Iraqi Journal of Agricultural Sciences –2026:57(7):1998-2011
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/d9mvbw42

Role of Melatonin, Boron and *Chlorella Vulgaris* on the Antioxidant Enzymes Of Date Palms in A Desert Environment

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ABSTRACT

This study aimed to evaluate the effects of melatonin, boron, and *Chlorella vulgaris* algae on the antioxidant enzyme activity and nutrient status of date palm trees under environmental stress conditions. The study was conducted during the growing season in 2024 at Fadak Date Palm Station, located in the Karbala desert, on *Phoenix dactylifera* L. ' var. Sayer' trees. With A total of 54 uniforms were seven -year -old palms chosen for the experiment. Melatonin was applied to three concentrations (0, 50 and 100 mg L^{-1}) as foliar spray, while boron (in the form of boric acid) were sprayed at two concentrations (0 and 500 mg L^{-1}). In addition, *Clorella vulgaris* algae were incorporated in the soil in three concentrations (0, 100 and 200 ml L^{-1}). The results demonstrated that melatonin and boron play an important and dynamic role in increasing the activity of antioxidant enzymes (peroxidase, catalase, and polyphenol oxidase) under drought-induced stress conditions, which improves the nutrition and physical condition of the palms and eventually reflected in fruit quality. In addition, the soil application of *Chlorella vulgaris* at varying levels increased sharply nitrogen and potassium content in palm leaves. These factors combined alleviate environmental pressures on date palms in desert conditions.

Key words: biofertilizer, catalase, combating desertification, peroxidase, polyphenol.

Received: 16/4/2025, Accepted: 13/7/2025, Published: 31/7/2026

INTRODUCTION

Date Palm (*Phoenix dactylifera* L.) The most important perennials known to humanity are one of the evergreen fruit trees. Related to the Arecaceae family and Palmae order, it is monocotyledonous species. The date palm has tremendous economic significance because of its very nutritious and commercially valuable fruits, globally especially in the Middle East. This makes it a key economic contributor, enhancing national income (Al-Khafaf,.1998). Boron is a vital micronutrient that plays a critical role in plant growth and vitality. Although required in trace amounts (Ismail *et al*., 2022) boron is essential throughout all plant developmental stages. It collaborates with calcium in forming and strengthening plant cell walls (Kumar *et al*., 2024). Notably, boron is the sole element regulating the translocation of endogenous hormones—such as auxins, cytokinins, and gibberellins—within the plant. These hormones govern cell division and elongation, with boron facilitating their transport from synthesis sites to active locations (Liu *et al*., 2019). Boron is particularly crucial in fruit development t(Arredondo and Bonomelli., 2023), as it directly influences pollination and fruit set. It enhances pollen viability and the viscosity of the stigmatic surface, improving pollination efficiency and subsequent fruit retention, thereby increasing overall yield (Pereira *et al*., 2021; Sayed and Gomaa., 2024). Additionally, boron is responsible for supplying pollen with borate-sugar complexes, formed by the binding of boron to photosynthetically derived sugars. This process is vital for sugar translocation from source to sink tissues (Abdel-Karim *et al*., 2015 ; El-Kosary *et al*., 2023). Melatonin, which is a sleep rhythm hormone in animals, has recently been found to be a growth regulator in many plant developmental pathways. Its multiple roles in plant growth and responses to both biotic

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abiotic stresses. In plants, melatonin modulates plant development by controlling the level of several plant hormones including auxins (AUXs), gibberellins (GAs), abscisic acid (ABA), and cytokinins (CKs), and their related signaling pathways. In this regulation, melatonin affects the different processes of physiological growth and development of plants in a prominent way (Altaf *et al*., 2022; Hu *et al*., 2021). In addition, melatonin increases root elongation, lateral root production, and improves the efficiency of photosynthesis, which when combined, promote total plant growth and productivity. It also acts to control the internal biological clock of plant (circadian rhythm), affecting the principal physiological events that determine plant screen growth (Li *et al*., 2022). Moreover, melatonin is a powerful antioxidant that is indispensable for plants with stress. It functions by scavenging the reactive oxygen species (ROS), reducing oxidative stress, and modulating the plant response to abiotic stresses. Melatonin further modulates stomatal opening, preserves cellular water, and increases stress resistance through the expression of stress-related genes. Remarkably, melatonin works in conjunction with nitric oxide to regulate heat stress responses, so that the plant can better withstand severe, dry habitats (Li *et al*., 2021).

The most common method of using microalgal biomass is through biofertilizers. Biofertilizers are living microorganisms or their derivatives that promote or increase the nutrition of the plant by improving the availability of nutrients in the soil through the colonization of rhizosphere, root surfaces or even rot interiors. Biofertilizers are classified for nitrogen fixing, potassium solubilizing and phosphorus-solubilizing type (Mitter *et al* ., 2021; Uysal *et al*., 2015). With single -celled algae and cyanobacteria, fungi and bacteria, which form the best layers of soil, which together are referred to as biological organic crust (BSC), which plays an important role in increasing the soil's fertility and growing tree productivity (Abinandan *et al*., 2019). Comparable, a wide range of algae shows an impressive potential to thrive in a wide range of soil, including sandy soils that are typically deficient in key nutrients as nitrogen, phosphorus and various micronutrients. Despite these harsh environmental conditions and the lack of surface moisture (Kraiser *et al*., 2011) The algae bio -feeder has been shown to improve the concentrations of nutrients in the soil (especially nitrogen, phosphorus and many other micronutrients nutrients), increase soil stability and help maintain some of the soil moisture content (Cakirsoy *et al*., 2022; Nosheen *et al*., 2021; Sadak and Şensoy., 2022). Desertification is considered a global environmental problem, as climate change and human settlement cause this deterioration in arable land. Therefore, date palms face several challenges at once, including drought, salinity and high temperatures. It is therefore necessary to know how date palms defend themselves against these stressful conditions, especially in the Middle East, where the desert is encroaching significantly with the significant change in temperatures.(Zittis *et al*., 2022). This study aimed to evaluate the effects of melatonin, boron, and *Chlorella vulgaris* algae on the antioxidant enzyme activity and nutrient status of date palm trees (*Phoenix dactylifera* L.) under environmental stress conditions.

MATERIALS AND METHODS

The experiment was conducted at the Fadak Date Palm Farm, situated west of the Karbala city desert according to the coordinates (32.722086, 43.876009), on Sayer cultivar date palm trees (7 years old) that were irrigated via a drip system during the 2024 growing season; for this purpose, 54 trees were selected to be as homogenous as possible, and the research area was divided into three replicates, with each replicate containing 18 palms, where each experimental unit consisted of a single palm. The experiment was implemented as a 3x3x2 factorial experiment in a randomized complete block design (RCBD). Urea was sprayed at a concentration of 0.2% one day before the application of the experimental treatments to facilitate the penetration of the applied solutions into the leaflets. Sampling was carried out in August, at the begining rutab (ripening) stage.

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Figure 1. The experiment site is located in the desert of Karbala, which is characterised by hard desert conditions, with temperatures reaching 55°C, soil salinity (EC) 5.75 ds/m, well water salinity (EC) 6.16 ds/m, pH of soil 7.5, and average of annual rainfall of 11 mm.

Study Factors:

1. Melatonin (0, 50, and 100 mg L^{-1}):

-The first spray was applied at the Kimri stage, the second spray was conducted 21 days after the first application, and the third spray was applied at the Khalal stage.

-Spraying was performed using an electric +sprayer until full coverage of the trees was achieved.

2.Boron (as boric acid) (0 and 500 mg L^{-1}):

-The first spray was applied at the beginning of March, the second spray at the Kimri stage, and the third spray at the Khalal stage.

3. Algal biofertilizer (*Chlorella vulgaris*) (0, 100, and 200 mL L^{-1}):

-Applied to the soil on five specific dates: 10/1, 10/2, 10/3, 10/4, and 10/5 (5 liters per tree for each application).

Measured Parameters:

1.Peroxidase Enzyme Activity:

Method described by (Whitaker and Bernhard .,1972).

2.Catalase Enzyme Activity:

Method described by (Goth ., 1991).

3.Polyphenol Oxidase Enzyme Activity:

method described by (Marri *et al* .,2003).

4.Nitrogen percentage (N %):

Method described by (Haynes et al.,1980).

5.Potassium percentage (K %):

method described by (Haynes et al.,1980).

6. bunch/means .kg

The mean Weight of a single bunches per palm was calculated by dividing the total amount of each treatment by the number of bunches.

The concentrations used in the experiment:

1. B0M0F0: (control, without any add-ons) (only water)
2. B0M0F1: Boric acid (0 mg L^{-1}) + Melatonin (0 mg L^{-1}) + *C. vulgaris* (100 ml L^{-1})
3. B0M0F2: Boric acid (0 mg L^{-1}) + Melatonin (0 mg L^{-1}) + *C. vulgaris* (200 ml L^{-1})
4. B0M1F0: Boric acid (0 mg L^{-1}) + Melatonin (50 mg L^{-1}) + *C. vulgaris* (0 ml L^{-1})
5. B0M1F1: Boric acid (0 mg L^{-1}) + Melatonin (50 mg L^{-1}) + *C. vulgaris* (100 ml L^{-1})
6. B0M1F2: Boric acid (0 mg L^{-1}) + Melatonin (50 mg L^{-1}) + *C. vulgaris* (200 ml L^{-1})
7. B0M2F0: Boric acid (0 mg L^{-1}) + Melatonin (100 mg L^{-1}) + *C. vulgaris* (0 ml L^{-1})

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8. B0M2F1: Boric acid (0 mg L^{-1}) + Melatonin (100 mg L^{-1}) + *C. vulgaris* (100 ml L^{-1})
9. B0M2F2: Boric acid (0 mg L^{-1}) + Melatonin (100 mg L^{-1}) + *C. vulgaris* (200 ml L^{-1})
10. B1M0F0: Boric acid (500 mg L^{-1}) + Melatonin (0 mg L^{-1}) + *C. vulgaris* (0 ml L^{-1})
11. B1M0F1: Boric acid (500 mg L^{-1}) + Melatonin (0 mg L^{-1}) + *C. vulgaris* (100 ml L^{-1})
12. B1M0F2: Boric acid (500 mg L^{-1}) + Melatonin (0 mg L^{-1}) + *C. vulgaris* (200 ml L^{-1})
13. B1M1F0: Boric acid (500 mg L^{-1}) + Melatonin (50 mg L^{-1}) + *C. vulgaris* (0 ml L^{-1})
14. B1M1F1: Boric acid (500 mg L^{-1}) + Melatonin (50 mg L^{-1}) + *C. vulgaris* (100 ml L^{-1})
15. B1M1F2: Boric acid (500 mg L^{-1}) + Melatonin (50 mg L^{-1}) + *C. vulgaris* (200 ml L^{-1})
16. B1M2F0: Boric acid (500 mg L^{-1}) + Melatonin (100 mg L^{-1}) + *C. vulgaris* (0 ml L^{-1})
17. B1M2F1: Boric acid (500 mg L^{-1}) + Melatonin (100 mg L^{-1}) + *C. vulgaris* (100 ml L^{-1})
18. B1M2F2: Boric acid (500 mg L^{-1}) + Melatonin (100 mg L-1) + C. vulgaris (200 ml L-1)

RESULTS AND DISCUSSION

Peroxidase enzyme activity. Unit mg $^{-1}$

Results in Table (1) reveal that application of boron (B_1), increased peroxidase enzyme activity significantly at 1.180 Unit mg $^{-1}$, compared to the control treatment which was 0.523 units mg^{-1}. This was 125.6% higher than the control. For melatonin treatments, M_2 expressed the most peroxidase activity (1.342 units mg^{-1}), while M_0 expressed the lowest (0.367 units mg^{-1}) with a 265.7% increase. Regarding *Chlorella vulgaris* soil application, F_1 and F_2 fertilized were respectively increased in activity with 0.893 and 0.878 units mg^{-1} compared to F_0 which represented the lowest activity with 0.783 units mg^{-1} resulting an enhancement of 14.04 and 12.1 % respectively. In the two-way B × M interaction, the combination of B_1M_2 exhibited the maximum peroxidase activity (1.658 units mg^{-1}) and was significantly higher over the B_0M_0 interaction, which recorded the least activity (0.234 units mg^{-1}) with the increase was 608.5%. Regarding three way interaction (B × M × F), the $B_1M_2F_1$ combination had the maximum level of peroxidase enzyme activity (1.710 units mg^{-1}), and the lowest (0.223 units mg^{-1}) was observed in the $B_0M_0F_0$, which to a highly significant extent was 666.8% higher.

Table 1. Effect of spraying melatonin, boron and adding Chlorella vulgaris to the soil on peroxidase enzyme activity. Unit mg $^{-1}$

B	**M**	**F**			**B x M**	**(B)**
		F0	**F1**	**F2**		**means**
B0	M0	0.223	0.257	0.224	0.234	0.523
	M1	0.293	0.313	0.317	0.308	
	M2	0.967	0.970	1.140	1.026	
B1	M0	0.307	0.627	0.563	0.499	1.180
	M1	1.350	1.480	1.317	1.382	
	M2	1.557	1.710	1.707	1.658	
(F) means		0.783	0.893	0.878	L.S.D 0.05 0.112	L.S.D 0.05 0.071
L.S.D 0.05 B x M x F		0.216				
F x B	B0	0.494	0.513	0.560	L.S.D 0.05 0.112	
	B1	1.071	1.272	1.196		
F x M	M0	0.265	0.442	0.393	0.367	(M) means
	M1	0.822	0.897	0.817	0.845	
	M2	1.262	1.34	1.423	1.342	
L.S.D 0.05						
F x M		F			M	
0.160		0.092			0.092	

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P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
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Catalase enzyme activity. Unit mg $^{-1}$

Data in Table (2) shows that application of boron (B1) increased catalase enzyme activity significantly at 34.15 units mg^{-1} compared to the control treatment which was 23.41 units mg^{-1}. This was 45.9% higher than the control. For melatonin treatments, M_2 expressed the most catalase activity (42.78 units mg^{-1}), while M_0 expressed the lowest (12.33 units mg^{-1}) with a 246.9% increase. Regarding *Chlorella vulgaris* soil application, F_1 and F_2 fertilized were respectively increased in activity with 29.50 and 30.78 units mg^{-1} compared to F_0 which represented the lowest activity with 26.06 units mg^{-1}, resulting in an enhancement of 13.20% and 18.11% respectively. In the two-way B × M interaction, the combination of B_1M_2 exhibited the maximum catalase activity (50.78 units mg^{-1}) and was significantly higher over the B_0M_0 interaction, which recorded the least activity (10.67 units mg^{-1}) with an increase of 375.9%. Regarding three-way interaction (B × M × F), the $B_1M_2F_2$ combination had the maximum level of catalase enzyme activity (52.67 units mg^{-1}), and the lowest (9.00 units mg^{-1}) was observed in the $B_0M_0F_0$, which to a highly significant extent was 485.2% higher.

Table 2. Effect of spraying melatonin, boron and adding Chlorella vulgaris to the soil on Catalase enzyme activity. unit mg

B	M	F			B x M	(B)
		F0	F1	F2		means
B0	M0	9.00	10.67	12.33	10.67	23.41
	M1	19.33	27.33	27.67	24.78	
	M2	32.67	35.33	36.33	34.78	
B1	M0	11.67	15.33	15.00	14.00	34.15
	M1	34.67	37.67	40.67	37.67	
	M2	49.00	50.67	52.67	50.78	
(F) means		26.06	29.50	30.78	L.S.D 0.05 4.2	L.S.D 0.05 4.28
L.S.D 0.05 B x M x F		7.44				
F x B	B0	20.33	24.44	25.44	L.S.D 0.05 4.2	
	B1	31.78	34.56	36.11		
F x M	M0	10.33	13.00	13.67	12.33	(M) means
	M1	27.00	32.50	34.17	31.22	
	M2	40.83	43.00	44.50	42.78	
L.S.D 0.05						
F x M		F			M	
5.38		3.10			3.10	

Polyphenol oxidase enzyme activity. Unit mg $^{-1}$: Table (3) reveals that application of boron (B_1) increased polyphenol oxidase enzyme activity significantly at 0.142 units mg^{-1} compared to the control treatment which was 0.101 units mg^{-1}. This was 40.6% higher than the control. For melatonin treatments, M_2 expressed the most polyphenol oxidase activity (0.171 units mg^{-1}), while M_0 expressed the lowest (0.074 units mg^{-1}) with a 131.1% increase. Regarding *Chlorella vulgaris* soil application F_2 fertilized were respectively increased in enzyme activity with 0.134 units mg^{-1} compared to F_0 which represented the lowest activity with 0.106 units mg^{-1}, resulting in an enhancement of 26.4%. In the two-way B × M interaction, the combination of B_1M_2 exhibited the maximum polyphenol oxidase enzyme activity (0.202 units mg^{-1}) and was significantly higher over the B_0M_0 interaction, which recorded the least activity (0.071 units mg^{-1}) with an increase of 184.5%. Regarding three-way interaction (B × M × F), the $B_1M_2F_2$ combination had the maximum level of polyphenol oxidase enzyme activity (0.213 units mg^{-1}), and the lowest (0.060 units mg^{-1}) was observed in the $B_0M_0F_0$, which to a highly significant extent was 255% higher.

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Table 3. Effect of spraying melatonin, boron and adding Chlorella vulgaris to the soil on polyphenol oxidase enzyme activity. Unit mg $^{-1}$

B	**M**	**F** F0	F1	F2	**B x M**	**(B) means**
B0	M0	0.060	0.083	0.070	0.071	0.101
	M1	0.087	0.097	0.097	0.093	
	M2	0.120	0.147	0.150	0.139	
B1	M0	0.060	0.077	0.093	0.077	0.142
	M1	0.120	0.143	0.180	0.148	
	M2	0.190	0.203	0.213	0.202	
(F) means		0.106	0.125	0.134	L.S.D 0.05 0.016	L.S.D 0.05 0.017
L.S.D 0.05 B x M x F		0.029				
F x B	B0	0.089	0.109	0.106	L.S.D 0.05 0.016	
	B1	0.123	0.141	0.162		
F x M	M0	0.060	0.080	0.082	0.074	(M) means
	M1	0.103	0.120	0.138	0.0121	
	M2	0.155	0.175	0.182	0.171	
L.S.D 0.05						
F x M		F			M	
0.021		0.012			0.012	

Nitrogen (%): Table (4),shows significant increases of the leaf nitrogen values in the examined treatments can be observed. Boron (B_1) sprayings constantly increased N formation up to 0.75%, mean value, compared to 0.68% of the control (B_0) untreated (10.3%). With respect to melatonin treatments, M_2 showed to be the most effective in increasing nitrogen content, averaging 0.79%. M_0, however, was the variety with the least nitrogen tin value (0.64%), Analyzing the effect of Chlorella vulgaris soil application, the F_2 treatment recorded the maximum nitrogen content (0.83%), followed by F_1 (0.73%) and F_0 (0.58%) with an enhancement of 43.1% and 25.9% over the untreated soil, respectively. Two-way interaction analysis (B × M) further revealed that B_1M_2 achieved the highest nitrogen percentage (0.83%), notably exceeding the lowest value observed in B0M0 (0.63%), Moreover, the three-way interaction (B × M × F) confirmed that the $B_1M_2F_2$ combination recorded the highest nitrogen percentage (0.92%), while the minimum was detected in the $B_0M_0F_0$ treatment (0.47%). This improvement amounted to a significant 95.7%, indicating a strong synergistic effect between melatonin, boron, and Chlorella vulgaris application.

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Table 4. Effect of spraying melatonin, boron and adding Chlorella vulgaris to the soil on Percentage of nitrogen %.

B	M	F			B x M	(B) means
		F0	F1	F2		
B0	M0	0.47	0.63	0.78	0.63	0.68
	M1	0.50	0.69	0.82	0.67	
	M2	0.62	0.76	0.84	0.74	
B1	M0	0.47	0.70	0.80	0.66	0.75
	M1	0.69	0.75	0.84	0.76	
	M2	0.73	0.84	0.92	0.83	
(F) means		0.58	0.73	0.83	L.S.D 0.05 0.04	L.S.D 0.05 0.01
L.S.D 0.05 B x M x F		0.07				
F x B	B0	0.53	0.70	0.81	L.S.D 0.05 0.04	
	B1	0.63	0.77	0.85		
F x M	M0	0.47	0.67	0.79	0.64	(M) means
	M1	0.60	0.72	0.83	0.72	
	M2	0.68	0.80	0.88	0.79	
L.S.D 0.05						
F x M		F			M	
0.05		0.03			0.03	

Potassium (%): Data in Table (5), shows significant increases of the leaf potassium values in the examined treatments can be observed. Boron (B_1) sprayings constantly increased K formation up to 1.600%, mean value, compared to 1.286% of the control (B_0) untreated, reflecting an increase of 40%. As for the effect of to melatonin treatments, M_2 showed to be the most effective in increasing Potassium content, averaging 1.642%. M_0, however, was the treatment with the least Potassium value (1.196%). Analyzing the effect of *Chlorella vulgaris* soil application, the F_2 treatment recorded the maximum Potassium content (1.548%), followed by F_1 (1.433%) and F_0 (1.348%), with enhancements of 8 % and 6 % over the untreated soil, respectively. Two-way interaction analysis (B × M) further revealed that B_1M_2 achieved the highest Potassium percentage (1.758%), notably exceeding the lowest value observed in B_0M_0 (1.028%). Moreover, the three-way interaction (B × M × F) confirmed that the B1M2F2 combination recorded the highest Potassium percentage (1.817%), while the minimum was detected in the $B_0M_0F_0$ treatment (0.923%). This improvement amounted to a significant 82.5%, indicating a strong synergistic effect between melatonin, boron, and *Chlorella vulgaris* application.

Table 5. Effect of spraying melatonin, boron and adding Chlorella vulgaris to the soil on percentage of Potassium %

B	M	F			B x M	(B) means
		F0	F1	F2		
B0	M0	0.923	0.997	1.163	1.028	1.286
	M1	1.073	1.380	1.460	1.304	
	M2	1.403	1.550	1.627	1.527	
B1	M0	1.383	1.237	1.470	1.363	1.600
	M1	1.623	1.660	1.753	1.679	
	M2	1.680	1.777	1.817	1.758	
(F) means		1.348	1.433	1.548	L.S.D 0.05 0.141	L.S.D 0.05 0.081
L.S.D 0.05 B x M x F		0.245				
F x B	B0	1.133	1.309	1.417	L.S.D 0.05 0.141	
	B1	1.562	1.558	1.680		
F x M	M0	1.153	1.117	1.317	1.196	(M) means
	M1	1.348	1.52	1.607	1.492	
	M2	1.542	1.663	1.722	1.642	
L.S.D 0.05						
F x M		F			M	
0.014		0.1			0.1	

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Bunch/ means (kg): As is shown in Table (6), significant increases of Weight of a single bunches values in the examined treatments. Boron (B_1) sprayings constantly increased Weight of a single bunches formation up to 4.78 kg mean value, compared to 4.25 kg of the control (B_0) untreated, reflecting an increase of 12%. As for the effect of to melatonin treatments, M_2 showed to be the most effective in increasing mean Weight of a bunch 4.87 kg .M_0, however, was the treatment with the least value 4.15 kg. Analyzing the effect of *Chlorella vulgaris* soil application, the F_2 treatment recorded the maximum mean Weight of bunch 5.08 kg, followed by F_1 4.48 kg and F_0 3.99 kg with enhancements of 27.3 % and 12.3 % over the untreated soil, respectively. Two-way interaction analysis (B × M) further revealed that B_1M_2 achieved the highest mean Weight of bunch 5.15 kg notably exceeding the lowest value in B_0M_0 3.90 kg . Moreover, the three-way interaction (B × M × F) confirmed that the $B_1M_2F_2$ combination recorded the highest mean Weight of bunch 5.70 kg while the minimum was detected in the $B_0M_0F_0$ treatment 3.23 kg , This improvement amounted to a significant 76.4%, indicating a strong synergistic effect between melatonin, boron, and *Chlorella vulgaris* application **.**

Table 6. Effect of spraying melatonin, boron and adding Chlorella vulgaris to the soil on Bunch/ means (kg)

B	M	F			B x M	(B)
		F0	F1	F2		means
B0	M0	3.23	3.70	4.76	3.90	4.25
	M1	4.03	4.20	4.56	4.26	
	M2	4.36	4.20	5.20	4.58	
B1	M0	3.70	4.76	4.76	4.41	4.78
	M1	3.933	4.96	5.50	4.80	
	M2	4.70	5.06	5.70	5.15	
(F) means		3.99	4.48	5.08	L.S.D	L.S.D
L.S.D 0.05		0.70			0.05	0.05
B x M x F					0.36	0.18
F x B	B0	3.87	4.03	4.84	L.S.D 0.05	
	B1	4.11	4.93	5.32	0.36	
F x M	M0	3.46	4.23	4.76	4.15	(M)
	M1	3.98	4.58	5.03	4.53	means
	M2	4.53	4.63	5.40	4.87	
L.S.D 0.05						
F x M		F			M	
0.52		0.30			0.30	

Table (1, 2 and 3) indicates that melatonin increases the activity of important antioxidative stress-active enzymes. These enzymes are highly important for detoxifying reactive oxygen species and reducing oxidative stress (Imran *et al*., 2022). The ability of melatonin to convert oxide radicals to hydrogen peroxide, and hydrogen peroxide in turn to be detoxified by peroxidase, catalase and polyphenol oxidase demonstrates the presence of a cooperatively elevated enzymatic defense system contributed by melatonin. In addition, melatonin modulates gene expression in the cells and is implicated in antioxidative defense mechanisms, as it can stimulate the transcription factors that regulate gene expression in the anti-oxidative response (Arnao and Hernández-Ruiz., 2021). This results in the upregulation of numerous antioxidative genes, and subsequently the anti-oxidative potential of the plant is enhanced. Melatonin also increases the production of secondary metabolites (e.g., phenolics) which play a supplementary antioxidative role to enzymes and strengthen plant defense systems, as well (Back., 2021). By preventing lipid peroxidation and stabilizing the cell membrane, as well as not allowing it to deteriorate, melatonin contributes to maintaining cellular homeostasis during

Iraqi Journal of Agricultural Sciences –2026:57(7):1998-2011
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/d9mvbw42

various stress conditions (Chang *et al* ., 2023). Under drought conditions, melatonin and boron have a significant impact in alleviating harmful effects, as stomata small pores on the leaf surface regulate gas exchange and water loss in plants. Under drought condition, melatonin controls stomatal closure and decreases transpiration rate and water loss from plants, resulting in maintain intracellular water content in plant during the drought period (Ding *el al*., 2017). Reactive free radicals may be generated by drought stress in plants, it in turn may cause oxidative stress. Thus, melatonin plays a crucial role in the antioxidant system in removing excess oxidative damage and drought-induced oxidative damage in plants. They tend to upregulate drought-responsive genes in response to drought stress. Drought stress caused by excessive transpiration disrupts water balance and osmotic relations in the plant. To protect plants from drought, osmotic and water balance is regulated through the accumulation of proline and soluble sugars (Tiwari *et al* .,2023). Plant hormones such as ABA and CKs play a vital role in the plant's physiology for coping with drought stress, and melatonin modifies the production of these hormones to enhance drought stress tolerance (Ahmad et al.,2022 ; Al-Asally and Al-Hijemy .,2023 ; Al-Hassany and Abbood., 2021). Table (4, 5 ,6) illustrates the effect of *Chlorella vulgaris* on the soil through atmospheric fixation (the photochemical conversion of nitrogen into nitrate) (4, Zeng et al., 2022 ; Stal., 2015; Tiwari et al., 2023; Alvarez et al., 2021).. In the algae, the nitrogen is part of the structure of the chlorophyll pigment so that when algae decompose, nitrogen is released directly to the soil and taken up by plants without any possible N loss (Dineshkumar et al., 2018; Jabbar and Hassan., 2020; Sadiq., 2023). Using living organisms which dissolve and aid in the uptake of Potassium, such as algae through the production of organic acids that increase solubility, the algae themselves has a mechanism for the uptake of Potassium whereby they have pools within the cell of the Potassium and this is released back into the soil once the algae die and decay (Sajid *et al.*, 2024). This indicates that bio-fertilizers are influencing the availability of macronutrients to the plants. In addition, it is found that B plays an important function in the absorption of Potassium function of the synergistic relation between B and K(Sharafi and Raina., 2021; Ödemiş and Uncu., 2022; Walli *et al*., 2022 ; Shireen *et a*l., 2018). Boron may also play a significant role in the transport of energy compounds to different parts of the plant, in cooperation with the activity of microorganisms that supply the plant with essential nutrients necessary for its growth and development, The combined effects of boron and melatonin on the plant and the effect of algae as an important bio-fertilizer in providing nutrients led to the improvement of all growth processes and this was reflected in the improvement of the quality of the fruit (Faisal *et al* ., 2024; Trivedi et al., 2016; Vera-Maldonado et al ., 2024; Zhang *et al*., 2024).

CONCLUSION

Results of the present study clearly indicate that exogenous application of melatonin, boron and *Chlorella vulagris* in combination significantly improved the antioxidant system in date palm through enhancement of the activity of the peroxidase, catalase and polyphenol oxidase enzymes. The treatments also enhanced the nutritional status of the trees, increasing nitrogen and Potassium contents in the leaves and especially in environmentally stressed conditions. The antioxidant- and regulatory-like actions of melatonin combined with the nutrient-supplying properties of boron and *Chlorella vulgaris* blended well to enhance the overall resilience and physiological performance of plants. The combined application of melatonin, boron, and algal bio-fertilizer could, therefore, be utilized as a potential management measure to improve date palm resilience to drought stress and growth promotion.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to the College of Agricultural Engineering Sciences, University of Baghdad, for providing the research facilities and support needed to complete this study. Appreciation is also extended to the staff of the Fadak Date

P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/d9mvbw42

Palm Farm in Karbala for their valuable assistance during fieldwork and data collection.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

AUTHOR/S DECLARATION

Mustafa E. Al-Asally designed and conducted the experiment, collected the data, and wrote the first draft of the manuscript. **Salah H. J. Al-Hchami** supervised the study, contributed to data analysis, interpretation, and manuscript revision. Both authors reviewed and approved the final version of the manuscript.

AUTHOR'S CONTRIBUTION STATEMENT

This research presents the first integrated study evaluating the combined effects of melatonin, boron, and Chlorella vulgaris algae on the physiological, biochemical, and yield characteristics of *Phoenix dactylifera* cv. Al-Sayer under desert stress conditions. The results demonstrate that applying these bioactive agents together can significantly enhance fruit quality, nutrient absorption, and overall yield in date palms, offering a sustainable strategy for improving production under harsh environmental conditions

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P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/d9mvbw42

دور الميلاتونين والبورون و *Chlorella vulgaris* في الإنزيمات المضادة للأكسدة لنخيل التمر في بيئة صحراوية

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المستخلص

تهدف هذه الدراسة إلى تقييم تأثيرات الميلاتونين والبورون وطحالب *Chlorella vulgaris* على نشاط الإنزيمات المضادة للأكسدة وحالة المغذيات في أشجار النخيل تحت ظروف الإجهاد البيئي. أجريت الدراسة خلال موسم النمو في عام 2024 في محطة فدك لنخيل التمر، الواقعة في صحراء كربلاء، على أشجارنخيل صنف الساير(*Phoenix dactylifera L.*) , تم اختيار 54 نخلة متجانسة عمرها سبع سنوات لإجراء التجربة .تم رش الميلاتونين بثلاثة تراكيز (0-50-100) ملغم لتر$^{-1}$، والبورون (حمض البوريك) بتركيزين (0 - 500) ملغم لتر$^{-1}$، وإضافة طحالب *Chlorella vulgaris* في التربة بثلاثة تراكيز (0-100-200) مل لتر$^{-1}$, أظهرت النتائج أن الميلاتونين والبورون يلعبان دوراً هاماً وحيوياً في زيادة فعالية الإنزيمات المضادة للأكسدة (peroxidase, catalase, polyphenol oxidase) تحت ظروف الإجهاد الناتج عن الجفاف، مما أدى إلى تحسين الحالة الغذائية والفسيولوجية للنخيل، الأمر الذي انعكس على جودة الثمار. وأدت إضافة الطحالب إلى التربة بمستويات مختلفة إلى زيادة تركيز النيتروجين والبوتاسيوم في وريقات النخيل. اذ تؤدي هذه العوامل مجتمعة من تخفيف الضغوط البيئية على نخيل التمر في ظروف الصحراء.

الكلمات المفتاحية: التسميد الحيوي، كاتاليز، مكافحة التصحر، البيروكسيديز، بولي فينول.