera* plant with different concentrations of melatonin caused significant, and decreases in MDA contents either in plant irrigated normally or drought-stressed plants compared with their corresponding untreated controls. A total of 100 mM melatonin was the most effective treatment as it caused the lowest decreases in MDA contents either at normal or drought-stressed conditions.

Changes in antioxidant enzymes

Activities of various specific antioxidant enzymes in drought-stressed and melatonin foliar-treated plants are presented in Fig. 4. Drought stress by skipping irrigation caused significant increases in SOD, CAT, and POX. The percentages of increases were 71.70, 64.09, and 33.65 of SOD, CAT, and POX, respectively, as compared with the control plant. Moreover, foliar treatment with different concentrations 50, 100, and 150 mM of melatonin increased significantly SOD, CAT, POX activities as compared with their corresponding untreated controls not only of drought-stressed plants but also of those plants which grow under normal irrigation conditions. One hundred millimolar melatonin treatment was the most effective treatment on increasing the studied antioxidant enzyme activities of *M. oleifera* plant as compared with other used concentrations.

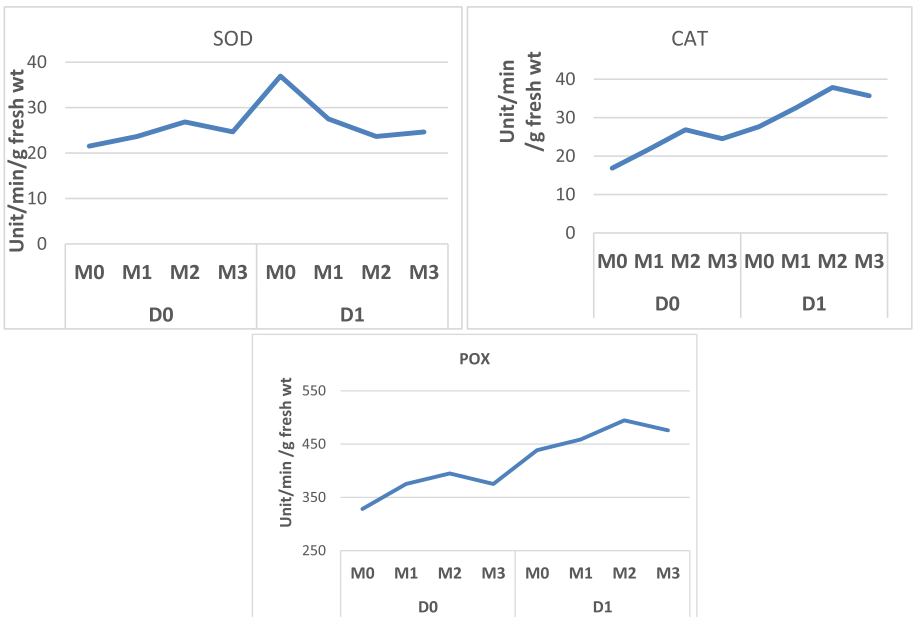

Fig. 4 Effect of melatonin (0, 50, 100, and 150 mM) on antioxidant enzymes (SOD, CAT, and POX unit/min/g fresh wt.) of *M. oleifera* plants subjected to drought stress (normal irrigation and skipping two irrigation times)

Changes in yield and yield components (foliage yield)

The effects of foliar treatments of melatonin with different concentrations on foliage yield and its components of *M. oleifera* plants subjected to drought stress are presented in Table 3. Subjecting *M. oleifera* plant to drought stress by skipping irrigation caused significant decreases in all studied foliage yield and yield components (plant height, number of leaves/plant, stem circumference, fresh and dry wt. of leaves and shoots). On the other hand, melatonin foliar treatment with different concentrations significantly increased all the abovementioned foliage yield and yield components at either normal irrigation or drought stress plants. The most effective treatment was 100 mM as it gave the highest increases in all these studied yield parameters.

Changes in element contents

The changes in element contents of *M. oleifera* plant in response to foliar treatment with different concentrations of melatonin (50, 100, and 150 mM) grown under drought stress are presented in Fig. 5. Nitrogen, phosphorus, potassium, calcium, and magnesium contents of *M. oleifera* plants decreased significantly under drought stress (skipping irrigation) as compared with plants irrigated normally. On the other hand, melatonin foliar treatment increased element contents of *M. oleifera* plants at either normal irrigated plants or at drought stressed plants (Fig. 5). The most effective concentration was 100 mM as it caused the highest increases in different studied element contents as compared with untreated controls at normal and drought stress conditions.

Changes in amino acid composition

Based on the obtained results in the first season, 100 mM melatonin was selected for the determination of amino acid constituents under normal and drought stress conditions as shown in Table 4. Seventeen amino acids were detected in *M. oleifera* leaves with different concentrations. Glutamic acid was the highest values (ranged from 45.87 to 53.13 mg/g dry wt.) followed by phenylalanine ranged from 35.65 to 46.52 mg/g dry wt., followed by threonine ranged from 33.65 to 38.35 mg/g dry wt., followed by proline, histidine, isoleucine, lycine, valine, arginine, and leucine. These various amino acids are considered the predominant amino acids, while other amino acids are considered as minor amino acids. Drought stress caused marked increases in total amino acid contents and total essential amino acid contents (threonine, valine, methionine, leucine, isoleucine, and phenylalanine). Meanwhile, the decreased ratio of essential to non-essential amino acids compared with control plant, with regard to melatonin foliar treatment, in most cases, increases in the content of total amino acids and essential amino acids, and the ratio of essential to non-essential amino acids was obtained compared with the corresponding drought levels (Table 4). It is worthy to mention that the application of melatonin before exposing *M. oleifera* plants to drought stress resulted in an observed accumulation of most of amino acids especially glutamic, proline, tyrosine, histidine, lysine, and arginine contents compared with those of control plants (Table 4).

Discussion

One of the most effective factors affecting not only plant growth and productivity but also the security of food is water (Garg et al. 2002). In our investigation, growth parameters and foliage yield and its components were significantly decreased in *M. oleifera* plants under drought stress (skipping irrigation) (Tables 2 and 3). In harmony with our results of drought stress, Dawood and Sadak (2014) stated that different growth criteria and yield parameters of canola shoots decreased with decreasing water holding capacity, and they referred these decreases to disorders induced by drought stress and generation of reactive oxygen species (ROS). Also, Abd Elhamid et al.

Table 3 Effect of melatonin (0, 50, 100, and 150 mM) on yield and yield components of *M. oleifera* plants subjected to drought stress (normal irrigation and skipping two irrigation times) (data are means of two seasons)

Drought	Melatonin	Plant height (cm)	Number of branches/plant	Stem diameter (cm)	Leaves fresh wt. (g)	Stem fresh wt. (g)	Leaves dry wt. (g)	Stem dry wt. (g)
D0	M0	150.00	17.67	11.67	59.75	198.73	18.65	53.25
	M1	163.33	18.33	11.33	108.09	293.13	27.39	102.80
	M2	183.33	21.33	14.17	176.68	437.61	34.70	123.90
	M3	173.67	19.67	13.67	137.83	402.51	22.60	81.20
D1	M0	110.33	12.33	9.00	28.14	176.12	12.63	39.85
	M1	143.67	16.67	10.67	69.50	380.92	21.87	73.00
	M2	176.00	17.00	12.33	147.31	386.91	24.69	91.50
	M3	173.33	13.33	13.33	56.52	292.91	18.90	74.20
LSD at 5%		15.352	1.987	1.758	11.658	13.135	1.524	1.152

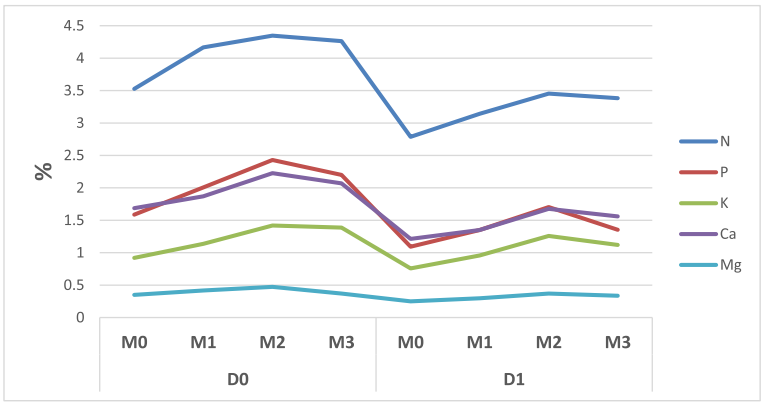

Fig. 5 Effect of melatonin (0, 50, 100, and 150 mM) on element contents of *M. oleifera* plants subjected to drought stress (normal irrigation and skipping two irrigation times)

(2016); Sadak (2016b); Elewa et al. (2017a); Sadiq et al. (2018); and Dawood et al. (2019) reported that drought stress reduced growth characters and yield components of fenugreek, quinoa, mung bean, flax, and sunflower plants. These decreases in plant height in response to drought stress (Table 2) might be due to the decreases in cell elongation, cell turgor, cell volume, and eventually cell growth (Banon et al. 2006). Also, drought affects plant–water relations, decreases shoot water contents, causes osmotic stress, and inhibits cell expansion and cell division consequently growth of plants as a whole (Bakry et al. 2012; Alam et al. 2014). It is worthy to mention that water availability to plant in various plant growth stages affects plant growth and development. The decreases in yield of *M. oleifera* plants resulted from the decreases in growth criteria (Table 2) and photosynthetic pigments (Fig. 1). The decreases in chlorophyll contents in drought stressed *M. oleifera* leaves which caused inhibition of photosynthetic enzyme activities. Thus, it leads to decreased carbohydrate accumulation in mature leaves and consequently might decrease the rate of transport of carbohydrate to the developing organs (Anjum et al. 2003). However, growth and yield reductions by drought were alleviated significantly by different concentrations (50, 100, and 150 mM) of melatonin (Tables 2 and 3). Earlier studies have confirmed that melatonin acted as a growth regulator of plant and thus could increase growth parameters in various plants (Li et al. (2015), Liu et al. (2015), Ye et al. (2016), Cui et al. (2017), Kabiri et al. (2018), and Huang et al. (2019)). Melatonin treatment could alleviate the adverse effects of drought stress on *M. oleifera* plant via improving their growth and physiological attributes. The dual function of melatonin on plant as a growth promoter enhancing growth (Arnao and Hernandez-Ruiz 2014) and as a protector against abiotic stress (Li et al. 2012; Manchester et al. 2000) were highlighted earlier. Moreover, the natural antioxidant capacity of melatonin makes it a useful tool for using as a biostimulant for agricultural goals and improves plant's ability to cope with environmental stresses (Arnao and Hernandez-Ruiz 2014). The obtained data showed that melatonin foliar treatment

Table 4 Effect of melatonin (0 and 100 mM) on amino acid composition of *M. oleifera* plants subjected to drought stress (normal irrigation and skipping two irrigation times)

Amino acid	Drought	D0		D1	
	Treatment	M0	M2	M0	M2
Aspartic acid		6.62	6.83	7.24	7.91
Threonine*		34.36	38.35	33.65	36.33
Serine		6.65	7.21	7.54	7.99
Glutamic		45.87	48.13	50.98	53.13
Proline		22.52	26.65	24.35	28.52
Glycine		5.52	5.99	6.36	6.96
Cystine		2.95	3.16	3.96	3.25
Alanine		12.65	14.85	14.65	13.85
Valine*		22.1	26.65	20.85	22.69
Methionine*		3.52	3.41	4.65	5.02
Leucine*		20.5	27.66	22.85	28.26
Isoleucine*		31.8	37.53	30.51	35.53
Phenylalanine*		36.8	46.52	35.65	37.65
Tyrosine		2.35	2.43	2.65	3.12
Histidine*		32.52	43.52	33.63	40.35
Lysine*		24.65	35.52	24.05	35.62
Arginine*		21.45	30.65	23.52	29.53
Essential AA		227.7	289.81	232.36	270.98
Non-essential AA		105.13	115.25	117.73	124.73
Total amino acids		332.83	405.06	340.09	395.71
Essential A /Non essential A A		2.17	2.51	1.91	2.17

* indicate essential amino acids

could enhance *M. oleifera* responses to drought stress in terms of morphological traits, biochemical, and physiological aspects. The enhancing role of melatonin treatment resulted from the increased growth criteria (Table 2) as well as increased photosynthetic pigments (Fig. 1) thus increased photosynthetic output so increased translocation from source to sink. Moreover, melatonin can mediate various biochemical and physiological processes in plants, i.e., growth regulation and ion homeostasis and increase vegetative growth in a number of plant species leading to increments in seed yield (Janas and Posmyk 2013; Sadak 2016b; El-Awadi et al. 2017a; Li et al. 2017, and Çolak 2018). The promotive effects of melatonin treatment on *Moringa oleifera* plants could be attributed to melatonin as a kind of endogenous plant growth regulator acting in trace amounts (Zhang et al. 2015).

Photosynthesis is the physico-chemical process which use light energy to drive the biosynthesis of different organic compounds and consequently plant production (Ye et al. 2016). Drought is a dangerous abiotic stress-reducing photosynthetic pigments in *M. oleifera* plant (Fig. 1). These obtained data are congruent with those obtained earlier on canola (Dawood and Sadak 2014), fenugreek (Sadak 2016b), quinoa (Elewa et al., 2017a and b), and Moldavian balm (Kabiri et al. 2018). One of the main visible symptoms of water stress in plant leaves is the loss of chlorophylls that are reflecting some disruption in chloroplasts (Sadak 2016b). These decreased resulted by drought stress on photosynthetic pigments could be attributed to the oxidation of pigments that damaged photosynthetic pigments and impaired their biosynthesis (Pandey et al. 2012). Moreover, these decreases of photosynthetic pigments might resulted from photo-oxidation of pigments that cause oxidative, photosynthetic system damaging which leads to reduces in photosynthetic carbon assimilation (Din et al. 2011). Moreover, the principle reason for decreasing photosynthetic rate is that water stress causes limitation of surrounding CO_2 diffusion to the site of carboxylation that is induced by stomatal closure (Liu et al. 2013). Melatonin treatment could alleviate the effect of drought on *M. oleifera* plant and increased photosynthetic pigment contents (Fig. 1). In agreement to these, the following results were obtained: Liu et al. (2015) on tomato, Sadak (2016a) on wheat, Ye et al. (2016) on maize, Cui et al. (2017) on wheat, and Kabiri et al. (2018) on Moldavian balm plants. The improved effect of melatonin on photosynthetic pigments resulted from delaying in loss of chlorophyll and leaf senescence and a reduction in chlorophyll degradation via the improvement of antioxidant enzyme activities that scavenge ROS (Wang et al. 2013). Earlier reports stated that treating an apple tree with melatonin could maintain a significantly higher assimilation rate of CO_2 and stomatal conductance under water stress (Li et al. 2015). Also, this enhancement role could be due to increasing antioxidant contents, antioxidant enzyme activities, and reducing the formation of ROS thus delaying the senescence process (Arnao and Hernandez-Ruiz 2006). Many authors referred the promotive effect of melatonin to the interactive effect of melatonin and other plant growth promoters such as kinetin and ABA on leaf senescence (Arnao and Hernandez-Ruiz 2017).

Subjecting *M. oleifera* plants to drought stress by skipping two irrigation times caused significant decreases in indole acetic acid contents, meanwhile increased significantly phenolic content relative to the control plant (Fig. 2). These decreases in IAA in response to drought stress were concurrent with reduced growth criteria of *M. oleifera* plants (Table 2). The obtained results are similar to those of Sadak (2016a) and Elewa et al. (2017a and b) on fenugreek and quinoa plants, respectively. These decreases in indole acetic acid contents under drought stress could be attributed to the increase in their degradation and/or decrease in their biosynthesis and increase in their transformation to inactive form (Elewa et al. 2017a). These enhancing roles of melatonin on IAA were confirmed by Dawood and El-Awadi (2015) on faba bean plant and Sadak (2016a) on wheat plant.

Phenolic compounds acts as strong antioxidant scavenger of free radicals through their high reactivity as electron or hydrogen donors and thus stabilize the unpaired electron (chain-breaking function) via their ability for chelate transition metal ions (Huang et al. 2005). The increased levels of phenolic contents under drought stress (Fig. 2) could be due to the disturbances in various metabolic processes in plant cells resulted from stress which leads to increases in phenolic compounds (Keutgen and Pawelzik 2009). Drought stress accelerates ROS (reactive oxygen species) production and increased their level in plant cells that are concurrent with the changes in net carbon gain that affects on carbon-based secondary metabolites biosynthesis particularly phenolic compounds (Dawood and El-Awadi 2015). Moreover, the enhancing role of melatonin on phenolic contents resulted from the induction of various metabolic pathways and promotes the formation of different compounds especially operating under stress (Tan et al. 2012b). El-Awadi et al. (2017a) and Çolak (2018) reported that melatonin treatment of different plant species increased phenolic compounds.

Drought stress triggers the accumulation of ROS and disturbs the balance between ROS production and detoxification (Gong et al. 2005). Increased ROS levels in plant cells caused changes in MDA which is an indicator of lipid peroxidation of plant cells. The malonyldialdehyde (MDA) is considered as a marker for evaluation of the degree of the lipid peroxidation or damage to

plasmalemma and organelle membranes that are associated with damages provoked by ROS due to environmental stresses (Ozkur et al. 2009). Drought stress caused a modification in the lipid matrix of the plasma membrane and changes in the physical organization of the membrane (Mirzaee et al. 2013). Meanwhile, foliar treatment of the moringa plant with different concentrations of melatonin caused significant decreases in MDA contents either in plant irrigated normally or in drought-stressed plants compared with their corresponding untreated controls (Fig. 3). Several studies have reported the role of melatonin in the prevention of lipid peroxidation is dependent on its ability to react with lipid peroxyl and lipid alcoxyl radicals and interrupt the peroxidation chain (Sarropoulou et al. 2012; Li et al. 2012; Zhang et al. 2013; Kabiri et al. 2018).

Plant possesses an efficient system for scavenging ROS. Antioxidative enzymes are not part of this system but are key elements in the defense mechanisms. Activities of antioxidant enzymes of plants under stress show many changes (Abdelhamid et al. 2013). Superoxide dismutase, catalase, and peroxidase are of enzymes that are responsible for ROS scavenging. These results are in agreement with Abdelgawad et al. (2014), El-Awadi et al. (2017b), and Kabiri et al. (2018). Higher levels of enzyme activities in *M. oleifera* plant under water deficient may be due to its resistance. NAD^+ recovering and CO_2 fixation at the Calvin cycle decrease under drought stress cause damage to the cell membrane due to the increases of free radicals. Adverse environmental stresses increase catalase activity in several cycles of physiological processes. At stress conditions, a higher content of ROS (especially H_2O_2) is detoxified by catalase (Dat et al. 2000). Superoxide dismutase (SOD) is the first defense enzyme that converts superoxide to H_2O_2, which can be scavenged by catalase (CAT) and different classes of peroxidases (POX). Shi et al. (2007) confirmed the essential role of antioxidant systems in plant tolerance of various environmental stresses especially in tolerant cultivars that had higher activities of ROS-scavenging enzymes than the susceptible ones. Melatonin-treated *M. oleifera* plant shows that MDA content decreased, and this could be related to antioxidant enzyme activities such as SOD, CAT, and POX (Fig. 4). In this concern, melatonin and some of its metabolites are considered as endogenous free radical scavenger and antioxidants (Zhang et al. 2013) that could directly scavenge ROS such as H_2O_2 (Cui et al. 2017). Moreover, one of the main functions of melatonin, along with the activities of SOD and CAT may be to preserve intracellular H_2O_2 concentrations at steady scale levels (Cui et al. 2017). Li et al. (2017) showed that melatonin, a potent long-distance signal, may be translocated from the treated leaves or roots of plant to distant untreated tissues via vascular bundles, leading to systemic induction of a different abiotic tolerance. Moreover, studies on how melatonin interacts with stress signaling mechanisms have identified a complex relationship with ROS. Results concluded that melatonin is a broad-spectrum direct antioxidant which can scavenge ROS with high efficiency. A detailed knowledge of melatonin chemistry and molecular interactions with ROS and with strong oxidants has been documented. As well as, melatonin treatments modulate the antioxidant enzymes by both upregulating the transcript level and increasing the activity levels (Zhang et al. 2014b). Improving plant antioxidant systems has been considered the primary function of melatonin in plant stress tolerance (Zhang et al. 2015). Zhao et al. (2011) had proposed that melatonin protected *Rhodiola crenulata* cells against oxidative stress during cryo-preservation by increasing SOD and CAT activities.

Nitrogen, phosphorus, potassium, calcium, and magnesium contents of *M. oleifera* plants decreased significantly under drought stress (skipping irrigation) as compared with plants irrigated normally (Fig. 5). These reduced effects of drought on element contents are in good agreement with those obtained earlier by several authors such as Peuke and Rennenberg (2011) on beech and Arjenaki et al. (2012) on wheat plant and Ramadan et al. (2019) on sunflower plant. The decreases in K and Mg caused decreases in photosynthetic pigment contents and thus the utilization of fixed carbon (Mengel and Kirkby 2001). Calcium is an essential component of membranes and cell wall that has large functions in metabolic processes such as signaling pathway and stress response. Ca is the most effective divalent and stabilizes cell membrane under abiotic stress via mediating membrane association such as the reduction of ion leakage, uptake of ions and amino acids, and maintenance of the configuration of enzyme binding sites in cells (Fu et al. 2006). On the other hand, melatonin foliar treatment increased element contents of *Moringa oleifera* plants either at normal irrigated plants or at drought-stressed plants (Fig. 5). These enhanced roles of melatonin treatment congruent with the results of Li et al. (2012 and 2017). Thus, the increase in leaf N, P, K, Ca, and Mg of *M. oleifera* plants under drought is likely owing to the promoting effect of melatonin on the accumulation and absorption of these elements. In agreement with previous findings, melatonin application led to an increase in Ca concentration under stressed and unstressed conditions. Similarly, we also found that melatonin application markedly decreased MDA (drought-induced decreases in levels of membrane damage indicators such as electrolyte leakage and lipid peroxidation). This improving effect of melatonin may be the direct physiological cause for the enhancement of *M. oleifera* drought tolerance by melatonin application. In addition, there is evidence

indicating that environmental stress can increase the level of endogenous melatonin in plants (Zhang et al. 2015). Enhanced Ca concentration in melatonin-treated seedlings suggests that melatonin might had achieved its protective effect on membranes by increasing the Ca concentration. These results are confirmed by earlier findings by Jiang et al. (2016) who found the increase in K level of maize plant by melatonin treatment under drought stress and Li et al. (2017) who show that melatonin treatment on rice plant increased K contents under salinity stress.

The patterns of changes in the amino acid composition of the yielded *M. oleifera* leaves treated with melatonin (50, 100, and 150 mM) grown under drought stress are shown in Table 4. Seventeen amino acids were detected in *M. oleifera* leaves with different concentrations. Drought stress caused marked increases in the total amino acid contents and total essential amino acid contents (threonine, valine, methionine, leucine, isoleucine, and phenylalanine), meanwhile, decreased the ratio of essential to non-essential amino acids compared to control plants. With regard to melatonin foliar treatment, in most cases, increases in the content of total amino acids, essential amino acids, and the ratio of essential to non-essential amino acids were obtained compared with the corresponding drought levels (Table 4). Amino acids are directly or indirectly involved in the regulation of plant responses to environmental signals related to environmental stress (Ashraf and Harris 2004). These obtained results are in agreement with the results obtained by the previous studies of Tammam et al. (2008), Kovács et al. 2012, and Abd Elhamid et al. (2014) on different plant species. The accumulation of total amino acids may be involved in osmotic adjustment, free radical scavenging, and maintenance of protein and membrane integrity (Keutgen and Pawelzik 2008). Among all individual free amino acids, proline content in drought-treated leaves increased significantly in *M. oleifera* plants. Proline serves as an important compatible osmolyte, and its accumulation is believed to reduce cellular water potential and avoid deleterious toxicity of high ionic strength; it has also been proposed to serve as reactive oxygen species scavenger (Verbruggen and Hermans 2008) and its accumulation can stabilize the structure of membranes and proteins to minimize the damage of cells under salt stress. In addition, a large increase in proline precursors, glutamate, and arginine was observed in *M. oleifera* leaves under drought stress. A part from the considerable accumulation of proline and its precursors in *M. oleifera* plant also induced an increase in isoleucine, leucine, and aspartate contents. The interactive effect of different salinity levels and foliar spraying of melatonin on *M. oleifera* plant had increases in total amino acid and essential amino acid content. These increases in amino acids, especially proline, can be one of these causes that the inhibitory effect of salinity was alleviated by melatonin foliar applications to *M. oleifera*.

Conclusion

It was concluded from the current field study that using different foliar applications of melatonin is beneficial to mitigate the adverse effects of drought stress in sandy soil under a wide range of field conditions. The results of this study highlight the role of melatonin in improving *M. oleifera* growth and yield under sandy soil conditions via enhancing different biochemical and physiological attributes to minimize the hazardous effects of drought as abiotic stress. In general, 100 mM melatonin encourages the farmers to use it as a new, natural, and low-cost stimulation to enhance *M. oleifera*, which is mediated via improvement in yield and yield components.

Abbreviations

CAT: Catalase; IAA: Indole acetic acid; MDA: Malondialdehyde; POX: Peroxidase; SOD: Superoxide dismutase; TSS: Total soluble sugar

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