Egypt. J. of Appl. Sci., 40 (7-8) 2025 **101-119**

AMELIORATION OF DROUGHT STRESS IN *Hordeum vulgare* (L.) BY MELATONIN AND *Bacillus megaterium*

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ABSTRACT

A greenhouse pot experiment was conducted to evaluate the efficacy of melatonin foliar application at 0.10 mM and seed inoculation with *Bacillus megaterium* (10^7-10^8 CFU ml^{-1}) on growth, yield, biochemical properties and soil enzymatic activity of barley cv. Giza 133 under drought stress (70 % of water requirements). The findings showed that grain and straw yields, chlorophyll content and relative water content were significantly reduced under drought conditions. However, electrolyte leakage percentage, peroxidase activity and proline content statistically increased because of water lack. Using melatonin and *B. megaterium* significantly improved growth and yield characters, total chlorophyll content, relative water content and soil biological properties. Melatonin and *B. megaterium* showed significant decreases in electrolyte leakage % and peroxidase activity under drought stress. This study recommends using melatonin as foliar spray and *B. megaterium* as seed inoculant to modulate abiotic stress-adaptive mechanisms, thereby protecting stressed plants and increasing plant resistance for drought conditions.

Key Words: Drought stress, Melatonin, Barley, *Bacillus*, PGPR

1- INTRODUCTION

Abiotic stressors are non-living environmental triggers that impede plant development and reduce agricultural production. Agricultural activities are significantly affected by water deficiency, salinity, high temperature, inundation, nutrient availability, toxicity of heavy metals, UV contamination and air pollution **(Gomes *et al.*, 2025)**. Diminished agricultural yields and quality may be the consequence of disruptions in physiological processes, nutrient assimilation, cellular integrity, and photosynthesis **(Sehgal *et al.*, 2018)**. Drought is a significant stressor that has a detrimental impact on the activity of antioxidant enzymes, chlorophyll levels, and morphological characteristics in a variety of plant species. The germination, growth, and yield of numerous plants are all negatively influenced by drought. The reactive O_2 species (ROS) production, such as superoxide anion (O_2), hydrogen peroxide, and hydroxyl radicals (OH), increased significantly during dehydration stress **(Abdou *et al.*, 2023 and Bhanbhro *et al.*, 2024)**. The antioxidant shield system, which comprises proline, catalase, peroxidase (POD), ascorbate peroxidase, and ascorbic acid, can be regulated to mitigate the increase in ROS levels **(Al-Shammari *et al.*, 2023)**.

Recent developments in sustainable agriculture have highlighted the importance of rhizobacteria as a successful means of reducing abiotic stress in crops. *Bacillus megaterium* has been the subject of much study for its N_2 fixation, phosphate solubilisation, and producing phytohormones including indole-3-acetic acid (IAA) **(Al-Shammari *et al.*, 2024)**. These mechanisms increase osmotic stress tolerance, enhanced water and nutrient acquisition, and root elongation **(Ferioun *et al.*, 2025)**. *B. megaterium* synthesizes exopolysaccharides (EPS) that protect root systems from desiccation, enhance soil aggregation, and preserve root-soil connections, particularly during drought conditions **(Peter *et al.*, 2024)**. According to **Meenakshi *et al.*, (2019)**, treatments with *Bacillus thuringiensis*, *Bacillus subtilis*, and *Bacillus megaterium* notably enhanced chlorophyll content in plants under stress. In barley plants inoculated with PGPR, the levels of H_2O_2 and MDA under diverse environmental conditions are much lower than those in uninoculated plants subjected to abiotic stressors **(Ferioun *et al.*, 2023 and Al-Shammari *et al.*, 2024)**. They also demonstrated a substantial improvement in antioxidant enzyme activity (catalase, peroxidase, superoxide dismutase) in barley plants inoculated with PGPR compared to the uninoculated counterparts.

Plant stress reduction through application of bio stimulants may be achieved by using melatonin as it stands out as both signaling molecule and strong antioxidant with multiple physiological functions. Melatonin is regarded as a crucial bioregulator in agricultural protection and in augmenting antioxidant defense mechanisms. Melatonin, a versatile signaling molecule, is extensively present in plants. Exogenous melatonin application could trigger various physiochemical processes in response to adverse abiotic stress across diverse plant systems **(Xu *et al.*, 2024 and Rezaei *et al.*, 2024)**. Plants show improved chlorophyll biosynthesis and stabilized activity of PSII proteins because of melatonin treatment under drought and salinity stress conditions **(Michałek *et al.*, 2025)**. As a result, photosynthetic performance and biomass accumulation increase. Melatonin improves plant resistance by directly clearing reactive oxygen species, and it provides so by modulating transcription factors associated with stress and enhancing antioxidant enzyme activity and photosynthetic efficiency **(Shaffique *et al.*, 2024)**.

Several studies indicate that combining melatonin and *Bacillus megaterium* can increase stress tolerance. **Peter *et al.*, (2024)** revealed that the combination of *Bacillus megaterium* and melatonin yields superior stress tolerance compared to the use of either substance alone. Plants can reach deeper soil moisture and nutrients levels through the *B. megaterium* and melatonin interaction which maximizes their adaptive abilities in water-depleted territories. However, little is known about the effect of melatonin on soil biological activities, especially under abiotic stress.

This research investigates the effects of combining *Bacillus megaterium* with melatonin treatments to enhance barley growth as well as photosynthetic efficiency together with antioxidant defense mechanisms and nutrient acquisition under drought conditions. We hypothesized that melatonin foliar spray combined with *B. megaterium* seed inoculation would synergistically improve drought tolerance in barley by enhancing physiological traits and soil enzymatic activity.

2. MATERIALS AND METHODS

2.1. Assessing Plant Growth-Promoting (PGP) Traits of *B. megaterium*

The performance of *B. megaterium* EW017 in improving plant growth in abiotic stress conditions was evaluated by analyzing numerous plant growth-promoting (PGP) characteristics, including the production of Indole-3-Acetic Acid (IAA), Hydrogen cyanide (HCN), and Siderophore production and phosphate (PO_4^{-3}) solubilization.

2.1.1. Phosphate Solubilization Assay

For assaying phosphate (PO_4^{-3}) solubilization, Pikovskaya's medium with insoluble tricalcium phosphate served as the platform to examine phosphate solubilization ability of bacterial strains. After spot-inoculation of the bacterial cultures, the dishes were incubated at 28 ± 2°C for five days. The development of clear halos around bacterial colonies signaled phosphate solubilization. The formula described by Al-Shammari et al. (2024) was used to get the solubilization index (SI):

SI = (Halo zone diameter + Colony diameter) / Colony diameter

Furthermore, liquid phosphate solubilization experiments were performed by inoculating bacterial strains into Pikovskaya's broth and incubated at 30°C for seven days with constant shaking at 150 rpm. After incubation, the supernatant was obtained using centrifugation at 4,000 rpm for 10 minutes, and the soluble phosphorus concentration was measured as described by **Kwon *et al.*, (2024)** using vanado-molybdate colorimetric technique at 420 nm.

2.1.2. Indole-3-Acetic Acid (IAA) Production

The research evaluated bacterial phytohormone production by performing IAA quantification on bacterial culture media extracts. The bacterium was cultured in Luria-Bertani medium enriched with 0.10 % L-tryptophan and incubated at 28 ± 2°C for 48 h, both with and without L-tryptophan. Following incubation, cultures were centrifuged at 6,000 rpm for 10 minutes, and the supernatant was combined with Salkowski reagent (1:2). The mix was incubated in dark for 10 min. **(Patten and Glick, 2002)**, the emergence of a pinkish hue signified the effective synthesis of IAA. The results were recorded by the IAA generation absorbance at 530 nm through spectrophotometer (Jenway 6105, UK). The IAA concentration was determined utilizing a standard curve derived from established IAA concentrations.

2.1.3. Siderophore production

The qualitative assessment of siderophore producing by *B. megaterium* EW017 was determined utilizing the Chromo Azurol S (CAS) evaluation, in accordance with the methodology established by **Alexander and Zuberer (1991)**. New bacterial cultures were introduced onto CAS medium and then cultured at 28 ± 2°C for 72 h. The presence of orange halos surrounding bacterial colonies proved siderophore creation which scientists measured through ruler calibration. The results were classified using a 0–3 rating scale, wherein 0 denoted the absence of a detectable halo, 1 indicated a halo of less than 1 mm, 2 represented a halo ranging from 1 to 5 mm, and 3 marked a halo of 6 mm or greater, as per the criteria set out by **Gopalakrishnan *et al.*, (2015)**.

2.1.4. Hydrogen cyanide (HCN) production

HCN production ability of *B. megaterium* EW017 were analyzed by King's B agar medium with added 4.4 g/L glycine according to the procedures outlined in **Geetha *et al.*, (2014)**. The inner surface of Petri plate lids received Whatman filter no. 1 which was pre-soaked in Na_2CO_3 solution combined with picric acid solution (0.05% in 2%) for a two-day (28 ± 2°C) cultivation period. The plates were sealed with Parafilm and incubated for an additional 48 hours at 28 ± 2°C. The formation of HCN was indicated by a color transition in the filter paper from deep yellow to reddish-brown. According to the criteria established by **Devarajan *et al.*, (2022)**, the extent of color alteration was employed to classify HCN production into three tiers: high (dark brown, score 3), moderate (medium brown, score 2), and low (light brown, score 1).

2.2. Preparation of inoculant and seed inoculation

Bacillus megaterium EW017 was acquired from Microbiological Resource Center (MIRCN), Ain Shams University, Cairo. For preparing the bacteria inoculation, a loopful from *B. megaterium* stock culture was cultured in 80 ml bottles containing 30 ml of nutrient broth medium, then incubated for 3 days (28 ± 2°C). The cell count ranged $10^7 – 10^8$ CFU ml^{-1}. For barley seed inoculation, about 20 g were sterilized using ethyl alcohol 70% for five min. then rinsed using sterilized water. Barley sterilized seeds were soaked in 250 ml of inoculant for 2 h prior to sowing.

2.3. Pot experiment

A pot trial was performed in the greenhouse of the Experimental Farm of Faculty of Agriculture, Suez Canal University, Ismailia, Egypt in the winter season of 2024 to evaluate the potential of *Bacillus megaterium* EW017 ($10^{7\ -8}$ CFU ml^{-1}) and/or melatonin (0.10 mM) in enhancing the tolerance of barley to drought stress (70% from full irrigation water requirement). Local potential evapotranspiration was computed by Penman-Monteith method. The climatological data used in current study were attained from CLIMWAT (version 2.0). Data were combined into CROPWAT programme (version 8.0) to estimate barley water requirements in Ismailia, Egypt **(Allen *et al.*, 1998)**.

The trial consists of 8 treatments as follows: irrigation with 100% of water requirement (control, C); 100% of water requirement + melatonin (M); 100% of water requirement + *B. megaterium* (B); 100% of water requirement + M + B; 70 % from water requirement (drought, D); D + M; D+B and D+M+B.

Initial soil sample and the utilized organic manure (compost) were analyzed for physicochemical properties as described by **Sparks (1996)** (**Table 1**). Soil samples were homogenously filled in pots, each of 29 cm height and 23.8 cm diameter at a rate of 15.0 kg pot^{-1}. Compost was mixed with the upper 10 kg of the soil in each pot. The trial was laid out in a randomized complete block design (RCBD) in a factorial arrangement with five replications. Seven barley seeds were sown in each pot and irrigated to almost soil field capacity with Ismailia canal water (0.35 dSm^{-1}). The seedlings were thinned to four uniform plants pot^{-1} after two weeks from cultivation. Barley seedlings were foliar sprayed with melatonin solution (0.10 mM) at five intervals: 30, 50,70, 90, 110 days after seed cultivation. The plants were fertilized in accordance with the Egyptian Ministry of Agriculture's recommendations.

2.4. Samples collection and determinations

Plant samples were collected after 135 d (ripeness stage) after cultivation, oven dried at 70°C to measure grain and straw dry weights. After 90 d from sowing date, five leaf discs were collected for the estimation of membrane permeability and leaf relative water content (RWC) according to the described method by **Lutts *et al.*, (1996) and Sade *et al.*, (2015)**, respectively. Membrane permeability was calculated as Electrolyte Leakage EL %. For determination of total phenolics compounds, 0.5 g of fresh leaves were soaked in acetone (80%)

following the procedure of **Julkunen-Tiitto (1985)** and were measured spectrophotometrically at 760 nm. Proline was assayed in 0.5 g dry leaves sample as stated by **Sanchez *et al.*, (2004)** using spectrophotometer at 520 nm. Total chlorophyll concentration was determined based on the methodology of meter readings using SPAD (502 plus-Minolta, Japan) at the anthesis stage. Peroxidase activity was determined as described by **Chance and Maehly (1955)** spectrophotometrically at 470 nm and was expressed µmol min^{-1} mg $plant^{-1}$. For processing soil determinations, samples were taken after 50, 90 and 120 d from sowing date and analyzed for alkaline phosphatase activity following the technique introduced by **Tabatabai (1994)**. After barley harvest, soil samples were air-dried and assessed for available phosphorus and pH as detailed by **Sparks (1996)**.

Table 1. Physio-chemical properties of soil and compost used in the current study.

Properties	Soil	Compost
Particle size distribution (%)		
Sand	93.25	-
Silt	3.45	-
Clay	3.30	-
Textural class	Sand	-
Bulk density (g/ cm^{-3})	1.53	-
Soil order	Aridisols	-
Field Capacity (%)	16.80	-
pH	7.91†	7.33‡
EC (dS/ m) ‡‡	2.55	8.35
Soluble cations (meq/ l) ‡‡		
Ca^{2+}	9.86	19.2
Mg^{2+}	6.14	16.2
Na^{+}	5.17	22.4
K^{+}	0.97	25.7
Soluble anions (meq/ l) ‡‡		
CO_3^{2-}	0.00	0.00
HCO_3^{-}	2.72	27.5
Cl^{-}	10.59	42.8
SO_4^{2-}	12.19	13.2
Organic C (g/ kg)	1.78	184
Total N (g/ kg)	0.128	16.1
Available N (mg/ kg)	7.82	176
Available P (mg/ kg)	9.04	152

Where: † pH value was measured in soil-water suspension (1:2.5); ‡ pH value was measured in compost-water suspension (1:5); ‡‡Electric conductivity (EC) and soluble cations and anions values were determined in soil or compost saturate paste extracts.

2.5. Statistical analysis

All data were processed for analysis of variance (ANOVA) and least significant difference (LSD) tests (P < 0.05) using IBM SPSS software version 27.0.

3. RESULTS

3.1. plant growth-promoting (PGP) traits

Data presented in **Table 2** displayed that PGP traits of *B. megaterium* were assessed under control conditions (0 MPa) and PEG6000-induced osmotic stress (-1.5 MPa). In both environments, the synthesis of HCN and siderophores remained consistently elevated (+++). The strain showed enhanced phosphate solubilization activity denoted by (+++) while the solution phosphate amounts slightly declined from 254 mg P L^{-1} under standard conditions to 243 mg P L^{-1} during stress conditions. Under stress, IAA synthesis increased somewhat from 6.76 to 6.81 µg mL^{-1} (without L-tryptophan) and from 12.45 to 13.03 µg mL^{-1} (with L-tryptophan). The studies confirmed by LSD ($P \leq 0.01$) show *B. megaterium* retains substantial PGP activity under extreme osmotic conditions thus pointing to its effective use as a biofertilizer for dry and arid environments **(Table 2)**.

Table 2 plant growth promoting (PGP) traits of *B. megaterium* under control (0 MPa) and PEG6000-induced stress (-1.5 MPa).

PGP traits	Siderophore production	P-solubilization		IAA production, µg ml^{-1}			HCN production
		On solid medium	mg P l^{-1}	On solid medium	Without L-TRP	With L-TRP	
Control (0 MPa)	+++	+++	254	+++	6.76	12.45	+++
Induced stress (1.5 MPa)	+++	+++	243	+++	6.81	13.03	+++
L.S.D $_{0.01}$	-	-	17.26	-	0.425	1.051	-

IAA: Indole acetic acid; L-TRP: L-tryptophan- = no production + = low production; ++ = moderate production and +++ = strong production

3.2. pot experiment

3.2.1. Barley yield

Regarding the results in **Table 3**, drought stress decreased grain, straw and biological yield significantly (20.4, 23.7 and 21.8 %) compared with control under normal conditions. Generally, the treatment with melatonin and/or *B. megaterium* statistically improved grain, straw and biological yields (**Table 3**) in the stressed barley plants when compared to plants without melatonin and *B. megaterium* treatment under the same stress conditions. The maximum grain and biological yields were recorded in the treatment of melatonin + *B. megaterium* without drought where the relative increases over the control conditions were 10.7 and 9.6 % correspondingly (**Table 3**). It is worth noting that the above-mentioned treatment did not differ significantly with the treatment of *B. megaterium* without drought. This finding emphasizes the potential efficacy of melatonin and *B. megaterium* co-application in enhancing stress tolerance mechanisms.

Table 3. Effect of melatonin and *B. megaterium* application on grain, straw and biological yields (ton ha^{-1}) of barley plants under drought stress.

Treatments	Grain yield	Straw yield	Biological yield
Control (C)	4.51	3.29	7.80
Melatonin (M)	4.82	3.40	8.22
B. megaterium (B)	5.01	3.60	8.61
M + B	5.05	3.58	8.63
Drought (D)	3.59	2.51	6.10
D + M	4.28	3.22	7.50
D + B	4.41	3.31	7.72
D + M + B	4.45	3.27	7.72
L.S.D. $_{0.05}$	0.226	0.198	0.214

3.2.2. Chlorophyll content by SPAD

Chlorophyll content showed a significant decrease reach to 5.1% under drought conditions compared with normal conditions. The treatment of melatonin + *B. megaterium* was recorded as the highest chlorophyll content under normal and under drought conditions. (**Fig. 1**).

3.2.3. Leaf relative water content (RWC)

RWC was assessed for its role as an indicator of stress and unfavorable conditions in plants. Results in **Fig (2)** show that bacterial inoculant and/or melatonin application significantly enhanced RWC when comparing with control. The maximum RWC value was detected with the treatment of *B. megaterium* with no statistically significant difference when melatonin was applied individually or in combination with *B. megaterium* under both normal and stress conditions.

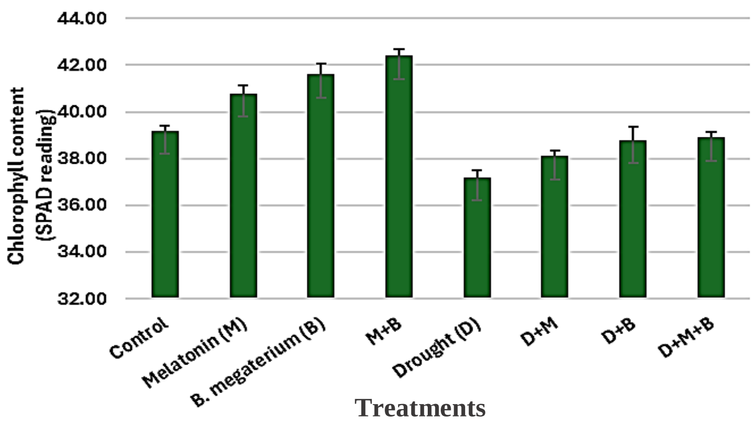

Fig. 1. Effect of melatonin and *B. megaterium* application on chlorophyll content (SPAD reading) of barley plants under drought stress.

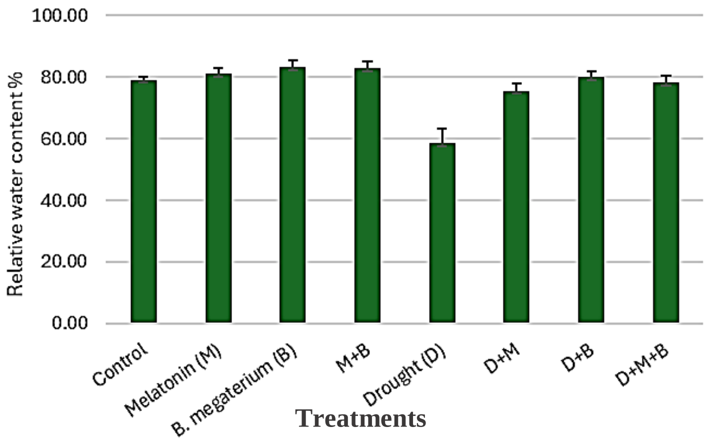

Fig. 2. Effect of melatonin and *B. megaterium* application on relative water content (%) of barley plants under drought stress.

3.2.4. Phenolic compounds

The data in **Fig. 3** reveal that drought stress led to a significant decline in phenolic compounds (38.2 %) in the stressed barley plants comparing to the plants under normal conditions. Application of melatonin or *B. megaterium* led to a significant increase in phenolic compounds in the stressed barley plants. Total phenolic compounds increased significantly (36.2 %) in stressed plants treated with melatonin and *B. megaterium* as compared to untreated stressed plants (**Fig. 3**).

3.2.5. Proline content

The concentration of proline was determined as a reliable biochemical indication reflecting plant exposure to drought stress. **Fig. 4** shows that proline content improved significantly (41.6%) in the stressed barley plants compared to the control. Utilizing *B. megaterium* and melatonin caused significant declines in proline concentrations (19.3 and 16.3%, respectively) under drought conditions.

3.2.6. Electrolyte leakage

Electrolyte leakage was assayed as an indication of oxidative harm in barley under drought stress. Drought conditions induced a statistical increase in electrolyte leakage (35.5 %) in the stressed plants compared to normal conditions. On the other hand, the exogenous application of melatonin and *B. megaterium* statistically declined electrolyte leakage (22.8 % and 29.8 %, respectively) in the barley plants under drought conditions in comparison to the plants in the same conditions without *B. megaterium* and melatonin application (**Fig. 5**).

3.2.7. Peroxidase activity

The activity of peroxidase enzyme was determined as an indicator for the antioxidant enzymes; **Fig. 6** indicates that the activity of peroxidase significantly increased (27.6%) in the drought-affect barley plants relative to control. Conversely, the use of *B. megaterium* and melatonin significantly reduced (17.6 and 12.2% respectively) the activity of peroxidase in the drought-affect plants relative to untreated stressed plants.

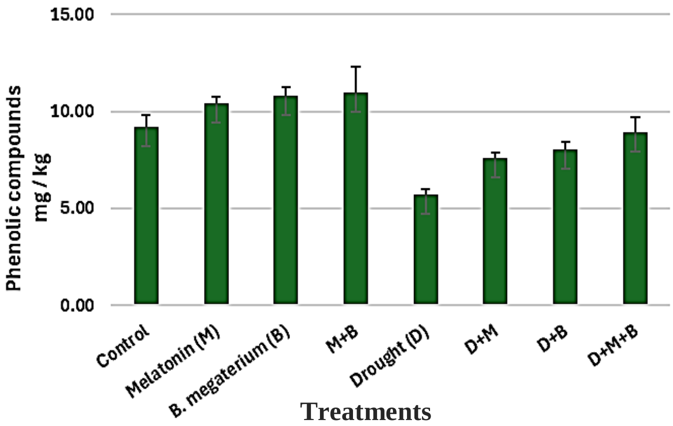

Fig. 3. Effect of melatonin and *B. megaterium* application on phenolic compounds (mg Kg^{-1}) of barley plants under drought stress.

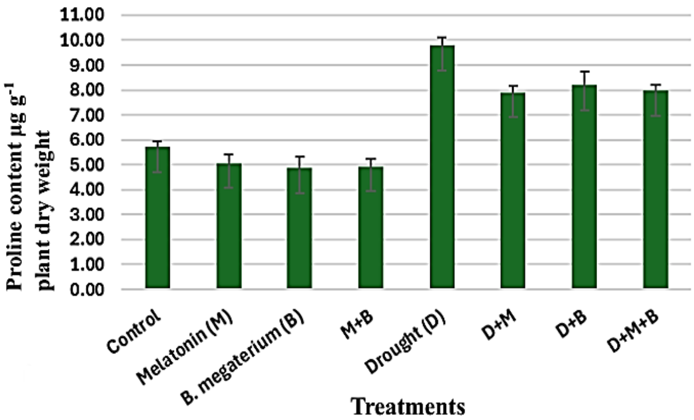

Fig. 4. Effect of melatonin and *B. megaterium* application on proline compounds (µg g^{-1}) of barley plants under drought stress.

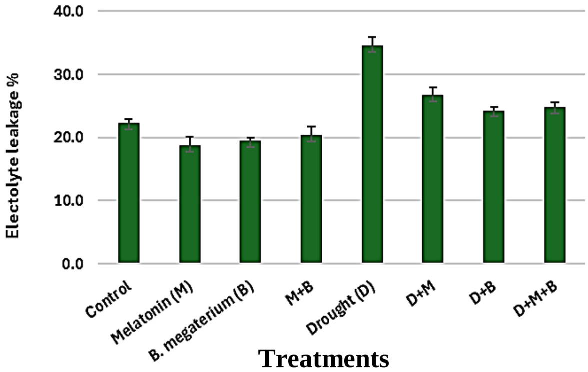

Fig. 5. Effect of melatonin and *B. megaterium* application on electrolyte leakage (%) of barley plants under drought stress.

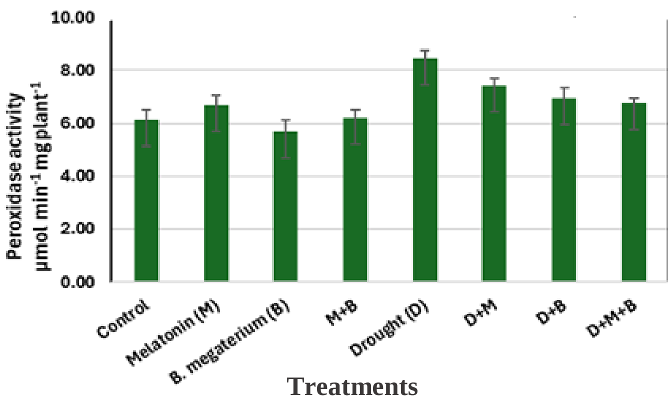

Fig. 6. Effect of melatonin and *B. megaterium* application on peroxidase activity (µmol min^{-1} mg $plant^{-1}$) of barley plants under drought stress.

3.2.8. Available phosphorus, pH and alkaline phosphatase in soil

Generally, it was noticed that soil pH values declined because of *B. megaterium* inoculation and sometimes melatonin foliar application compared to the control (**Table 4**). The pH values exhibited their lowest readings of 7.66 and 7.68, in the treatments: *B. megaterium*, and melatonin + *B. megaterium* respectively. Results presented in **Table (4)** show that compared to the initial soil pH value of 7.91 (**Table 1**), the reductions in pH values in above-mentioned treatments were 0.25 and 0.23, respectively. Consistently, available phosphorus levels in the soil followed a similar trend to that of pH values, exhibiting an inverse relationship. The highest concentrations of available phosphorus were recorded in treatments *B. megaterium* and melatonin + *B. megaterium*, which also showed the lowest pH values under both normal and stress conditions (**Table 4**).

The activity of alkaline phosphatase was determined for its roles in P cycle in soil. The activity of the alkaline phosphatase reached its peak after 50 d from barley sowing and subsequently declined to 120 d. Results in Table (4) show that *B. megaterium* and/or melatonin application significantly improved the enzyme activity compared to control. The enzyme activity reached the maximum level in the soil in the treatment of melatonin + *B. megaterium* under both normal and stressed conditions after 30, 50 and 95 d from sowing (**Table 4**).

Table 4. Effect of melatonin and *B. megaterium* application on soil available phosphorus (P), pH and the activity of alkaline phosphatase after 50, 90 and 120 d from barley cultivation under drought stress.

Treatments	Available soil P (mg kg^{-1})	Soil pH	Alkaline phosphatase, (µg ρNP/ g soil/ h)		
			50 d	90 d	120 d
Control (C)	9.11	7.81	76.4	122	94.5
Melatonin (M)	9.23	7.82	79.2	128	98.7
B. megaterium (B)	10.52	7.66	88.4	133	101
M + B	10.55	7.68	89.5	135	104
Drought (D)	9.24	7.88	66.0	107	83.3
D + M	9.31	7.80	72.5	119	92.8
D + B	10.92	7.69	74.4	117	93.8
D + M + B	9.96	7.64	77.1	119	94.3
L.S.D. $_{0.05}$	0.574	0.120	9.82	5.64	6.43

4. DISCUSSION

Although barley plants exhibit relative tolerance to drought stress, grain and straw yields were harmfully influenced under drought conditions. This negative influence may be attributed to the effect of water lake on the uptake of nutrients which might decrease cell expansion and division **(Ghanem *et al.*, 2024)**. The damaging influence of drought on plants were extensively reported in prior research **(Ferioun *et al.*, 2023; Peter *et al.*, 2024 and Said *et al.*, 2025)**.

Melatonin was previously shown to regulate morphological properties of many crops **(El-Yazied *et al.*, 2022 ; Li *et al.*, 2022 and Rezaei *et al.*, 2025)**. *Bacillus* spp. was numerously reported as effective plant growth promoting bacteria which could assist plants in alleviating the harm of abiotic stress by several mechanisms such as secreting IAA and siderophore compounds, reducing soil pH, increasing phosphatase activity and solubilizing inorganic phosphate. The current tested inoculant was proved to solubilize inorganic phosphate, synthesis of HCN, IAA and siderophores under both normal (0 MPa) and extreme osmotic conditions (-1.5 MPa) thus confirmed its potential effectiveness as biofertilizer under drought stress. Additionally, rhizobacteria play great roles in improving drought resistance by releasing auxins, gibberellins and ACC-deaminase, which contribute to plant cell division, promotion of seed germination and increase plant growth through many growth phases **(Kumar *et al.*, 2022; Ajijah *et al.*, 2023; Al-Shammari, *et al.*, 2024 and Kamal *et al.*, 2025)**.

Emam *et al.*, (2023), reported that deficit irrigation water significantly decreased the chlorophyll content which may be attributed to many reasons such as declining the closing of stomata and significant damage of leaf transpiration thus inhibiting some photosynthetic process. The essential role of melatonin as a plant growth regulator and *B. megaterium* as a plant growth promoting bacteria, could explain the marked increases in total chlorophyll concentration under drought conditions. Many previous studies displayed that proline concentration was greater in stressed plants than in unstressed plants **(Abdelaal *et al.*, 2020 and Said *et al.*, 2025). Mohammad-khani and Heidari (2008)** observed that proline concentration in drought-affect plants was about 2.25 times higher than normal plants. This result may be attributed to the role of proline as an osmoregulatory and metabolite, which plays vital role in protecting cell membrane to save plant metabolism under abiotic stress **(Said *et al.*, 2025)**. The reductions in the total phenolic compounds and relative water content in the stressed barley plants may be due to the harmful effect of water scarcity on cell membrane stability which cause structural disruption of the cell wall and consequently decrease relative water content **(Imam *et al.*, 2023)**. The significant increase in membrane permeability may be attributed to abiotic stress, which manifests as oxidative damage to plant organelles, thereby increasing electrolyte leakage. Several studies indicated that electrolyte leakage levels were significantly increased under stress conditions **(Omara and Abdelaal 2018)**. Peroxidase plays a pivotal role in stress resistance; hence its concentration increased in stressed plants compared to normal conditions to elevate the oxidative damage in plants caused by abiotic stress; **(El-Yazied *et al.*, 2022).**

Reduction of soil pH values due to *B. megaterium* was reported by several investigators (Singh et al., 2019; El-Kharbotly and Ghanem 2020; Alaskar and AL-Shwaiman 2023). Recent research reported that the significant reductions in values of soil pH may be due to the secreting of organic acids and probable increase in the pressure of CO_2 in the rhizosphere by the metabolic activities of microorganisms **(Kumar *et al.*, 2022 and Jiang *et al.*, 2025).** Soil alkaline phosphatase was higher at the anthesis stage than the vegetative and ripening stages, which could be attributed to many aspects which affect the secretion rate of the extracellular enzymes. Soil microorganisms is believed to be the key for soil enzymes due to its high metabolic activities, short lifetimes and large biomass which allow them to secrete large amounts of extracellular enzymes **(Jiang *et al.*, 2025)**. The significant enhancement in soil available phosphorus after seed inoculation of *B. megaterium* may be due to the increase in phosphatase activity and the reduction in soil pH **(Ughamba *et al.*, 2025).**

CONCLUSION

The current study proved that drought stress detrimentally influences the physiological, biochemical and yield properties of barley plants. Conversely, using of melatonin or *B. megaterium* enhanced all tested qualities such barley yield, total chlorophyll concentration, relative water content and the total phenolic compounds in the stressed barley plants. Additionally, Electrolyte leakage, proline content and peroxidase activity significantly declined with melatonin and *B. megaterium* under drought stress. Likewise, soil available phosphorus, pH and alkaline phosphatase activity were enhanced with the treatment with melatonin or *B. megaterium*. Thus, the application of melatonin or *B. megaterium* presents protection to stressed barley plants under water-limited conditions.

Ethics approval : Not applicable.
Conflict of interests : The authors have no conflicts of interest to declare that are relevant to the content of this article.
Funding: Not applicable.
Authors' contributions: Conceptualization, O.M.G. and A.F.S.; Roles/Writing-original draft, O.M.G., M.A.E. and A.F.S.; Data curation, O.M.G., M.A.E. and A.F.S.; Formal analysis, M.A.E. and A.F.S.; Investigation, O.M.G., M.A.E. and A.F.S.; Methodology, O.M.G., M.A.E. and A.F.S.; Software, M.A.E. Supervision, O.M.G. and A.F.S.; Visualization, O.M.G., and M.A.E.; Validation, O.M.G. and A.F.S. All authors read and agree for submission of manuscript to the journal.

Acknowledgments:

The authors gratefully acknowledge the technical staff of Soil and Water, Agronomy and Agricultural Botany Departments, Faculty of Agriculture, Suez Canal University, for their help.

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تحسين تحمل الإجهاد المائي في الشعير باستخدام الميلاتونين وبكتريا

Bacillus megaterium

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تم اجراء تجربة أصص لتقييم تأثير اضافة الميلاتونين رشاً علي النباتات (0.10 مللي مولار) وتلقيح البذور ببكتريا *Bacillus megaterium* على نمو وإنتاجية الشعير (جيزة 133) تحت ظروف الإجهاد المائي (70% من الاحتياجات المائية). وكانت المعاملات تشمل الكنترول (100% من الاحتياج المائي)، والميلاتونين، وبكتريا *Bacillus megaterium* ، والتبادلات المختلفة بينهما تحت ظروف الإجهاد والظروف العادية. أدى الإجهاد المائي إلى انخفاض كبير في إنتاج الحبوب (20.4%)، والقش (23.7%) مقارنة بالظروف العادية. كما انخفض محتوى الكلوروفيل والمحتوى المائي النسبي والمركبات الفينولية الكلية بشكل كبير بسبب الإجهاد المائي. لوحظ أن المعاملة بالميلاتونين أو بكتريا *Bacillus megaterium* تؤدي إلى تحسين إنتاجية الحبوب والقش في النباتات

المعرضة للإجهاد. كما ان محتوى النبات من الكلوروفيل والمحتوى المائي النسبي تحسن معنوياً مع المعاملة بالميلاتونين أو بكتريا *Bacillus megaterium* مقارنة بالنباتات المعرضة للإجهاد غير المعاملة. وزاد محتوى البرولين(41.6%) وتسرب الالكتروليتات (35.5%) ونشاط إنزيم البيروكسيديز (27.6%) في النباتات المعرضة للإجهاد لكنه انخفض مع معاملات الميلاتونين و *Bacillus megaterium*.. ولوحظ وجود انخفاض معنوي في درجة حموضة التربة وزيادة مستويات الفوسفور الميسر معنوياُ مع معاملات الـ *Bacillus megaterium* وكذلك زيادة نشاط إنزيم الفوسفاتيز القلوي في التربة معنويا والذي بلغ ذروته عند 50 يومًا من الزراعة وتحسن بشكل كبير نتيجة التلقيح البكتيري تحت ظروف الاجهاد المائي.

خلاصه هذه الدراسة أن الإجهاد المائي يؤثر سلبًا على الخصائص الفسيولوجية والبيوكيميائية والإنتاجية لنبات الشعير. ولكن المعاملة ببكتريا *Bacillus megaterium* والميلاتونين يمكن أن تحسن النمو والإنتاج والخصائص البيوكيميائية للشعير تحت الإجهاد المائي. وتوصي الدراسة باستخدام الميلاتونين و بكتريا *Bacillus megaterium* لتحسين مقاومة الشعير للإجهاد في حالة نقص الاحتياجات الميائية بمقدار 30%.