

Mitigation of salinity adverse effects of on wheat by grain priming with melatonin

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Abstract: A pot experiment was conducted during (2013/2014 & 2014/2015) at the National Research Centre, Dokki, Giza, Egypt, to study the effect of soaking wheat grains with melatonin (ME) (100 μ M and 500 μ M) on growth, photosynthetic pigments, IAA, yield quantity and quality in fever of nutritional and antioxidant compounds in the yielded grains of wheat plants irrigated with diluted seawater at 3.85 dS/m and 7.69 dS/m. Salinity stress caused marked decreases in wheat plant growth parameters (shoot height, number of leaves/plant, fresh and dry weights of shoot) with significant decreases in photosynthetic pigments and indole acetic acid (IAA) contents. Yield and yield attributes, carbohydrates, protein, nitrogen, phosphorous and potassium contents were decreased in response to different salinity levels. Meanwhile flavonoids and phenolic contents increased by salinity stress. Antioxidant activity at 50 and 100 μ g/l showed significant increases in response to salinity stress. On the other hand, ME treatments proved to be effective in enhancing growth parameters, photosynthetic pigments and IAA contents of salinity stressed plants. Melatonin treatments at different levels caused significant increases in yield and yield attributes, carbohydrate, protein, nitrogen, phosphorous, potassium, flavonoids, phenolic contents, and antioxidant activity of the yielded seeds either in non stressed and salinity stressed plants relative to their corresponding controls. Generally, 500 μ M ME was the most pronounced and effective treatment in alleviating the deleterious effect of salinity stress on wheat plants.

Key words: Antioxidant activity, flavonoids, melatonin, phenolics, protein, salinity, Wheat yield.

Introduction

In Egypt, wheat is the strategic cereal. Wheat plant can grow in different environmental conditions so this permits large-scale cultivation as well as long-term storage of food. Around 70 % of this crop for food, 19 % for animal feed and 11% is for industrial applications, including biofuels. Wheat has high nutritional value and this is important because it gives it an important value among the few crop species so it grown as staple food sources¹. Moreover, due to its high antioxidant contents wheat crop may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion².

Due to increased competition of human activities and industrial uses, fresh water resources becoming limited. So, for saving fresh water resources, sea water can be diluted and used in irrigation and this can be used to grow crops under certain conditions³. Cultivation of crops is limited by salt stress due to excess uptake of salts by plants and is an unavoidable consequence of high ion concentrations. High salt concentrations in soil, which result from irrigation with saline water, have detrimental effects on plant growth⁴. Ion toxicity, osmotic stress and production of ROS resulted from high salt stress⁵ caused oxidative damage and cell death mainly from

the imbalance of ROS occurred⁶. Moreover, photosynthesis, cell membranes, and some enzyme activities of plants all affected by salinity stress⁵. Decreasing of salt content of plant, ion compartmentation, osmotic adjustment and induction of antioxidant enzymes might cause improvement of salinity tolerance⁶. Many attempts were evaluated to improve salinity tolerance of plant by using antioxidant compounds as seed soaking or foliar treatment different growth stages⁷. One of these antioxidants is melatonin,

Melatonin (*N*-acetyl-5-methoxytryptamine), is an indoleamine synthesized throughout the plant kingdom. Melatonin is distributed in many parts of plant as leaves, roots, stems, fruits, and seeds of different species⁸. It was known that melatonin has some auxin- like effects so may act as a regulatory molecule in plants and rooting agent⁹. Melatonin act as a universal hydrophilic and hydrophobic antioxidant as it is soluble in both water and lipid¹⁰. This amphiphilic character enables it to cross cell membranes easily and enter subcellular compartments¹¹. Moreover, it is considered as an antioxidant and a modulator in multiple plant developmental processes and various stress responses¹². This antioxidative melatonin effect was reported in many plant species such as apple, rice, and grape^{13,14&15}. Improvement of resistance of plant against different stressors may be by exogenous application of melatonin¹⁶. In addition, melatonin possess the ability to regulate plant growth and to enhance crop production. So, this investigation aimed to study the effect of seed priming with melatonin in ameliorating the harmful effects of diluted seawater levels on the performance of wheat plants.

Materials and Methods

Wheat grains (*Triticum aestivum* L.) Giza 168 cultivar were sown on November 24, 2013 and November 25, 2014 at the wire house of National Research Centre, Dokki, Giza, Egypt. Wheat grains were obtained from the Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Wheat grains were selected for uniformity, then washed with distilled water, sterilized with 1 % sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water then grains were divided into three groups and soaked for 4 hours, the first group were soaked in dist water, the second in 100 μ M ME and the third in 500 μ M ME as (ME0, ME1, or ME2) and left to dry at room temperature for approx. 1 h. Ten uniform air-dried wheat grains were sown in pots 30 mm depth, each filled with about 7.0 kg clay soil mixed with sandy soil in a proportion of 3:1(v:v), respectively, in order to reduce compaction and improve drainage. Before planting calcium super phosphate (15.5% P₂O₅) and potassium sulfate (48% K₂O) in the rate of 3.0 and 1.50 g/pot were added, respectively. Ammonium sulfate (20.5% N) in the rate of 6.86 g/pot was added in two equal doses, the first one was add after two weeks from sowing and the 2nd two weeks later. The experiment was arranged in a factorial arrangement, with three levels of seawater (S0, S1, or S2). Four replicates were used. Seawater was dissolved in fresh water, and the plants were watered with an equal volume of 0.23, 3.85 dSm⁻¹ and 7.69 dSm⁻¹. 3 weeks after sowing (treatments S0, S1, and S2, respectively). Concentration of EC, pH, cations and anions of irrigation water and soil used were determined according¹⁷ and shown in Table 1. Soil water capacity was estimated by saturating the soil in each pot with water and weighing the soil after the soil had drained for 48 h. Water capacity of the soil in each pot was 0.36 kg kg⁻¹. Soil water contents were maintained at approx. 90% of the pot water capacity. Ten days after sowing (DAS), wheat seedlings were thinned to five seedlings per pot and irrigated with equal volumes of tap water until 30 DAS. Starting from 45 DAS plants were irrigated with either tap water (0.23 dS m⁻¹) or differently diluted seawater (3.85 dSm⁻¹ and 7.69 dSm⁻¹). At 75 DAS, samples were taken to study some growth parameters as plant height (cm), no of leaves/plant, fresh and dry weight of shoot/plant (g). photosynthetic pigments of leaves, endogenous indole acetic acid (IAA). Yield and its components as plant height, spikes number/plant, spikes weight /plant (g), grains weight/ plant, grains number/plant, grains number/spikes and 1000 grains weight. as well as nutritive value of the yielded grains as total carbohydrates%, protein%, flavonoids, phenolic contents, nitrogen, phosphorus and potassium percentages, in addition to antioxidant activities %.

Table 1. EC, pH, and concentration of cations and anions of irrigation water and soil used in the pot

	EC dSm ⁻¹	pH	Cations meq l ⁻¹				Anions meq l ⁻¹			
			Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	HCO ₃ ⁻	CO ₃ ⁻	SO ₄ ²⁻	Cl ⁻
Soil:										
Sandy	0.15	8.21	2.58	2.45	1.35	0.20	1.12	0.00	4.20	0.71
Clay	1.42	7.69	5.63	1.94	5.86	0.33	1.52	0.00	6.79	5.52
Water:										
Tap water	0.23	7.34	1.01	0.52	2.42	0.21	0.11	0.00	1.31	2.69
Sea water	51.25	7.74	43.17	15.14	454.79	1.52	6.07	0.00	76.37	432.14

Biochemical analysis:

Relative water content (RWC) was measured in the first fully-expanded leaf (from the top) using the method¹⁸.

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Chlorophyll a, chlorophyll b and carotenoids concentrations were estimated using the method¹⁹. Indole acetic acid was determined according to the method²⁰. Total carbohydrates were determined according²¹. The protein content was determined by microkjeldahl method²². Total flavonoid contents were measured by the aluminum chloride colorimetric assay²³. Total phenolic compounds were determined according to the method²⁴. The free radical scavenging activity was determined using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent²⁵.

Statistical analysis

The data were statistically analyzed on complete randomized design system according²⁶. Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability.

Results**Growth parameters:**

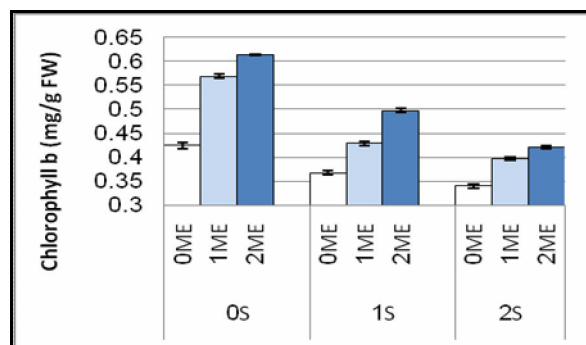
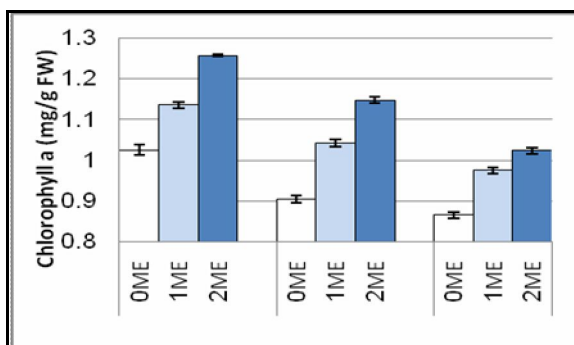
Table (2) show the soaking wheat grains effect in melatonin (ME0, ME1 and ME2) grown under different salinity levels on growth parameters. Different levels of irrigation diluted sea water (S1ME0 and S2ME0) decreased the studied growth parameters as well as relative water content (RWC) relative to control plants (S0ME0). These decreases were significant at S2ME0 treatment. With respect to melatonin effect, both melatonin concentrations (ME1 and ME2 as 100 µM and 500 µM) increased growth parameters of plants irrigated with tap water. These increases were significant at 500 µM melatonin (ME2), meanwhile, melatonin at 100 µM (ME1) caused non-significant increases in all parameters except RWC that showed significant increase relative to control plants (S0ME0). Under salinity stress (S1 and S2), 100 µM and 500 µM melatonin (ME1 and ME2) increased all the examined growth parameters relative to corresponding controls, where the increases in plant dry weight and RWC were significant.

Table 1. Effect of melatonin at 100 μM (ME1) and 500 μM (ME2) on growth parameters of wheat plants irrigated with tap water (S0) or seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). Results are means of two successive seasons.

Salinity (dS/m)	Melatonin (ME) (μM)	Plant height (cm)	Leaves no/plant	Shoot fresh weight /plant (g)	Shoot dry weight/plant (g)	RWC%
S0	ME0	56.31	4.67	4.57	1.35	70.44
	ME1	60.00	5.00	5.22	1.51	71.10
	ME2	63.67	5.33	5.73	1.62	71.67
S1	ME0	53.67	4.33	3.88	1.28	67.16
	ME1	56.11	4.67	4.14	1.28	69.12
	ME2	60.67	5.00	4.42	1.31	70.39
S2	ME0	49.33	3.00	3.12	1.11	64.47
	ME1	52.67	3.67	3.89	1.22	68.81
	ME2	54.67	4.33	4.25	1.31	69.17
LSD at 5%		4.021	0.615	0.687	0.278	5.147

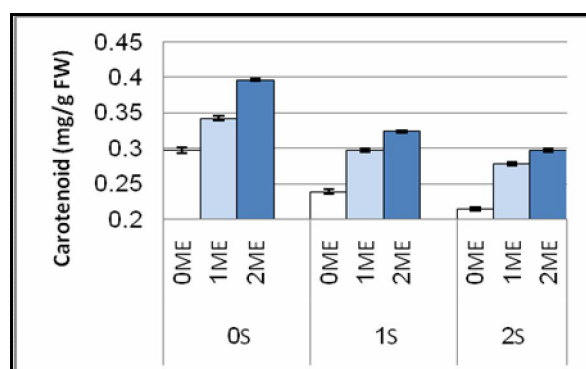
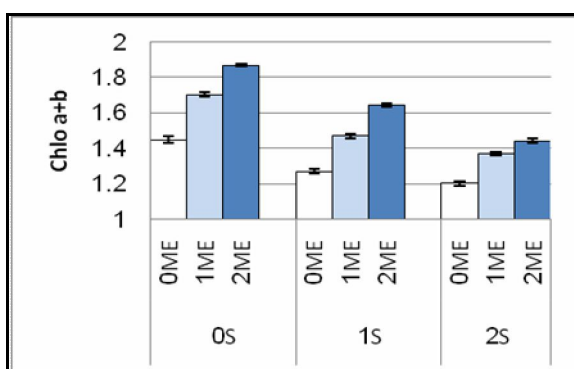
Photosynthetic Pigments:

Irrigation of wheat plants with the low salinity level (S1ME) caused marked decreases in photosynthetic pigments compared with control plants (S0ME0) (Fig 1). Meanwhile, high salinity level S2ME0 significantly decreased them. Meanwhile, chlorophyll a/b ratio were increased in plants irrigated with S1 and S2 as compared with control plants. Photosynthetic pigments were markedly increased in response to 100 μM melatonin under tap water, meanwhile significant increases were obtained at 500 μM melatonin relative to the control (S0ME0). Interaction between the two salinity levels (3.85 dS/m and 7.69 dS/m) and melatonin (100 μM and 500 μM) showed that both melatonin concentrations caused marked increases in different photosynthetic pigments relative to their corresponding controls. In the same time chlorophyll a/b were decreased as compared with the corresponding salinity levels. Higher concentration of melatonin (ME2) was more pronounced than lower melatonin (ME1), either in the plants irrigated with tap water (S0) or diluted seawater (S1 and S2).



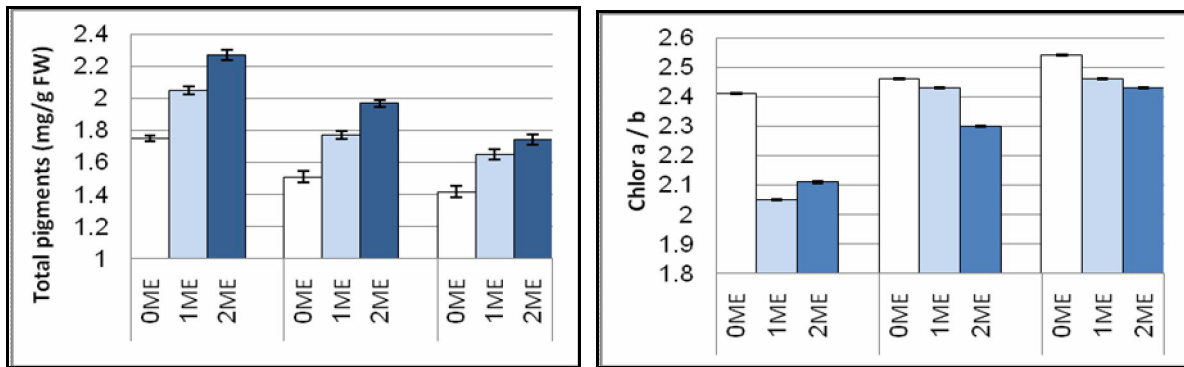
LSD at 5%: 0.157

LSD at 5%: 0.047



LSD at 5%: 0.242

LSD at 5%: 0.024



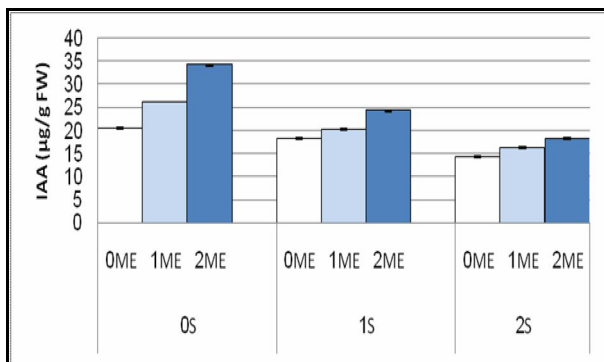
LSD at 5%: 0.328

LSD at 5%: 0.240

Fig 1. Effect of melatonin at 100 μ M (ME1) and 500 μ M (ME2) on photosynthetic pigments (mg/g fresh weight) of wheat plants irrigated with tap water (S0) or seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). Results are means of two successive seasons.

Changes in IAA contents:

Data presented in Fig (2) demonstrated that IAA were significantly reduced in wheat leaves with increasing levels of sea water stress (S1ME0 and S2ME0) when compared with control those of the control (S0ME0) plants. The percentages of decreases reached to 10.58% and 30.07% in plants irrigated with S1ME0 and S2ME0, respectively. On the other hand, soaking wheat grains in melatonin ME1 & ME2 alone or in combination with salinity stress caused significant increases in IAA as compared with untreated plants (ME0). 500 μ M melatonin (ME2) was more effective than 100 μ M melatonin (ME1), either in the plants irrigated with S0 or S1 and S2. These increases reached 66.91 %, 32.70 % and 27.87 % at S0ME2, S1ME2 and S2ME2 compared with S0ME0, S1ME0 and S2ME0, respectively.



LSD at 5%: 1.250

Fig 2. Effect of melatonin at 100 μ M (ME1) and 500 μ M (ME2) on indole acetic acid (μ g/g fresh weight) of wheat plants irrigated with tap water (S0) or seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). Results are means of two successive seasons.

Yield, yield Components:

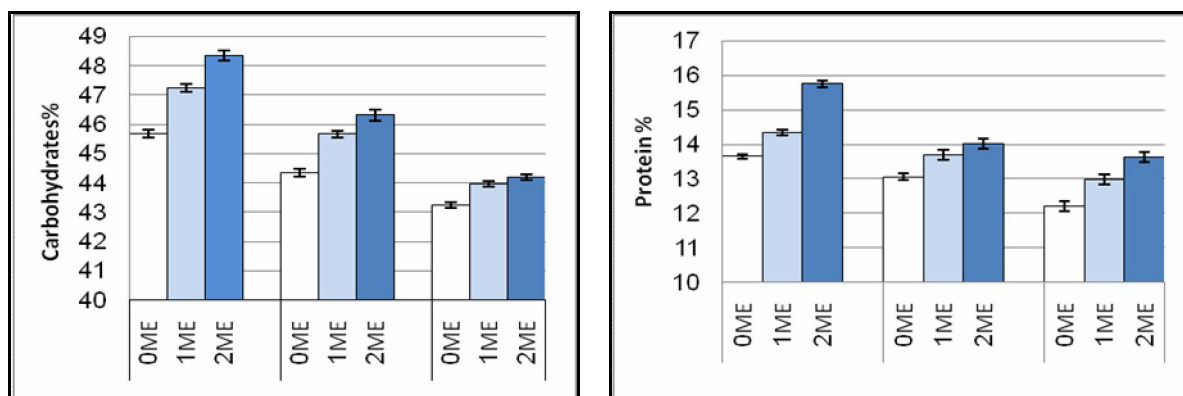
At harvest, plant height (cm), spikes number/plant, spikes weight /plant (g), grains weight/ plant (g), grains number/plant, grains number / spikes and 1000 grains weight (g) of wheat plant were decreased by irrigation of different diluted sea water S1ME0 & S2ME0 as compared to plant irrigated with tap water. For instance, the reduction in seed weight and 1000 seeds weight reached to 19.91%, 18.52%, respectively as compared with tap irrigated plants. On the other hand, soaking wheat grains in different concentrations of melatonin ME1 & ME2 (100 & 500 μ M) under normal and salinity stressed conditions caused significant increases in all parameters of yield components as compared to their corresponding control plants, the most prominence concentration was 500 μ M it increased all yield attributes more than 100 μ M melatonin.

Table 3. Effect of melatonin at 100 μ M (ME1) and 500 μ M (ME2) on yield & yield components of wheat plants irrigated with tap water (S0) or seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). Results are means of two successive seasons.

Salinity (dS/m)	Melatonin (ME) μ M	Plant height (cm)	Spikes No/plant	Spikes weight /plant (g)	Grains weight/plant (g)	Grains No/plant	Grains No/spike	1000 grains wt (g)
S0	ME0	66.33	4.00	5.765	4.248	82.35	21.34	42.35
	ME1	69.00	4.38	6.248	4.685	89.35	22.68	44.68
	ME2	75.33	4.93	6.754	5.324	94.35	23.04	46.35
S1	ME0	58.67	3.67	4.687	4.014	78.35	20.54	40.35
	ME1	63.33	4.00	5.214	4.357	84.25	21.06	42.35
	ME2	66.67	4.24	5.765	4.547	89.35	22.72	43.35
S2	ME0	52.33	3.33	3.759	3.175	71.35	19.92	38.35
	ME1	58.67	3.67	4.215	3.835	79.36	20.53	40.25
	ME2	50.67	4.00	4.579	4.128	83.65	21.16	41.35
LSD at 5%		6.384	0.415	0.352	0.218	5.351	1.456	5.41

Carbohydrate and protein percentage in yielded grains:

Fig (3) shows that different seawater salinity caused significant decreases in total carbohydrate and total crude protein contents of grains of wheat plant compared with control plant. These decreases were gradually with increased salinity level from S1 to S2. Meanwhile, different concentrations of melatonin treatment ME1 and ME2 caused significant increases in carbohydrates and crude protein contents of the yielded grains of wheat plant compared with their corresponding controls. Higher concentrations of melatonin was more effective in increasing the above mentioned parameters than lower concentration either under normal and stressed conditions.



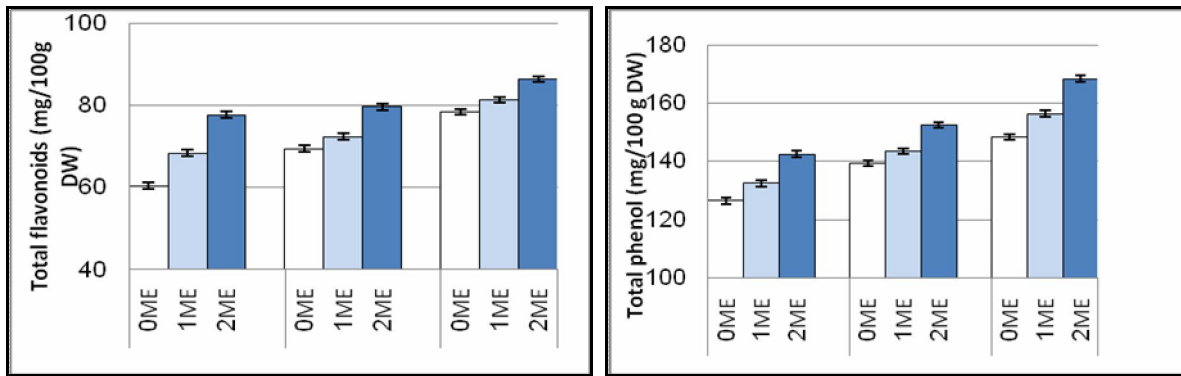
LSD at 5%: 1.125

LSD at 5%: 0.554

Fig 3. Effect of melatonin at 100 μ M (ME1) and 500 μ M (ME2) on carbohydrates% & protein% of wheat grains of wheat plant irrigated with tap water (S0) or seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). Results are means of two successive seasons.

Total flavonoids and total phenols content:

The two applied salinity levels (S1ME0 and S2ME0) caused significant and gradual increases in (S0ME0) (Fig 4) flavonoids and phenolic content relative to control plant (S0ME0). Melatonin concentrations (ME1 and ME2) caused more gradual increases in flavonoids and phenolic content relative to their corresponding controls.



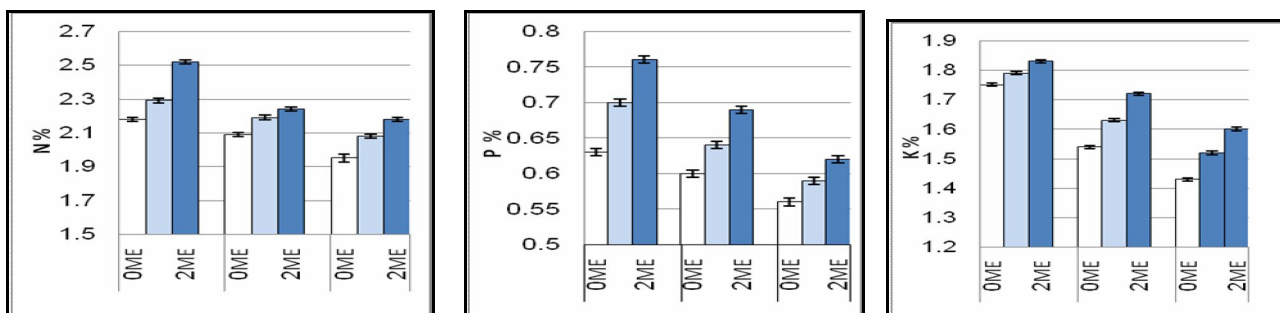
LSD at 5%: 3.322

LSD at 5%: 1.365

Fig 4. Effect of melatonin at 100 μ M (ME1) and 500 μ M (ME2) on total flavonoids & total phenolic (mg/100 gDW) of grains of wheat plants irrigated with tap water (S0) or seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). Results are means of two successive seasons.

Mineral contents:

The two applied salinity levels (S1ME0 and S2ME0) decreased significantly & gradually in nitrogen, phosphorus and potassium percentages contents of the yielded grains relative to the control plants (S0ME0) (Table 4). Both melatonin concentrations caused gradual increases in N, P and K relative to corresponding controls. 500 μ M melatonin was more effective than 100 μ M in increasing N, P & K of wheat grains at 3.85 dS/m & 7.69 dS/m.



LSD at 5%: 0.102

LSD at 5%: 0.023

LSD at 5%: 0.078

Fig 5. Effect of melatonin at 100 μ M (ME1) and 500 μ M (ME2) on mineral contents of grains of wheat plants irrigated with tap water (S0) or seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). Results are means of two successive seasons.

Antioxidant activity:

The radicals scavenging activity of wheat grains extracts of wheat plant irrigated with different sea water levels (S1ME0 and S2ME0) and treated with two concentrations of melatonin (ME1 & ME2) expressed the percentage reduction of the initial DPPH absorption and melatonin treatment are presented in Table 4. Different salinity levels increased antioxidant activity at 50 and 100 μ g/ml. Melatonin treatment with different concentrations caused more significant increases in antioxidant activity. 500 μ M was more effective than 100 μ M melatonin under normal and different salinity irrigation water.

Table 4. Effect of melatonin at 100 μ M (ME1) and 500 μ M (ME2) on antioxidant activity at 50 & 100 μ g/l of grains of wheat plants irrigated with tap water (S0) or seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). Results are means of two successive seasons.

Salinity (dS/m)	Melatonin (ME) μ M	Antioxidant activity	
		50 μ g/l	100 μ g/l
S0	ME0	35.29	56.35
	ME1	38.35	57.36
	ME2	40.21	60.42
S1	ME0	37.32	58.36
	ME1	40.21	62.42
	ME2	42.36	64.54
S2	ME0	39.35	61.24
	ME1	41.35	63.25
	ME2	43.25	65.25
LSD at 5%		2.245	3.014

Discussion

Salinity stress results in considerable decreases in all growth parameters such a reduction in growth could be due to the effect of high osmotic stress and ion toxicity^{27&28} or the induced alteration of cell wall structure²⁹. Further, cell division, cell enlargement and expansion might be inhibited by salinity stress³⁰. With respect to melatonin treatment Table 2 show the promotive effect of melatonin on growth parameters of wheat plant under normal and salinity stress conditions. Melatonin can act as a potential modulator of plant growth and development in a dose-dependent manner^{31,32,33&34}. Melatonin affect plant growth by effecting on growth regulation and ion homeostasis³⁵. In addition, ME may play auxin-IAA role as its chemical structure is similar to this hormone³⁶.

Photosynthetic pigments were reduced under salinity stress conditions (Fig 1). These reductions may be due to the toxic effects of salt on the biosynthesis of pigments, increasing their degradation and/or causing damage to chloroplast thylakoids. Salinity stress caused the shape of thylakoids to be swollen and disorganized. Salt stress enhanced the activity of chlorophyllase and interfered with the *de-novo* synthesis of proteins, such as those that bind chlorophyll³⁷. Moreover, these decreases in chlorophyll content in stressed leaves of wheat was mainly due to a decrease of ALA (5-aminolinolic acid) synthesis which is a protochlorophyllide precursor, that converts to chlorophyll when exposed to light³⁸. Leaves exposed to severe saline stress not only reduced their chlorophyll concentrations, but their chlorophyll *a/b* ratios increased, due to more rapid degradation of chlorophyll *b* than chlorophyll *a* (Fig 1). This might be due to that the first step in the degradation of chlorophyll *b* involves conversion to chlorophyll³⁹ *a*. An increase in the chlorophyll *a/b* ratio has been linked to changes in the pigment composition of the photosynthetic apparatus that contains lower levels of light harvesting proteins⁴⁰. In agreement with our results of melatonin effect, pretreatment of *Malus hupehensis* Rehd and *Cucumis sativus* L. with melatonin improved plant growth and photosynthetic capacity under high salinity conditions^{35&41}. The promotive effect of melatonin on wheat plant photosynthetic pigment might be due to melatonin improvement of the ultrastructure of chloroplasts. Moreover, this role of Me may be due to raising the antioxidant enzyme activities and antioxidant contents and thus inhibiting the production of reactive oxygen species⁴².

Data presented in Fig (2) demonstrated that IAA were significantly reduced in wheat leaves with increasing levels of sea water stress (S1ME0 and S2ME0) when compared with those of the control (S0ME0) plants. IAA as a phytohormone regulate the protective responses of plants against both biotic and abiotic stresses by means of synergistic or antagonistic actions with other hormones as GA₃, BA and ABA referred to as signaling crosstalk⁴³. Salinity, drought and heat stresses on flax, canola and wheat plants gradually declined the contents of IAA (auxin) contents below those of the unstressed controls^{44,45,45&47}. These decreases might be due to increase the destruction of IAA by increasing the activity of IAA oxidase⁴⁸. On the other hand, exogenous application of melatonin ME1 & ME2 alone or in combination with salinity stress caused significant

increases in IAA as compared with untreated plants (ME0). The promotive effect of melatonin on IAA contents were confirmed on many plant species^{49&50}.

Different levels of diluted sea water caused marked decreases in yield and yield components (Table 3). Increasing sea water levels reduced yield & yield components of wheat plant in comparison with control plants. In accordance with our results, those obtained on sunflower, flax, wheat and faba bean plant^{51,52,53&54}. These decreases might be due to the decreases in plant growth, photosynthetic pigments and disturbance in the nutrient balance⁵⁵. On the other hand, treatments of wheat plant with the melatonin could alleviate the harmful effect of salinity stress on yield and yield components (Table 4). These obtained data are in accordance with those obtained^{36,12,36,48&56}. Melatonin treatment enabling plants to maintain a robust root system and improved photosynthetic activity so could alleviate growth inhibition and increased crop yield⁵⁷.

Regarding to the nutritional values of the yielded grains, different levels of sea water caused significant decreases in total carbohydrates and crude protein contents. As the photosynthetic pigments reduced of wheat leaves under salinity stress (Fig 1) thus might led to photo-assimilate decrease mainly total carbohydrates (Fig 3). These reduction in carbohydrates may be due to the reduction in the biosynthesis of carbohydrates and this might be due to the inhibitory effect of salinity on chlorophyll synthesis⁵⁸. The disturbance in nitrogen metabolism or inhibition of nitrate absorption might result from sea water stress thus led to decreased nitrogen contents of grains⁵⁹. Meanwhile, melatonin improvement effect on photosynthetic pigments (Fig 1) might caused the increases in carbohydrates and protein contents.

Fig 4 shows the enhancement effect of S and/or ME treatments on flavonoids and phenolic contents. This metabolic process disturbances induced by saline water leading to the increase in the flavonoids and phenolic synthesis compounds⁶⁰. The importance of flavonoids was known to possess significant antimicrobial activities and was utilized as natural plant protectants⁶¹. It could be suggested that flavonoids content may be an alternative to conventional fungicides in the control of storage grains against some fungi. The increased levels of ROS under stress is accompanied by changes in net carbon gain which may affect the biosynthesis of carbon-based secondary compounds, particularly leaf polyphenols³⁹. The signaling role of melatonin induced different metabolic compounds and this stimulate production of various substances²¹. Me increased synthesis of phenolics⁶² in *Vigna radiata* L.

The decreases in N, P and K in wheat grains under salinity are presented in Fig 5. These obtained results in accordance with results on flax⁵⁷ and faba plants⁵⁴. It was showed that when faba bean plants take up high amounts of Na, whereas the uptake of K, P and N is significantly reduced in leaves and this decreases affect on the mineral contents of seeds decreased N, P, K contents⁶³. Increased levels of Na⁺ cation has suppressive effect on N, P, K uptake and the transport of these ions⁶⁴. Melatonin presoaking treatments increased N, P, K content of grains of wheat plant (Fig 5). These enhancement effect may be due to that, melatonin might contribute to the maintenance of ion homeostasis via the control of the expression of ion-channel genes under salinity⁴⁴.

Results showed that different salinity levels increased activity against DPPH radical (Table5) the two used salinity levels S1 & S2 as compared with normal irrigated water S0. The 1, 1 diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with maximum absorbance at 517 nm and can readily undergo reduction by an antioxidant. Due to the ease and convenience of this reaction, it now has widespread use in the free radical-scavenging activity assessment³⁰. The reduction of DPPH radical (purple colour) to a yellow coloured compound, diphenyl picrylhydrazine in wheat grains extracts depends on hydrogen donating ability of the studied antioxidants and a dose dependent scavenging of DPPH free radical by grain extracts from 50 to 100 µg/ml. Also, different treatments increased antioxidant activity as to untreated controls. These results using different antioxidants were also supported on different plant species, they realized that the antioxidant scavenging potential was directly proportional with the concentration of the used samples^{65&66} (12.5 to 100µl).

Conclusion

We can concluded that melatonin treatments (100 µM and 500 µM) partially alleviated the reduced effects of sea water stress on growth parameters, relative water content, photosynthetic pigments, indole acetic acid, in leaf tissues of wheat plants. Also, yield and yield components and the nutritional values of the yielded

grains were improved. 500 mM ME was more effective in mitigating the adverse effects of salinity stress on wheat plants.

References

1. Abd Allah M MSh, El-Bassiouny HMS, Bakry BA, Mervat Sh Sadak. Effect of *Arbuscular Mycorrhiza* and Glutamic Acid on Growth, Yield, Some Chemical Composition and Nutritional Quality of Wheat Plant Grown in Newly Reclaimed Sandy Soil. Res. J. Pharmaceutical, Biol. & Chem. Sci., 2015, 6(3):1038-1054.
2. Yu L, Perret J, Davy B, Wilson J, Melby CL. Antioxidant properties of cereal products. J. of Food Sci., 2002, 67: 2600-2603.
3. Zeid IM, Alleviation of seawater stress during germination and early growth of barley. Inter. J Agric Res Rev., 2011, 1(2):59-67.
4. Sobhanian H, Razavizadeh R, Nanjo Y, Ehsanpour AA, Jazil FR, Motamed N, Komatsu S. Proteome analysis of soybean leaves, hypocotyls and roots under salt stress. Proteome Science, 2010, 8, 19–33.
5. Sreenivasulu N, Grimm B, Wobus U, Weschke W. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). Physiol. Plantarum, 2000, 109: 435-442.
6. Sadak Mervat Sh, Abdelhamid MT, El-Saad AM. Physiological responses of faba bean plant to ascorbic acid grown under salinity stress. Egypt J Agron., 2010, 32(1) : 89-106.
7. Ashraf M, Foolad M. Pre-sowing seed treatment -a shotgun approach to improve germination, growth and crop yield under saline and non-saline conditions. Adv. Agro., 2005,88: 223-271. Doi: [http://dx.doi.org/10.1016/S0065-2113\(05\)88006-X](http://dx.doi.org/10.1016/S0065-2113(05)88006-X).
8. Zhao Y, Tan DX, Lei Q, Chen H, Wang L, Li QT, Gao Y, Kong J. Melatonin and its potential biological functions in the fruits of sweet cherry. J. of Pineal Res., 2013, 55, 79–88.
9. Kolár J, Macháková I. Melatonin in higher plants: occurrence and possible functions. J Pineal Res., 2005, 39(4) :333-341. Doi: <http://dx.doi.org/10.1111/j.1600-079X.2005.00276.x>.
10. Janas KM, Posmyk MM. Melatonin, an under estimated natural substance with great potential for agricultural application. Acta Physiol. Plant., 2013, 35(12): 3285 - 3292. Doi: <http://dx.doi.org/10.1007/s11738-013-1372-0>
11. Shida C, Castrucci A, Lamyfreund M, High melatonin solubility in aqueous-medium. Journal of Pineal Research, 1994, 16, 198–201.
12. Tan DX, Hardeland R, Manchester LC, Korkmaz A, Ma S, Rosales-Corral S, Reiter RJ. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. J. of Exp. Bot., 2012, 63: 577–597.
13. Wang P, Yin L, Liang D, Li C, Ma F, Yue Z. Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate–glutathione cycle. J. of Pineal Res. 2012, 53, 11–20.
14. Park S, Lee DE, Jang H, Byeon Y, Kim YS, Back K. Melatonin rich transgenic rice plants exhibit resistance to herbicide-induced oxidative stress. J. of Pineal Res., 2013. 54, 258–263.
15. Vitalini S, Gardana C, Simonetti P, Fico G, Iriti M. Melatonin, melatonin isomers and stilbenes in Italian traditional grape products and their antiradical capacity. J. of Pineal Res., 2013, 54, 322–333.
16. Weeda S, Zhang N, Zhao XL, Ndip G, Guo YD, Buck GA, Fu CG, Ren SX. *Arabidopsis* transcriptome analysis reveals key roles of melatonin in plant defense systems. PLoS One, 2014, 9, e93462.
17. Chapman, HD, Pratt PE. Methods of analysis for soils, lands and waters. Univ. of Calif., Div. Agric. Sci., USA, 1978, 3043. pp: 162-165.
18. Yamasaki S, Dillenburg LC. Measurements of leaf relative water content in *Araucaria angustifolia*. R Bras Fisiol Veg. 1999, 11(2):69 -75.
19. Moran R. Formula for determination of chlorophyllous pigments extracted with N.N. dimethylformamide. Plant Physiol.,1982, 69: 1371-1381.
20. Larsen PA, Harbo S, Klungron, Ashein TA. On the biosynthesis of some indole compounds in *Acetobacter xylinum*. Physiol. Plant, 1962, 15: 552-565.
21. Dubois M, Guilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal. Chem., 1956, 28: 350-356.
22. A.O.A.C., Official methods of analysis. 20th edition. Association of Official Analytical Chemists, Arlington, Virginia, U.S.A. 1990, (No 984.13).

23. Ordoñez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chem.*, 2006, 97: 452-458.
24. Danil AD, George CM. Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. *J. Amer. Soc. Hort. Sci.*, 1972, 17: 621-624.
25. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaften und Technologi*, 1995, 28: 25-30.
26. Snedecor GW, Cochran WG. *Statistical Methods* 7th ed., the Iowa State Univ., Press. Ames, IA 1980.
27. Hasanuzzaman MK, Nahar and Fujita M. 2013. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad P, Azooz MM, Prasad MNV, editors. *Ecophysiology and responses of plants under salt stress*. New York: Springer; 2013p. 25- 87. Doi: http://dx.doi.org/10.1007/978-1-4614-4747-4_2
28. Tawfik, MM, Mervat Sh Sadak and Ibtihal M Abd Elhamid, Role of antioxidants in mitigating the negative impact of salinity., 2015, LAP LAMBERT Academic Publishing. Deutschland/Germany.
29. Sweet WJ, Morrison JC, Labaritch JM, Matthews MA. Altered synthesis and composition of cell wall of grapevines *Vitis vinifera* L. during expression and growth inhibiting water deficits. *Plant Cell Physiol.* 1990, 31:407-414.
30. Radi AA, Farghaly FA, Hamada AM. Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. *J. Biol Earth Sci.*, 2013, 3(1):72-88.
31. Afreen F, Zobayed SM, Kozai T. Melatonin in *Glycyrrhiza uralensis*: response of plant roots to spectral quality of light and UV-B radiation. – *J. Pineal Res.* 2006, 41: 108-115.
32. Arnao MB, Hernández-Ruiz J. Melatonin promotes adventitious and lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L. – *J. Pineal Res.* 2007, 42: 147-152.
33. Hernandez-Ruiz J, Arnao MB. Melatonin stimulates the expansion of etiolated lupin cotyledons. – *Plant Growth Regul.* 2008, 55: 29-34.
34. Wang, LY, Liu JL, Wang WX, Sun Y. Exogenous melatonin improves growth and photosynthetic capacity of cucumbers under salinity-induced stress. *Photosynthetica*, 2015, 53 (X): XXX-XXX.
35. Li C, Wang P, Wei Z, Liang D, Liu C, Yin L et al. The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*. *J Pineal Res.*, 2012, 53(3):298-306. Doi: <http://dx.doi.org/10.1111/j.1600-079X.2012.00999.x>
36. Sarropoulou VN, Therios IN, Dimassi-Theriou KN. Melatonin promotes adventitious root regeneration *in vitro* shoot tip explants of the commercial sweet cherry rootstocks CAB-6P (*Prunus cerasus* L.), Gisela 6 (*P. cerasus* x *P. canescens*), and MxM 60 (*P. avium* x *P. mahaleb*). *J Pineal Res.*, 2012, 52(1):38-46. Doi: <http://dx.doi.org/10.1111/j.1600-079X.2011.00914.x>
37. Rady M, Mervat Sh Sadak, Safaa R El-Lethy, Ebtihal M Abd Elhamid, Abdelhamid MT. Exogenous α -tocopherol has a beneficial effect on *Glycine max* (L.) plants irrigated with diluted sea water. *J. of Hort. Sci. & Biot.*, 2015, 90 (2) 195–202.
38. Santos CV. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Scient Horticult.*, 2004, 103(1):93-99. Doi: <http://dx.doi.org/10.1016/j.scienta.2004.04.009>
39. Fang Z, Bouwamp J, Solomos T. Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild-type of *Phaseolus vulgaris* L. *Journal of Experimental Botany*, 49, 1998, 503–510.
40. Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F. Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiology*, 119, 1999, 1091–1099.
41. Zhang N, Zhao B, Zhang HJ, Weeda S, Yang Ch, Yang ZC, et al. Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* L.). *J Pineal Res.* 2013;54(1):15-23. Doi: 10.1111/j.1600-079X.2012.01015.x
42. XD X, Sun Y, Sun B, Zhang J, Guo XQ. Effects of exogenous melatonin on active oxygen metabolism of cucumber seedlings under high temperature stress. *Ying Yong Sheng Tai Xue Bao.*, 2010 ;21(5):1295-1300.
43. Abass SM and Mohamed, HM, Alleviation of adverse effects of drought stress on common bean (*Phaseolus Vulgaris* L.) by exogenous application of hydrogen peroxide. *Bangladesh J. Bot.*, 2011, 41(1): 75-83.
44. El Hariri DM, Sadak Mervat Sh, El-Bassiouny HMS, Response of flax cultivars to ascorbic acid and α tocopherol under salinity stress conditions. *Inter. J. Acad. Res.*, 2010, 2: 101-109.

44. Dawood MG, Sadak Mervat Sh. Physiological Role of Glycinebetaine in Alleviating the Deleterious Effects of Drought Stress on Canola Plants (*Brassica napus* L.). Middle East Journal of Agriculture Research, 2014, 3(4): 943-954.
45. Sadak Mervat Sh, Orabi Salwa A. Improving thermo tolerance of wheat plant by foliar application of citric acid or oxalic acid. Inter. J. of Chem Tech Res. 2015, 8(1): 111-123.
46. Orabi, Salwa A. and Mervat Sh. Sadak. Alleviation of adverse effects of salinity stress on wheat (*Triticum aestivum* L.) by exogenous application of hydrogen peroxide. J. of Basic and Appl. Res. Inte., 2015, 8(4): 287-303.
47. Bano A, Samina Y. Role of phytohormones under induced drought stress in wheat. Pak. J. Bot., 2010, 42: 2579-2587.
48. Chen Q, Qi WB, Reiter RJ, Wei W, Wang BM, Exogenously applied melatonin stimulates root growth and raises endogenous indole acetic acid in roots of etiolated seedlings of *Brassica juncea*. J. Plant Physiol. 2009, 166(3):324-328. Doi: <http://dx.doi.org/10.1016/j.jplph.2008.06.002>
49. Posmyk MM, Bałabusta M, Wieczorek M, Sliwinska E, Janas KJ. Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling. J. Pineal Res. 2009, 46(2):214-223. Doi: <http://dx.doi.org/10.1111/j.1600-079X.2008.00652.x>
50. Sadak Mervat Sh, Abd El-Monem AA, El-Bassiouny HSM, Badr Nadia M. Physiological response of sunflower (*Helianthus annuus* L.) to exogenous arginine and putrescine treatments under salinity stress. J. of Appl. Sci. Res., 2012, 8(10): 4943-4957.
51. Sadak Mervat Sh, Abd Elhamid Ebtihal, M. Physiological response of flax cultivars to the effect of salinity and salicylic acid. J. of Appl. Sci. Res., 2013, 9(6): 3573-3581.
52. Sadak Mervat Sh, Abd Elhamid Ebtihal M, Mostafa HA. Alleviation of adverse effects of salt stress in wheat cultivars by foliar treatment with antioxidants I. Changes in growth, some biochemical aspects and yield quantity and quality. Amer-Eur J. Agric. & Environ. Sci., 2013, 13 (11): 1476-1487.
53. Sadak, Mervat Sh, Abdelhamid MT, Schmidhalter U. Effect of foliar application of aminoacids on plant yield and some physiological parameters in bean plants irrigated with seawater. Acta Biol. Colomb., 2015, 20(1):141-152
54. Taffouo VD, Kouamou JK, Ngalangue LMT, Ndjeudji B, Akoa A. Effects of salinity stress on growth, Ions partitioning and yield of some cowpea (*Vigna unguiculata* L. Walp.) Cultivars. Inter J. Bot., 2009, 5(2):135-143. DOI 10.3923/ijb.2009.135.143.
55. Zhang N, Sun Q, Zhang H, Cao Y, Weeda S, Ren Sh, Yang-Dong G. Roles of melatonin in abiotic stress resistance in plants. J. of Exper. Bot.,2014, doi:10.1093/jxb/eru336
56. Posmyk MM, Janas KM. Melatonin in plants. Acta Physiol Plant 20009, 31:1-11
57. Sadak, Mervat Sh, Dawood Mona G. Role of ascorbic acid and atocopherol in alleviating salinity stress on flax plant (*Linum usitatissimum* L.). J. Stress Physiol. Biochem. 2014, 10:93-111.
58. El-zeiny HA, Abou LB, Gaballah MS, Khalil S. Antitranspirant application to sesame plant for salinity stress Augmentation. Res. J. Agric. Biologic. Sci., 2007, 3: 950-959.
59. Keutgen AJ, Pawelzik E. Impacts of NaCl stress on plant growth and mineral nutrient assimilation in two cultivars of strawberry. Environ Exper Bot.,2009, 65(2-3):170-176. Doi: <http://dx.doi.org/10.1016/j.envexpbot.2008.08.002>
60. Weidenbomer M, Hindorf H, Weltzien HC, Jha HJ. An effective treatment of legume seeds with flavonoids and isoflavonoids against storage fungi of the genus *Aspergillus*. Seed Sci. and Techn., 1992, 20, 447-463.
61. Szafranska K, Glinska S, Janas KM. Changes in the nature of phenolic deposits after re-warming as a result of melatonin pre- sowing treatment of *Vigna radiata* seeds. J Plant Physiol. 2012, 169(1):34-40. Doi: <http://dx.doi.org/10.1016/j.jplph.2011.08.011>
62. Abdelhamid MA, Sadak Mervat Sh, Schmidhalter U, El-Saady, AM. Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters of faba bean plant. Acta Biol. Colomb., 2013, 18(3):499-510.
63. Asik BB, Turan MA, Celik H, Katkat AV. Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. Salihli) under conditions of salinity. Asian J. Crop Sci. 2009, 1:87-95. Doi: [http:// dx.doi.org/10.3923/ajcs.87.95](http://dx.doi.org/10.3923/ajcs.87.95)
64. Rekha C, Poornima G, Manasa M, Abhipsa V, Devi PJ, Kumar VHT, Kekuda PTR. Ascorbic Acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe Citrus fruits. Chem. Sci. Transactions, 2012, 1(2): 303-310.

65. Sadak Mervat Sh, Salwa A Orabi, Bakry AB. Antioxidant properties, secondary metabolites and yield as affected by application of antioxidants and banana peel extract on Roselle plants. Amer – Eur J. of Sustainable Agric, 2015 9 (4):93-104.
