ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 25(3) 2026, 13–22

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2026.5575

RESEARCH PAPER

Received: 24.07.2025
Accepted: 11.03.2026
Online first: 6.05.2026
Issue published: 22.06.2026

EFFECT OF CYCLIC 3-HYDROXYMELATONIN (3-OHM) ON PEPPER SEEDLING EMERGENCE UNDER DROUGHT CONDITIONS

Aygül Karaca[1], Gökçen Yakupoğlu[2], Ahmet Korkmaz[3]

ABSTRACT

Cyclic 3-hydroxymelatonin (3-OHM), a significant metabolite derived from melatonin (MEL) through its interaction with oxygen-containing compounds, is believed to play a crucial role in enhancing plant resistance to various abiotic stresses. Despite its importance, research on 3-OHM remains limited. Therefore, this study aimed to investigate the effects of exogenous 3-OHM treatments on the drought stress tolerance of pepper seedlings during the emergence phase. The application of 3-OHM to seeds at various concentrations (0, 10, 50, and 100 µM) notably improved seedling emergence performance under drought conditions compared to untreated controls. Furthermore, 3-OHM treatments significantly reduced oxidative stress markers such as hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS), while simultaneously enhancing the activities of key antioxidant enzymes including peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT). Additionally, seedling proline and total chlorophyll contents increased significantly by 3-OHM application. Among the concentrations tested, 50 µM 3-OHM consistently showed the most pronounced beneficial effects across multiple parameters. These results underscore the potential utility of 3-OHM as a natural bioactive compound to mitigate the detrimental impacts of abiotic stress in agricultural crops. To further elucidate the physiological mechanisms and confirm the efficacy of 3-OHM, future studies should focus on comparative analyses with MEL, employing the optimal 3-OHM concentration identified herein.

Keywords: melatonin metabolite, abiotic stress, water stress, seedling growth, antioxidant activity

INTRODUCTION

Drought is a significant constraint on plant growth, development, and productivity, making the study of plant responses to drought stress an important focus in environmental research [Ahmad et al. 2009]. By decreasing imbibition and increasing the osmotic potential of the growth medium, drought stress leads to reduced germination rates and seedling vigor [Anjum et al. 2017]. Polyethylene glycol (PEG), synthetic, water-soluble, and non-ionic polymer with a wide range of molecular weight [Chazen et al. 1995], is frequently used to induce drought stress in experiments involving higher plants [Hajihashemi and Sofo 2018]. The use of PEG-6000 effectively elevates the osmotic potential of the growth medium, consequently limiting the imbibition capacity of seeds. Under drought conditions, plants experience an overproduction of reactive oxygen species (ROS), which can lead to the oxidation of vital cellular macromolecules, disrupt physiological and metabolic processes, and, in severe cases, result in plant death [Bewley 1997]. To counteract the damaging effects of oxidative stress, plants have developed a sophisti-

[1] Vocational School of Food, Agriculture and Husbandry, Department of Crop and Animal Production, Bingol University, Bingol, Türkiye, https://orcid.org/0000-0001-9142-9678; corresponding author: akaraca@bingol.edu.tr
[2] Department of Horticulture, Faculty of Agriculture, Yozgat Bozok University, Yozgat, Türkiye, https://orcid.org/0000-0003-4921-0925
[3] Department of Horticulture, Kahramanmaras Sutcu Imam University, Kahramanmaras, Türkiye, https://orcid.org/0000-0002-3886-5953

© Copyright by the Author(s), 2026
Licensee University of Life Sciences in Lublin – Publishing House, Lublin, Poland
This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution 4.0 International license.

cated antioxidant defense system that plays a crucial role in scavenging excess ROS and maintaining intracellular redox equilibrium [Ahmad et al. 2009]. This defense mechanism comprises both enzymatic and non-enzymatic antioxidants. The enzymatic component includes a range of enzymes such as peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), which collectively function to neutralize ROS and preserve redox stability within the cell. For example, SOD facilitates the conversion of superoxide radicals into hydrogen peroxide (H_2O_2) and molecular oxygen, after which CAT and APX further detoxify H_2O_2 to prevent cellular damage [Anjum et al. 2017].

In plants, MEL (N-acetyl-5-methoxytryptamine) plays a vital role in defending against oxidative stress by mitigating the damaging effects of ROS. Beyond its antioxidant properties, MEL also functions as a biostimulant, contributing to enhanced plant productivity through its involvement in cellular signaling pathways [Rehaman et al. 2021]. MEL biosynthesis in plants follows a metabolic pathway: tryptophan → tryptamine → serotonin → N-acetylserotonin → MEL. This process occurs under normal physiological conditions, particularly in actively growing tissues, and involves four key enzymatic steps. The enzymes responsible for each step include TDC (tryptophan decarboxylase), T5H (tryptophan 5-hydroxylase), SNAT (serotonin N-acetyltransferase), ASMT (N-acetylserotonin methyltransferase), COMT (caffeic acid O-methyltransferase), respectively [Back 2021].

An important branch of plant MEL research focuses on its metabolites. Metabolites resulting from MEL degradation—such as N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), N-acetyl-5-methoxykynuramine (AMK), and the hydroxylated derivatives 2-OHM (2-hydroxymelatonin), 3-OHM (3-hydroxymelatonin), 4-OHM (4-hydroxymelatonin), and 6-OHM (6-hydroxymelatonin)-originally identified in animals, have also been detected in plant systems [Tan and Reiter 2020, Hardeland 2015]. These metabolites possess potent antioxidant properties and contribute substantially to MEL's broader antioxidant network [Reiter et al. 2016]. Notably, in terms of endogenous content, MEL metabolites represent the predominant forms of MEL present in plant tissues [Lee et al. 2016]. Among MEL derivatives in plants, 2-OHM and 3-OHM are recognized as the principal metabolites. These metabolites are generated through hydroxylation processes catalyzed by melatonin 2-hydroxylase (M2H) and melatonin 3-hydroxylase (M3H), respectively [Byeon and Back 2015, Lee et al. 2016]. Both enzymes are classified as members of the 2-oxoglutarate-dependent dioxygenase (2-ODD) protein family [Bugg 2003]. Interestingly, M2H and M3H are absent in animals, suggesting that the corresponding hydroxylated metabolites are specific to terrestrial plants [Lee and Back 2016]. The detection of MEL and its metabolites across plant species has spurred extensive research into their physiological functions and regulatory mechanisms. 2-OHM, in particular, has been shown to accumulate at higher levels than MEL itself in plant tissues [Byeon et al. 2015]. It is recognized not only for its antioxidant capacity but also for its pro-oxidant function, capable of inducing ROS production through respiratory burst oxidase homolog-mediated pathways. Korkmaz et al. [2023] reported that exogenous application of 2-OHM enhances seed germination and seedling emergence in pepper under abiotic stress. This dual role may be attributed to its ability to stimulate both enzymatic and non-enzymatic antioxidant systems [Shah et al. 2020a], thereby conferring broad protective effects against oxidative stress and environmental challenges [Shah et al. 2020b]. In addition, The cytochrome P450 monooxygenase enzyme CYP1A1, which participates in MEL metabolism, catalyzes the biosynthesis of 6-OHM. When the mouse-derived *MmCYP1A*1 gene was ectopically expressed in transgenic *Arabidopsis* and apple calli, both MEL and 6-OHM levels increased. This enhancement was associated with greater ROS-scavenging capacity and improved abiotic stress tolerance, suggesting a synergistic role of MEL and its hydroxylated metabolites in plant defense responses [Wang et al. 2023]. Álvarez-Diduk et al. [2016] reported that certain MEL derivatives, such as N-acetylserotonin (N-Ser) and 6-OHM, exhibit stronger peroxyl radical scavenging capacity than MEL itself. In a related study, Lee and Back [2019] showed that foliar application of 2-OHM significantly improved tolerance to cold and drought stress in cucumber and tomato seedlings, whether the stresses were applied individually or simultaneously. Moreover, 2-OHM treatment in rice seedlings under combined stress conditions led to increased proline accumulation, which contributed to enhanced stress resilience [Lee and Back 2016]. According to Korkmaz et al. [2023], application of 2-OHM under various abiotic stresses reduced the levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), while elevating the concentrations of proline and antioxidant enzymes. Interestingly, the enzymatic activity responsible for MEL degradation-yielding metabolites such as 2-OHM and 3-OHM has been reported to be greater than that of enzymes involved in MEL biosynthesis. As a result, MEL and its metabolites often co-occur in plant tissues [Back 2021]. This co-existence has led to the hypothesis that MEL's protective functions under stress are not solely attributed to the parent molecule, but are also significantly influenced by its downstream metabolites, particularly 2-OHM. In support of this, Byeon and Back [2015]

successfully cloned M2H genes from rice, which encode 2-oxoglutarate-dependent dioxygenases that catalyze the conversion of MEL to 2-OHM. High levels of 2-OHM were observed in the roots of MEL-treated plants. Choi and Back [2019a, 2019b] further reported that both 2-OHM and 3-OHM, similar to MEL, follow a diurnal rhythm, with elevated concentrations detected at night. In addition, 3-OHM was found to significantly enhance tiller formation in rice by upregulating the expression of the *MOC1* gene. Lee and Back [2016] also noted that 2-OHM and 3-OHM improved plant tolerance to multiple abiotic stresses, such as drought and cold, through the upregulation of stress-responsive genes. Lee and Back [2022] reported that exogenous application of 3-hydroxymelatonin in *Arabidopsis thaliana* enhanced both vegetative growth and the initiation of flowering. Similarly, 4-OHM, another melatonin-derived metabolite, has been identified in plants alongside 2-OHM and 3-OHM [Hardeland 2016], and is thought to play a crucial role in modulating ROS levels under stress conditions [Pérez-González et al. 2017]. In this context, Shah et al. [2021] demonstrated that 4-OHM alleviated the adverse effects of nickel toxicity on the root and shoot systems of eggplant seedlings, thereby enhancing their stress resilience.

Although research on MEL metabolites remains limited, existing evidence suggests that these compounds may exert biological functions similar to MEL, particularly in plants exposed to environmental stresses. Their potential contribution to abiotic stress tolerance has drawn increasing attention, underscoring the importance of investigating their physiological roles and underlying mechanisms. Gaining a clearer understanding of these metabolites is also essential for elucidating the broader functional dynamics of their precursor, MEL. Especially, few studies have revealed the physiological functions of this molecule in plants 3-OHM, a MEL metabolite. Pepper is one of the most important vegetable crops worldwide, and its production is significantly affected by environmental stresses such as drought, with seedling emergence representing one of the most sensitive developmental stages. Accordingly, the present study was designed to examine the effects of 3-OHM, a key MEL-derived metabolite, on pepper (*Capsicum annuum* L.) seedling emergence in drought-stressed environments.

MATERIALS AND METHODS

Seed sterilization and treatments. Seed-borne microorganisms on pepper seeds from a uniform seed lot were eliminated through a 15 min treatment with sodium hypochlorite solution (1%). Sterilized pepper seeds were placed between two layers of blotting paper moistened with 3-OHM solutions at concentrations of 0, 10, 50, and 100 µM. After that, the seeds were incubated at 20 °C in darkness for 24 hours, followed by a 1 min rinse under running tap water. 24-hour incubation period was sufficient for compound absorption, and washing step was performed only to remove residual solution from the seed surface. After treatment, seeds were air-dried on paper towels for one day before drought stress emergence testing.

Emergence test. Following the application of 3-OHM, the experiment was conducted using a completely randomized design with four replications, each consisting of 40 seeds. In total, 1280 seeds were used in the experiment (4 concentrations of 3-OHM × 2 PEG levels × 4 replications × 40 seeds). The seeds were seeded in plastic containers, filled with a growth medium composed of perlite and peat in a 1:3 ratio. After sowing, each container received 200 mL of either distilled water (0 g L^{-1} PEG 6000, non-stress control) or a polyethylene glycol (PEG 6000) solution at 75 g L^{-1} (–0.41 MPa) to simulate drought stress. The containers were kept in a climate-controlled chamber (Memmert IPP110) set to 25 °C. The chamber was illuminated with white LED lights providing a photoperiod of 16 hours per day at an intensity of 225 µmol m^{-2} s^{-1}. During the emergence period, supplementary irrigation with respective PEG solutions was provided as needed to prevent the drying of the substrate surface. Seedling emergence was monitored daily until stabilization of the emergence count. A seedling was considered emerged upon the visible formation of a hypocotyl arc above the substrate. The emergence test was carried out over a 30-day period under drought stress conditions. Emergence parameters including FEP (final emergence percentage), $E_{10\text{-}90}$ (emergence uniformity), and MET (mean emergence time) were determined using on the total number of emerged seedlings. Upon completion of the experiment, seedlings were harvested and their SFW (shoot fresh weight) was recorded. Immediately after, biochemical analyses were performed.

Determining chlorophyll, thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2), and proline contents. Chlorophyll content was quantified using the method reported by Gunes et al. [2007]. For this, leaf samples randomly collected from each replicate were ground in 80% acetone. After filtration, chlorophyll concentrations were determined by measuring absorbance at 645 and 663 nm with a spectrophotometer

(Shimadzu UV-1800, Kyoto, Japan). The concentration of TBARS in pepper seedlings was quantified based on the approach by Posmyk et al. [2009] with minor alterations. Samples were ground in a 10% TCA (trichloroacetic acid) solution, and the clear extract was mixed with 0.6% TBA (thiobarbituric acid). The mixture was then incubated in a water bath for 20 min, and absorbance values were measured using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at wavelengths of 532 nm, 600 nm, and 450 nm. H_2O_2 levels were determined by adapting the method from Ozden et al. [2009]. Shoots were fragmented in 0.1% (w/v) TCA, followed by mixing with 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide. Subsequently, the absorbance was measured at 390 nm to determine the hydrogen peroxide content. Proline levels in the tissue were determined using the approach by Bates et al. [1973]. Fresh shoot samples were homogenized in 3% sulfosalicylic acid and filtered. An aliquot of 0.5 mL filtrate was reacted with glacial acetic acid and acid ninhydrin in test tubes, then incubated for one hour in a water bath. Once at room temperature, the reaction mixture was extracted with toluene and absorbance was measured at 520 nm.

Extraction and analysis of antioxidant enzymes. The activities of essential antioxidant enzymes-CAT (EC 1.11.1.6), POD (EC 1.11.1.7), and SOD (EC 1.15.1.1) were determined using the extraction methods outlined by Ozden et al. [2009]. Protein concentration in the extracts was determined via the Bradford assay [Bradford 1976]. Enzyme activities were subsequently quantified based on established methods reported by Gong et al. [2001] for CAT, Dolatabadian et al. [2008] for POD, and Agarwal and Pandey [2004] for SOD, and expressed as units per milligram of protein (unit mg^{-1} protein).

Statistical analysis. The trials was conducted using a completely randomized design with four replicates per treatment. Data processing and calculation of means were performed using Microsoft Excel (Office XP). Statistical analyses were carried out with SPSS version 20.0. To assess the significance of differences among treatment means, two-way analysis of variance (ANOVA) was applied, followed by Duncan's multiple range test at a 5% significance level.

RESULTS

Effect of 3-OHM application on drought stress. The data presented in Table 1 indicate that 3-OHM treatments influenced MET, FEP, E_{10-90}, and SFW of seedlings under both optimal and drought stress conditions. Drought stress significantly reduced seedling emergence, with FEP decreasing from 92.3% under optimal conditions to 81.1% under stress. Seeds treated with 3-OHM at all concentrations showed higher emergence rates compared to the untreated control. Under drought conditions, the highest emergence percentage (85.6%) was observed in seeds treated with 50 µM 3-OHM. While drought stress delayed emergence, increasing MET from 9.38 to 10.30 days, 3-OHM application improved emergence speed, lowering MET from 10.15 days in untreated seeds to 9.50 days at 50 µM treatment. Emergence uniformity (E_{10-90}) also increased from 3.60 days under optimal conditions to 5.15 days under drought stress. Additionally, drought stress significantly reduced seedling fresh weight, from 14.54 g to 6.13 g on average. However, seeds treated with 50 µM and 100 µM 3-OHM yielded seedlings with greater fresh weights (10.93 g and 10.68 g, respectively) compared to the untreated group (9.56 g).

Table 2 presents proline, H_2O_2, TBARS, and chlorophyll levels in pepper seedlings after 3-OHM seed treatments under drought and optimal conditions. Chlorophyll content increased from 59.7 mg g^{-1} under optimal conditions to 70.3 mg g^{-1} under drought stress. Seedlings derived from seeds treated with 3-OHM exhibited chlorophyll levels that were consistently higher, though comparable, to those of the untreated control. TBARS content rose from 0.35 nmol g^{-1} in optimal conditions to 0.77 nmol g^{-1} under drought stress. However, 3-OHM treatments at 10 µM, 50 µM, and 100 µM significantly reduced TBARS levels compared to the control. The interaction analysis between drought stress and 3-OHM treatments showed that the lowest TBARS content was detected in seedlings grown under optimal conditions with 3-OHM application, indicating reduced lipid peroxidation. In contrast, the highest TBARS accumulation was noted in seedlings that were exposed to drought stress without 3-OHM, suggesting that the absence of treatment exacerbated oxidative damage under stress conditions. Similarly, H_2O_2 content increased from 0.29 nmol g^{-1} under optimal conditions to 0.34 nmol g^{-1} under stress, but 3-OHM treatments at all tested concentrations significantly decreased H_2O_2 accumulation. Proline content was markedly elevated from 117.6 nmol g^{-1} in optimal conditions to 473.1 nmol g^{-1} under drought stress. Notably, all 3-OHM treatments enhanced proline accumulation compared to the untreated seeds (201.8 nmol g^{-1}), with the highest proline level observed in seedlings from seeds treated with 10 µM 3-OHM (344.6 nmol g^{-1}).

Table 3 summarizes the activities of antioxidant enzymes CAT, POD, and SOD in pepper seedlings following treatment with 3-OHM. Under optimal conditions, CAT activity was measured at 0.93 U mg^{-1} protein and increased to 1.88 U mg^{-1} protein when plants were exposed to drought stress. While 3-OHM treatment resulted in an increase of CAT activity to approximately 1.50 U mg^{-1} protein, this change did not reach statistical significance. POD activity showed a modest rise from 0.009 U mg^{-1} protein in optimal conditions to 0.01 U mg^{-1} protein under drought. Importantly, POD activity was significantly enhanced by 3-OHM application, with the 50 µM and 100 µM treatments yielding higher enzyme activity (0.011 U mg^{-1} protein) compared to the untreated group. Similarly, SOD activity increased from 2.01 U mg^{-1} protein in well-watered plants to 2.55 U mg^{-1} protein under drought conditions. Notably, seedlings treated with 50 µM 3-OHM showed a marked increase in SOD activity, reaching 4.29 U mg^{-1} protein, compared to 1.12 U mg^{-1} protein in untreated controls.

Table 1. Effect of pre-sowing seed treatments with 3-OHM on pepper seed final emergence percentage (FEP), mean emergence time (MET), emergence uniformity (E_{10-90}) and seedling fresh weight (SFW) under optimum and drought stress conditions. Values are means (n = 4)

Treatments	Doses	FEP (%)	MET (day)	E_{10-90} (day)	SFW (g $plant^{-1}$)
PEG					
0 gL^{-1}	–	92.3 ±3.35a	9.38 ±0.26b	3.60 ±0.35b	14.54 ±0.65a
75 gL^{-1}	–	81.1 ±4.06b	10.30 ±0.22a	5.15 ± 0.41a	6.13 ±0.44b
3-OHM (µM)					
0	–	80.9 ±5.94c	10.15 ±0.39a	4.81 ±0.68	9.56 ±4.09c
10	–	88.1 ±4.38b	9.89 ±0.43ab	4.46 ±0.83	10.17 ±3.99b
50	–	90.6 ±5.00a	9.50 ±0.44b	3.97 ±0.48	10.93 ±4.24a
100	–	87.2 ±7.19b	9.83 ±0.58ab	4.26 ±1.09	10.68 ±4.48a
PEG × 3-OHM					
0 gL^{-1}	0 µM	86.9 ±1.08c	9.77 ±0.44	4.12 ±0.95	13.65 ±0.12
	10 µM	92.5 ±0.00b	9.46 ±0.18	3.63 ±0.29	14.16 ±0.49
	50 µM	95.6 ±1.08a	9.06 ±0.50	3.48 ±0.79	15.17 ±0.11
	100 µM	94.4 ±1.07ab	9.26 ±0.16	3.17 ±0.12	15.16 ±0.30
75 gL^{-1}	0 µM	75.0 ±1.77f	10.54 ±0.17	5.49 ±1.00	5.46 ±0.24
	10 µM	83.8 ±2.80 d	10.32 ±0.35	5.30 ±0.84	6.19 ±0.61
	50 µM	85.6 ±2.07cd	9.94 ±0.38	4.45 ±1.02	6.69 ±0.36
	100 µM	80.0 ±1.76e	10.41 ±0.24	5.35 ±1.35	6.20 ±0.30
Significance					
PEG	–	***	***	***	***
3-OHM (µM)	–	***	*	N.S.	***
PEG × 3-OHM	–	*	N.S.	N.S.	N.S.

NS – not significant; *, **, *** significant at P < 0.05, 0.01 or 0.001, respectively

Table 2. Effect of pre-sowing seed treatments with 3-OHM on chlorophyll, TBARS, H_2O_2, and proline contents of pepper seedlings under optimum and drought stress conditions. Values are means (n = 4)

Treatments	Doses	Chlorophyll (mg g^{-1} FW)	TBARS (nmol g^{-1} FW)	H_2O_2 (nmol g^{-1} FW)	Proline (nmol g^{-1} FW)
PEG					
0 gL^{-1}	–	59.7 ±2.95b	0.35 ±0.09b	0.29 ±0.08b	117.6 ±22.1b
75 gL^{-1}	–	70.3 ±0.39a	0.77 ±0.13a	0.34 ±0.07a	473.1 ±100a
3-OHM (μM)					
0	–	66.8 ±3.61a	0.75 ±0.24a	0.42 ±0.01a	201.8 ±110c
10	–	63.4 ±7.34c	0.48 ±0.18b	0.26 ±0.03c	344.6 ±243a
50	–	65.7 ±3.93b	0.49 ±0.19b	0.24 ±0.03c	322.0 ±175b
100	–	64.0 ±6.46c	0.51 ±0.23b	0.36 ±0.01b	313.0 ±181b
PEG × 3-OHM					
0 gL^{-1}	0 μM	63.2 ±0.68b	0.51 ±0.02d	0.40 ±0.02	91.2 ±3.0e
	10 μM	56.0 ±0.73d	0.30 ±0.03e	0.23 ±0.02	101.6 ±2.0e
	50 μM	61.8 ±0.86b	0.30 ±0.03e	0.21 ±0.01	146.3 ±1.9d
	100 μM	57.6 ±0.68c	0.28 ±0.02e	0.35 ±0.02	131.5 ±1.2d
75 gL^{-1}	0 μM	70.4 ±1.02a	0.99 ±0.07a	0.44 ±0.04	312.3 ±22.2c
	10 μM	70.7 ±0.96a	0.66 ±0.04c	0.30 ±0.03	587.7 ±14.9a
	50 μM	69.7 ±0.79a	0.68 ±0.02c	0.27 ±0.04	497.8 ±8.7b
	100 μM	70.5 ±0.92a	0.74 ±0.02b	0.37 ±0.02	494.5 ±9.8b
Significance					
PEG	–	***	***	***	***
3-OHM (μM)	–	***	***	***	***
PEG × 3-OHM	–	***	**	N.S.	***

NS – not significant; *, **, *** significant at P < 0.05, 0.01 or 0.001, respectively

Table 3. Effect of pre-sowing seed treatments with 3-OHM on catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) enzyme activities of pepper seedlings under optimum and drought stress conditions. Values are means (n = 4)

Treatments		CAT (U mg^{-1} protein)	POD (U mg^{-1} protein)	SOD (U mg^{-1} protein)
PEG				
0 gL^{-1}	–	0.93 ±0.10b	0.009 ±0.0b	2.01 ±1.42b
75 gL^{-1}	–	1.88 ±0.07a	0.010 ±0.0a	2.55 ±1.14a
3-OHM (μM)				
0	–	1.33 ±0.47	0.009 ±0.0b	1.12 ±0.39c
10	–	1.50 ±0.39	0.009 ±0.0b	2.51 ±0.33b
50	–	1.44 ±0.56	0.011 ±0.0a	4.29 ±0.02a
100	–	1.35 ±0.48	0.011 ±0.0a	1.20 ±0.36c

Treatments		CAT (U mg^{-1} protein)	POD (U mg^{-1} protein)	SOD (U mg^{-1} protein)
PEG × 3-OHM				
0 gL^{-1}	0 µM	0.86 ±0.03	0.009 ±0.0	0.74 ±0.10
	10 µM	1.11 ±0.15	0.009 ±0.0	2.18 ±0.24
	50 µM	0.88 ±0.06	0.010 ±0.0	4.27 ±0.62
	100 µM	0.87 ±0.03	0.010 ±0.0	0.84 ±0.08
75 gL^{-1}	0 µM	1.81 ±0.18	0.009 ±0.0	1.51 ±0.11
	10 µM	1.88 ±0.07	0.009 ±0.0	2.84 ±0.50
	50 µM	1.99 ±0.06	0.012 ±0.0	4.31 ±4.30
	100 µM	1.83 ±0.63	0.011 ±0.0	1.55 ±0.17
Significance				
PEG	–	***	*	***
3-OHM (µM)	–	N.S.	***	***
PEG × 3-OHM	–	N.S.	N.S.	N.S.

NS – not significant; *, **, *** significant at P < 0.05, 0.01 or 0.001, respectively. SD values for POD enzyme activity were rounded to one decimal place and presented as 0.0

DISCUSSION

MEL, known as both a circadian rhythm regulator and a potent antioxidant, has prompted extensive research into its metabolites. In terrestrial plants, the primary MEL metabolites are 2-OHM, 3-OHM, and 4-OHM [Byeon et al. 2015]. MEL and its metabolites possess MEL-like functions and play an active role in improving stress tolerance by acting as antioxidants [Lee and Back 2019]. Studies have demonstrated that 2-OHM application reduces cadmium uptake and polyamine oxidase activity in pepper seedlings. Furthermore, 2-OHM has been reported to alleviate cadmium toxicity in cucumber seedlings, enabling cultivation in cadmium-contaminated soils [Shah et al. 2020a, b]. In the work of Lee and Back [2016] high proline accumulation was observed in rice seedlings treated with 100 µM 2-OHM under combined cold and drought stress, which contributed to increased capacity to withstand stress. Additionally, treatment with 50 µM 2-OHM positively influenced germination and seedling emergence in pepper seeds exposed to different types of stress, effects attributed to the stimulation of the antioxidant system [Korkmaz et al. 2023]. The metabolite 3-OHM has been documented to counteract oxidative stress under adverse environmental conditions by increasing antioxidant activity to mitigate stress-induced damage [Reiter et al. 2016, Choi and Back 2019a, 2019b]. In rice plants, increased expression of M3H genes, which facilitate the conversion of MEL into 3-OHM, was detected in MEL-treated leaves, accompanied by significant accumulation of 3-OHM. These findings were supported by cloning studies of 2-oxoglutarate-dependent dioxygenase (2-ODD). Collectively, these results indicate that MEL is metabolized to 3-OHM via M3H genes in plants; however, the precise physiological role of 3-OHM remains to be fully elucidated [Lee et al. 2016].

The findings of this research clearly demonstrate that 3-OHM has a mitigating effect on the adverse influence of drought stress during the emergence stage of pepper seedlings. Pre-sowing application of 3-OHM notably enhanced both emergence rate and final emergence percentage under water-deficit conditions (Table 1). Following 3-OHM application, stressed seedlings exhibited marked reductions in H_2O_2 and TBARS levels, while proline content, total chlorophyll concentration (Table 2), and activities of antioxidant enzymes (Table 3) were significantly enhanced. Comparable findings have been reported in rice seedlings [Lee and Back 2016] and *Arabidopsis* subjected to chilling stress, where 3-OHM treatment promoted proline biosynthesis and upregulated genes such as *Myb4* and *AP37*, which are crucial for abiotic stress tolerance. Additionally, Lee and Back [2022] demonstrated that 3-OHM stimulated growth and development in *Arabidopsis* and exhibited antioxidant activity approximately 15 times greater than MEL itself. In rice, 3-OHM levels displayed a circadian rhythm similar to MEL and notably increased tiller numbers via upregulation of the *MOC1* gene [Choi and Back 2019a]. Furthermore, under cadmium exposure, 3-OHM content decreased while 2-OHM remained stable in rice plants, implicating 3-OHM in the oxidative stress response [Choi and Back, 2019b]. These observations align with previous reports on the crucial role of 3-OHM in MEL-related physiological effects [Galano et al. 2014] and support its potential as an antioxidant com-

parable in efficacy to MEL [Tan et al. 2014]. Furthermore, it is suggested that 3-OHM also has MEL-like functions and plays an active role in seedling emergence under stress factors. Nonetheless, further research is warranted to elucidate the precise mechanisms by which 3-OHM exerts its physiological functions in plants and to confirm these preliminary findings.

CONCLUSIONS

Seed treatment with 50 µM 3-OHM significantly enhanced pepper seedling emergence under drought stress. This metabolite reduced oxidative damage by lowering H_2O_2 and TBARS levels while increasing proline content and antioxidant enzyme activities, thereby stabilizing cell membranes. These findings suggest that 3-OHM functions as an effective antioxidant, improving drought tolerance similarly to MEL. However, further comparative studies with MEL at this concentration are necessary to elucidate the precise physiological mechanisms. Continued research on MEL metabolites will advance understanding of their role in stress mitigation and clarify MEL's overall function in plants.

ACKNOWLEDGMENTS

The authors wish to express their thanks to TUBITAK for providing the support.

SOURCE OF FUNDING

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) [grant number 121C409].

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

A. Karaca: investigation, conceptualization, data curation, funding acquisition, formal analysis, writing-review and editing. G. Yakupoğlu: investigation, conceptualization, data curation, funding acquisition, formal analysis, writing-review and editing. A. Korkmaz: writing-review and editing.

REFERENCES

Agarwal, S., Pandey, V. (2004). Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol. Plant., 48(4), 555–560. https://doi.org/10.1023/B:BIOP.0000047152.07878.e7

Ahmad, S., Ahmad, R., Ashraf, M.Y. et al. (2009). Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. Pak. J. Bot., 41(2), 647–654.

Álvarez-Diduk, R., Galano, A., Tan, D.X. et al. (2016). The key role of the sequential proton loss electron transfer mechanism on the free radical scavenging activity of some melatonin-related compounds. Theor Chem. Acc., 135(2), 38. https://doi.org/10.1007/s00214-015-1785-5

Anjum, S.A., Ashraf, U., Zohaib, A. et al. (2017). Growth and development responses of crop plants under drought stress: a review. Zemdirbyste-Agriculture, 104(3), 267–276. http://dx.doi.org/10.13080/z-a.2017.104.034

Back, K. (2021). Melatonin metabolism, signaling and possible roles in plants. Plant J., 105(2), 376–391. https://doi.org/10.1111/tpj.14915

Bates, L.S., Waldren, R.P.A., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39, 205–207. https://doi.org/10.1007/BF00018060

Bewley, J.D. (1997). Seed germination and dormancy. Plant Cell, 9(7), 1055–1066. https://doi.org/10.1105/tpc.9.7.1055

Bugg, T.D.H. (2003). Dioxygenase enzymes: catalytic mechanisms and chemical models. Tetrahedron, 59(36), 7075–7101. https://doi.org/10.1016/S0040-4020(03)00944-X

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Byeon, Y., Back, K. (2015). Molecular cloning of melatonin 2-hydroxylase responsible for 2-hydroxymelatonin production in rice (*Oryza sativa*). J. Pineal Res., 58, 343–351. https://doi.org/10.1111/jpi.12220

Byeon, Y., Tan, D.X., Reiter, R.J. et al. (2015). Predominance of 2-hydroxymelatonin over melatonin in plants. J. Pineal Res. 59(4), 448–454. https://doi.org/10.1111/jpi.12274

Chazen, O., Hartung, W., Neumann, P.M. (1995). The different effects of PEG 6000 and NaCl on leaf development are associated with differential inhibition of root water transport. Plant. Cell Environ., 18(7), 727–735. https://doi.org/10.1111/j.1365-3040.1995.tb00575.x

Choi, G.H., Back, K. (2019a). Cyclic 3-hydroxymelatonin exhibits diurnal rhythm and cyclic 3-hydroxymelatonin overproduction increases secondary tillers in rice by upregulating MOC1 expression. Melatonin Res., 2(3), 120–138. https://doi.org/10.32794/mr11250034

Choi, G.H., Back, K. (2019b). Suppression of melatonin 2-hydroxylase increases melatonin production leading to the enhanced abiotic stress tolerance against cadmium, senescence, salt, and tunicamycin in rice plants. Biomolecules, 9(10), 589. https://doi.org/10.3390/biom9100589

Dolatabadian, A., Sanavy, S.M., Chashmi, N.A. (2008). The effects of foliar application of ascorbic acid (vitamin C) on antioxidant enzymes activities, lipid peroxidation and proline accumulation of canola (*Brassica napus* L.) under conditions of salt stress. J. Agron. Crop Sci., 194(3), 206–213. https://doi.org/10.1111/j.1439-037X.2008.00301.x

Galano, A., Tan, D.X., Reiter, R.J. (2014). Cyclic 3-hydroxymelatonin, a key metabolite enhancing the peroxyl radical scavenging activity of melatonin. RSC Adv., 4(10), 5220–5227. https://doi.org/10.1039/C3RA44604B

Gong, Y., Toivonen, P.M., Lau, O.L. et al. (2001). Antioxidant system level in 'Braeburn' apple is related to its browning disorder. Bot. Bull. Acad. Sin., 42, 259–264

Gunes, A., Inal, A., Bagci, E.G. et al. (2007). Silicon mediates changes to some physiological and enzymatic parameters symptomatic for oxidative stress in spinach (*Spinacia oleracea* L.) grown under B toxicity. Sci. Hortic., 113(2), 113–119. https://doi.org/10.1016/j.scienta.2007.03.009

Hajihashemi, S., Sofo, A. (2018). The effect of polyethylene glycol-induced drought stress on photosynthesis, carbohydrates and cell membrane in *Stevia rebaudiana* grown in greenhouse. Acta Physiol. Plant., 40, 142. https://doi.org/10.1007/s11738-018-2722-8

Hardeland, R. (2016). Melatonin in plants – diversity of levels and multiplicity of functions. Front. Plant Sci., 7, 198. https://doi.org/10.3389/fpls.2016.00198

Hardeland, R. (2017). Taxon- and site-specific melatonin catabolism. Molecules, 22(11), 2015. https://doi.org/10.3390/molecules22112015

Korkmaz, A, Sözeri, E., Ardıç, Ş.K. et al. (2023). 2-hydroxymelatonin (2-OHM), a major melatonin metabolite, confers multiple stress tolerance in pepper at seed germination stage. S. Afr. J. Bot., 162, 830–837. https://doi.org/10.1016/j.sajb.2023.09.056

Lee, K., Zawadzka, A., Czarnocki, Z. et al. (2016). Molecular cloning of melatonin 3-hydroxylase and its production of cyclic 3-hydroxymelatonin in rice (*Oryza sativa*). J. Pineal Res*., 61*(4), 470–478. https://doi.org/10.1111/jpi.12361

Lee, H.J., Back, K. (2016). 2-Hydroxymelatonin promotes the resistance of rice plant to multiple simultaneous abiotic stresses (combined cold and drought). J. Pineal Res., 61(3), 303–316.https://doi.org/10.1111/jpi.12347

Lee, H.J., Back, K. (2019). 2-Hydroxymelatonin confers tolerance against combined cold and drought stress in tobacco, tomato, and cucumber as a potent anti-stress compound in the evolution of land plants. Melatonin Res., 2(2), 35–46. http://dx.doi.org/10.32794/mr11250020

Lee, H.Y, Back, K. (2022). The antioxidant cyclic 3-hydroxymelatonin promotes the growth and flowering of *Arabidopsis thaliana*. Antioxidants, 11(6), 1157. https://doi.org/10.3390/antiox11061157

Ozden, M., Demirel, U., Kahraman A. (2009). Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. Sci. Hortic., 119(2), 163–168. https://doi.org/10.1016/j.scienta.2008.07.031

Pérez-González, A., Galano, A., Alvarez-Idaboy, J.R. et al. (2017). Radical-trapping and preventive antioxidant effects of 2-hydroxymelatonin and 4-hydroxymelatonin: contributions to the melatonin protection against oxidative stress. BBA Gen. Subjects, 1861(9), 2206–2217. https://doi.org/10.1016/j.bbagen.2017.06.016

Posmyk, M.M., Bałabusta, M., Wieczorek, M. et al. (2009). Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress. J. Pineal Res., 46(2), 214–223. https://doi.org/10.1111/j.1600-079X.2008.00652.x

Rehaman, A., Mishra, A.K., Ferdose, A. et al. (2021). Melatonin in plant defense against abiotic stress. Forests, 12(10), 1404. https://doi.org/10.3390/f12101404

Reiter, R.J., Mayo, J.C., Tan, D.X. et al. (2016). Melatonin as an antioxidant: under promises but over delivers. J. Pineal Res., 61(3), 253–278. https://doi.org/10.1111/jpi.12360

Shah, A.A, Ahmed, S., Ali, A. et al. (2020a). 2-Hydroxymelatonin mitigates cadmium stress in cucumis sativus seedlings: modulation of antioxidant enzymes and polyamines. Chemosphere, *243*, 125308. https://doi.org/10.1016/j.chemosphere.2019.125308

Shah, A.A., Ahmed, S., Yasin, N.A. (2020b). 2-Hydroxymelatonin induced nutritional orchestration in *Cucumis sativus* under cadmium toxicity: modulation of non-enzymatic antioxidants and gene expression. Int. J. Phytoremediat., 22(5), 497–507. https://doi.org/10.1080/15226514.2019.1683715

Shah, A.A., Yasin, N.A, Ahmed, S. et al. (2021). 4-Hydroxymelatonin alleviates nickel stress, improves physiochemical traits of *Solanum melongena*: regulation of polyamine metabolism and antioxidative enzyme. Sci. Hortic., 282, 110036. https://doi.org/10.1016/j.scienta.2021.110036

Tan, D.X., Hardeland, R., Manchester, L.C. et al. (2014). Cyclic-3-hydroxymelatonin (C3HOM), a potent antioxidant, scavenges free radicles and suppresses oxidative reactions. Curr. Med. Chem., 21 (13), 1557–1565. http://dx.doi.org/10.2174/0929867321666131129113146

Tan, D.X., Reiter, R.J. (2020). An evolutionary view of melatonin synthesis and metabolism related to its biological functions in plants. J. Exp. Bot., 71(16), 4677–4689. https://doi.org/10.1093/jxb/eraa235

Wang, X.N., Yang, F., Zhang, J.C. et al. (2023). Ectopic expression of *MmCYP1A1*, a mouse cytochrome P450 gene, positively regulates stress tolerance in apple calli and Arabidopsis. Plant Cell Rep., 42(2), 433–448. https://doi.org/10.1007/s00299-022-02969-5