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Melatonin nanoparticles improve the growth and biochemical activities of *Ocimum basilicum* L. plant *In vitro*

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Abstract

Melatonin (MEL) plays important roles in various plant physiology, particularly regulation of plant growth, development, and metabolism. The present study was carried out on *Ocimum basilicum* L. plant growing in a half-strength Murashige and Skoog media supplemented with 5, 10, 20, and 30 µM MEL-CS nanoparticles (NPs). After four weeks, growing *O. basilicum* plants were harvested for measuring the changes in growth, physiological, and biochemical attributes. Our results revealed that MEL-CS nanoparticles treatment markedly increased the shoot, and root's length, fresh, and dry weight, photosynthetic pigments, carbohydrates fractions, total soluble proteins, free amino acids, and antioxidant enzymes activities (CAT, SOD, POD, and PPO). However, the accumulation of the superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), as well as malonaldehyde (MDA) reduced in basil leaves. The most efficient treatment was 10 µM MEL-CS nanoparticles. At the same time, the concentration of 30 µM was the lowest effective compared to untreated control, normal MEL, and CS treatments. The positive results of current study proved the effective role of MEL-CS nanoparticles in improving plant growth, the measured metabolites, and thereby reducing the oxidative stress ($O_2^{\bullet -}$, H_2O_2, and MDA) through stimulating the antioxidant defense system.

Keywords: *Ocimum basilicum* L.; Nanoparticles (NPs); Melatonin (MEL); Chitosan (CS).

1.Introduction

Basil (*Ocimum basilicum* L.) is one of the Lamiaceae family's most popular herbs. *Ocimum basilicum* L . is cultivated worldwide. *O. basilicum* L contains high nutritional value, many secondary metabolites, and essential oils [1]. It has been applied in numerous fields such as food, agriculture, and pharmacology. Its constituents showed anti-inflammatory, anti-

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spasmodic, anti-diabetic, immunomodulatory, anti-bacterial, anti-fungal, and anti-oxidant effects [2].

Recently, researchers have become more concerned about bioactive components from natural plants such as melatonin. Melatonin (MEL) is a low molecular weight indole amine (*N*-acetyl-5-methoxytryptamine), that was first identified in plants in 1995. It has been recognized as a pleiotropic molecule involved in a number-physiological processes, including plant growth, development, and defense against both biotic and abiotic stressors [3]. Researchers suggested that the application of melatonin exogenously shows changes in physiological, biochemical, and molecular characteristics [4].

MEL had various vital roles in different plant growth and development [4], [5] photosynthesis process [6], [7], [8] and carbohydrate accumulation [9], [10], [7]. Various research papers stated that MEL has a vital role in enhancing the accumulation of secondary metabolites, such as carotenoid, and anthocyanin, as well as stimulates the phenylpropanoid metabolism [11], [14]. In numerous studies, melatonin has a vital role in reducing ROS oxidative damage through direct neutralization of $O_2^{\bullet -}$, H_2O_2, and consequently reduces membrane lipid peroxidation (MDA) or via increasing the antioxidant enzymes activities. In addition, it regulated the expression of antioxidant related genes [6], [7], [11], [15], [20].

Melatonin is incorporated into different nanocarriers as a drug-delivery system. Melatonin incorporated into nanosized materials exhibits superior effectiveness [21]. One of the most superb polysaccharides is chitosan due to it is nontoxic, biodegradable, biocompatible, and biopolymer which provides improvements in the release of encapsulated ingredients. Chitosan (CS) is a natural polysaccharide compound produced from the deacetylation of chitins [22]. The characteristics of nanoscale materials are distinctive and differ from the bulk materials in their chemical, physical, and biological properties based on their size [23].

Consequently, the current study aimed to explore the role of melatonin nanoparticles incorporated in chitosan (MEL-CS NPs) in *Ocimum basilicum* L plant *in vitro* to enhance-plant growth, physiology, and antioxidant systems.

2.Materials and methods

The basil seeds were supplied by the National Gene Bank of Egypt (NGB), Ministry of Agriculture, Giza, Egypt. The prepared MEL-CS nanoparticles using the ionic gelation method were 83.45 nm in size with a 78% EE percentage and the zeta potential was +42 mV.

2.1. Growth conditions and treatments

Ocimum basilicum L. seeds were surface sterilized using a 30% (v/v) solution of commercial Clorox (Clorox®, 5.4% sodium hypochlorite) for 20 min [24]. Consequently, in a laminar flow hood. Sterilized seeds were cultured in 50 % Murashige and Skoog (MS) medium with vitamins, 3% sucrose, after solidified by 8.1 g l^{-1} agar. Seven seeds of *Ocimum basilicum* L were planted in eachglass jars. The inoculated flasks were maintained in 16 h lighting, at 25±2°C in a growth room, supplied by white-fluorescent light. This experimentation was conducted in 210 jars and was divided into seven groups (30 jars/ group), which were treated as follows. The treatments include: (1) untreated control, (2) normal MEL (100 µM) (Treated control), (3) normal CS (0.1%) (Treated control), (4) nano melatonin (MEL-CS NPs) (5 µM), (5) MEL-CS NPs (10 µM), (6) MEL-CS NPs (20 µM), (7) MEL-CS NPs (30 µM). Seven seeds of *Ocimum basilicum* L were planted on 14 August and harvested on 13 September. After four weeks in culture, the plants were removed from the jars and used to measure the alteration in growth, physiological, and biochemical parameters.

2.2. Extraction and estimation of photosynthetic pigment

Extraction of photosynthetic pigment from fresh leaves by acetone 80% (v/v) according to Arnon [25]. The absorbance was measured with a spectrophotometer (UV/Vis spectrophotometer T80^{+}, UK) at 646, 663, and 470 nm. Concentrations of pigments were determined using equations published by Lichtenthaler and Buschmann [26].

Chlorophyll a (µg ml^{-1}) =12.25 $A_{663.2}$ – 2.79 $A_{646.8}$

Chlorophyll b (µg ml^{-1}) .=21.5 $A_{646.8}$ – 5.1 $A_{663.2}$

Carotenoids $_{(x+c)}$ (µg ml^{-1}) = (1000 A_{470} – 1.82Chl_a –85.02Chl_b)/198

2.3. Extraction and estimation of carbohydrate fractions

2.3.1.Total carbohydrate

Polysaccharides were extracted from 0.5 g of dried leaf samples were hydrolyzed with 10 ml of 2.5N HCl. The mixture was then boiled for 2 h in a water bath. According to Dubois *et al*. [27], the phenol-sulfuric acid technique was used to assess the total carbohydrate content. The absorbance was measured at 490 nm by using a spectrophotometer (UV/Vis spectrophotometer T80^{+}, UK). The total sugar content was assayed and calculated using a standard curve prepared using 100 µg/ml of glucose.

2.3.2.Total soluble sugar

The method employed for the extraction of soluble sugar was done according to Mocready *et al*. [28]. Total soluble sugar was estimated by the phenol-sulfuric acid method according to Dubois *et al*. [27].

2.4. Extraction and estimation of nitrogen fractions

2.4.1.Total free amino acids

Total free amino acids content was determined by using ninhydrin reagent as described by Li *et al*. [29]. The absorbance was measured at 580 nm by spectrophotometer (UV/Vis spectrophotometer T80^{+}, UK). Arginine was used for making a standard curve.

2.4.2. Total soluble protein

The total soluble protein was extracted by homogenizing the 0.1 g of sample in 50 mM phosphate buffer (pH 7). The total soluble protein quantitively estimated by Bradford method [30]. The sample absorption was measured at 595 nm. Bovine serum albumin was used for performing protein standard curve.

2.5. Oxidative stress

2.5.1. Reactive oxygen species

A. Determination of superoxide radical ($O_2^{\bullet -}$) content

Superoxide radicals were attacked by NBT staining. The tissue was immersed in a staining solution including 0.1% NBT in 10 potassium phosphate buffer (pH 7.8) containing sodium azide. Then, the mixture was incubated at room temperature for 20 minutes. The quantification of superoxide was established as a method described by Ramal *et al*. [31]. The absorbance was recorded at 630 nm, and the content of $O_2^{\bullet -}$ was given as µmole g^{-1}FW.

B. Determination of hydrogen peroxide (H_2O_2) content

Trichloroacetic acid (0.1%) was used to homogenize the samples. For five minutes, the homogenate was centrifuged at 13,000 rpm. The supernatant was measured spectrophotometry at 390 nm, after reacting with potassium iodide [32]. Thus, the content of H_2O_2 was set as µmole g^{-1}FW.

2.5.2. Lipid peroxidation.

Determination of malondialdehyde (MDA) content

The malonaldehyde (MDA) level in plant cells is used as an indication for lipid peroxidation. Thiobarbituric acid (TBA) was used to determine the MDA content [33]. At 532 and 600 nm, the absorbance was concluded. The extinction coefficient of 155 $mM^{-1}cm^{-1}$ was used to calculate the MDA content.

2.6. Assay of antioxidant enzyme activity

The extraction of antioxidant enzymes was done according to the method described by Grace and Logan [34]. After centrifugation, the supernatant was used for the assay of the following enzymes.

The activity of catalase (CAT; EC 1.11.1.6) was assayed according to Beers and Sizer [35]. The decomposition of H_2O_2 was recorded at 240 nm by decrease in absorbance. The CAT activity calculation was performed using the extension coefficient = 0.039 mM^{-1} cm^{-1} and expressed as U (µmol H_2O_2 decomposed per minute) /g FW.

The ability of superoxide dismutase (SOD; EC 1.15.1.11) to inhibit the reduced nitroblue tetrazolium chloride (NBT) was used to measure its activity [36] . The absorbance was

determined by spectrometry at 560 nm. The unit of SOD activity was U g^{-1} FW. The one unit was stated as the amount of enzyme (µmol) needed to achieve a 50% inhibition of the NBT necessary photoreduction rate.

Chance and Maehly [37] protocol was used to measure the activity of peroxidase (POX; EC 1.11.1.7). The expression for POX activity was U g^{-1} FW. The extinction coefficient used in the calculations was 2.47 mM^{-1} cm^{-1}.

The activity of polyphenol oxidase (PPO; 1.14.18.1) was evaluated by Soffan *et al*. [38]. PPO activity is determined as a change in absorbance at 490 nm and expressed as (U g^{-1} FW). The extinction coefficient used in the calculations was 25.5 mM^{-1} cm^{-1}.

2.7. Statistical analysis

All of the data were analyzed by exposing them to one-way analysis of variance (ANOVA) using the SPSS software (version 20, USA). Duncan's test was utilized for significance according to Neter *et al.* [39].

3.Results and discussion

The Change in growth of basil seed at the age of 30 days treated with normal melatonin (MEL), normal chitosan (CS), or different concentrations of Nano-melatonin (MEL-CS NPs) (5, 10, 20, 30, 40, 50, 80, 100 µM) were presented in Table 1. It was found that there were significant differences in the length, fresh and dry weights of the basil shoot and root when compared to untreated control. The highest increments in all measurement growth parameters of shoot and root were recorded at 10 µM Nano-melatonin treated plants. It markedly increased shoot length, fresh weight, and dry weight over the untreated control by 186.88%, 169.13%, and 566.67%, respectively, while these parameters increased in the root by 199.61%, 364.10%, and 683.33%, respectively. Melatonin boosts the growth of seedlings by enhancing plant metabolism. For instance, MEL increased the chlorophyll content of the barley leaves to twice that in the control [40]. Correspondingly, the soil treated with melatonin led to a rise in protein content and photosynthetic rate in apples [41]. Furthermore, melatonin improved the morphological characteristics (shoot fresh weight, stem length, and number of leaves) and boosted stevia seed germination [42]. In contrast, high MEL concentrations inhibited the photosynthesis process and phloem transfer of sucrose while accelerating the accumulation of sucrose, hexose, and starch [43]. Also, high concentrations of MEL were toxic for the growth of red cabbage seedlings [44].

Posmyk *et al.* [45] stated the high concentrations of MEL inhibited protein synthesis and stimulated protein oxidations.

Melatonin plays an important role in various root growth and development including tap, lateral, and adventitious roots. It also contributes to rhizosphere signaling and interactions. Previous research papers support that the MEL has positive effects on root development [5]. Various plants treated with melatonin showed greater root length and biomass [46], [47]. Under normal conditions, melatonin treatments by foliar and root irrigation led to an increase in soybean ~~in~~ length and biomass of shoot and root compared to untreated plants [10].

Chang *et al*. [48] examined the different MEL concentrations (0, 50, 200, and 400 µM) on physiological,

and biochemical characteristics as well as *Prunella vulgaris* biomass production. Also, the results showed that MEL treatment at 100 µM significantly increased the growth and development of roots as well as the biomass of whole plants. Furthermore, the foliar spray of nano-silica and melatonin caused promotion in growth and yield of pea plants Also, the protein, and chlorophyll content significantly improved [8]. According to Jiang *et al*. [49] study, there was a significant rise in in roots (18%) and leaves (20.3%) growth as compared to the control in plant treated with melatonin-gold nanoparticles (Mel-Au NPs). The effects of melatonin and selenium (Se) nanoparticles on *Brassica napus* growth were evaluated by Farooq et al. [20]. They found significantly enhanced plant growth, leaf chlorophyll, biomass accumulation.

Table 1. Change in shoot and root growth of *Ocimum basilicum* L. (after 30 days) treated with either conventional MEL, CS, or different concentrations of Nano-melatonin.

Treatments	Shoot			Root		
	Length (cm)/plant	Fresh weight (g)/plant	Dry weight (g)/plant	Length (cm)/plant	Fresh weight (g)/plant	Dry weight (g)/plant
Control	3.522±0.07 ^{e}	0.149±0.03 ^{f}	0.009±0.07 ^{e}	3.072±0.02 ^{f}	0.039±0.07 ^{e}	0.0072±0.05 ^{f}
MEL (100 µM)	4.611±0.10 ^{c}	0.196±0.04 ^{cd}	0.036±0.03 ^{cd}	4.161±0.05^{d}	0.086±0.04 ^{cd}	0.0342±0.07 ^{d}
CS (0.1%)	5.442±0.04 ^{bc}	0.198±0.07 ^{cd}	0.038±0.06 ^{cd}	4.992±0.04 ^{c}	0.088±0.02 ^{c}	0.0365±0.04 ^{c}
MEL-CS NPs (5 µM)	4.123±0.02 ^{d}	0.171±0.04 ^{e}	0.031±0.05 ^{d}	3.673±0.08 ^{e}	0.061±0.07 ^{d}	0.0288±0.05 ^{e}
MEL-CS NPs (10 µM)	6.582±0.07 ^{a}	0.252±0.06 ^{a}	0.051±0.08 ^{a}	6.132±0.05 ^{a}	0.142±0.08 ^{a}	0.0492±0.03 ^{a}
MEL-CS NPs (20 µM)	5.912±0.05 ^{b}	0.231±0.02 ^{b}	0.044±0.07 ^{b}	5.462±0.03 ^{b}	0.121±0.04 ^{b}	0.0419±0.07 ^{b}
MEL-CS NPs (30 µM)	2.231±0.07^{f}	0.042±0.01 ^{g}	0.001±0.09 ^{f}	1.781±0.04 ^{g}	0.004±0.05 ^{f}	0.0006±0.07 ^{g}
F-value	106.133***	54.919**	14.471***	26.243***	14.832***	9.976***

Different letters indicate a significant difference at **: P<0.01, ***<0.001.

The content of photosynthetic pigments (chlorophyll a, chlorophyll b, chlorophyll a and b, catenoids, and total pigments,) changed in leaves of basil treated with either conventional MEL, CS, or different concentrations of Nano-melatonin (Figure 1). The results appeared that, in comparison to the untreated control, treatment with normal melatonin, chitosan, and Nano-melatonin at 10 and 20 µM significantly increased the photosynthetic pigments content. The content of these photosynthetic pigments decreased in the leaves of basil treated with 30 µM Nano-MEL as compared with all treatments and untreated control. Furthermore, the contents of carotenoids slightly changed between all treatments. It is obvious that the highest concentrations of chlorophyll a, chlorophyll b, chlorophyll a and b, catenoids, and total pigments were assayed in basil leaves treated with 10 µM Nano-melatonin. Such increments were about 189.23, 196.15, 191.55, 220.11, and 192.93, respectively as compared with untreated control. Many researchers assessed the impact and mechanism of melatonin on pigments involved in photosynthetic processes. Melatonin enhanced the synthesis of porphyrin, the head structure of chlorophyll, by increasing the activity of key enzymes such as (D-aminolevulinate synthase) which evolved in photosynthetic pigments synthesis [50]. Furthermore, Jahan *et al.* [51] noted that melatonin upregulated the gene expiration of chlorophyll synthesis. Also, MEL gathered in guard cells and might participate in stomal movements [7]. Furthermore, related research conducted by Cherono *et al*. [52] reported that improved photosynthetic efficiency in Coffea by suppressing the gene expression of chlorophyll degradation and up-regulated the gene expression of photosynthetic *RBCS2* encoding rubisco (ribulose-1,5-bisphosphate) protein. However, Chlorophyll content decreased with increasing MEL concentration. Chloroplast peroxidation and the deterioration of their structure and function may be brought on by excess MEL. Furthermore, it may enhance the activity of several enzymes that evolved in the degradation of chlorophyll, including pheophorbide a-oxygenase and chlorophyllase as Sarropoulou *et al*. [50]. Certain results showed that the melatonin application did not significantly alternate in catenoid content. Zhu *et al*. [53] confirmed that there were no relations between treatments with different concentrations of melatonin and carotenoid content. On the other hand, the results obtained in maize [54] and apple [55] treated with MEL showed a markedly change in carotenoid content.

Similar, results have been reached by Farooq *et al*. [20], Karaca and Cekic [56], Askari *et al*. [57], and Sardar *et al*. [9] on *Brassica napus*, *Solanum lycopersicum*, bean, and broccoli plants that demonstrated the exogenous MEL application has positively positive effect on the photosynthetic process by increasing chlorophyll a, chlorophyll b, and β-carotene content.

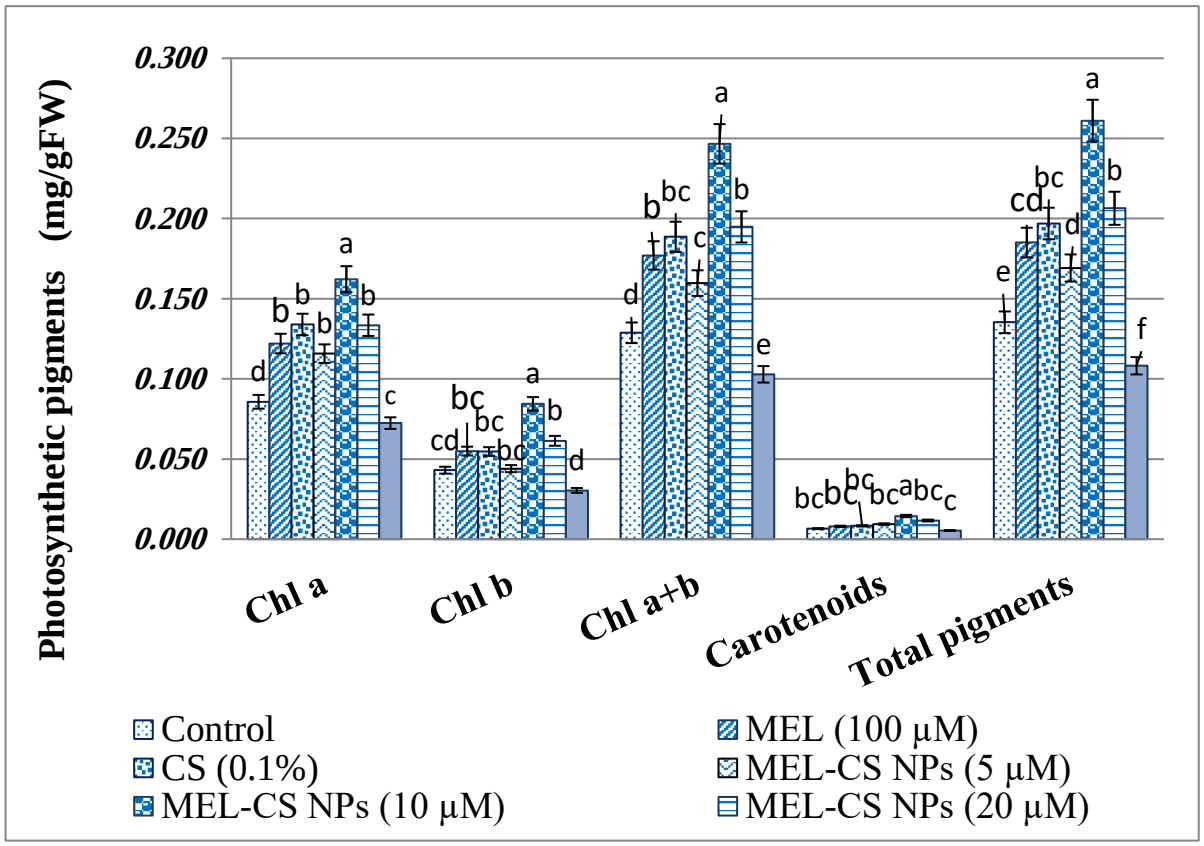


Figure 1. Change in the content of photosynthetic pigments (mg g^{-1} FW) in *Ocimum basilicum* L. leaves (after 30 days) treated with either conventional MEL, CS, or different concentrations of Nano-melatonin. Vertical bars represent the standard division (±SD). Different letters above the bars indicate significant differences using Duncan's multiple range tests.

Results in Table 2 showed that the change in the content of carbohydrate fractions (soluble sugar, insoluble sugar, and total carbohydrate) and nitrogen fractions (FAA and TSP) in basil leaves that growing in half-strength media supplemented with normal melatonin (100 µM), chitosan (0.1%), or a different concentration of Nano-melatonin (5, 10, 20, and 30 µM). It appears that the application of basil with either normal melatonin or chitosan caused significant increase in the carbohydrate contents and nitrogen fractions in basil leave as compared with untreated control. The highest accumulation of carbohydrate and nitrogen fractions was recorded in 10 and 20 µM Nano-MEL treatments and the lowest values of these fractions were observed in 30 µM Nano-MEL treatment as compared with the other treatments. Thus, the trend of treatments was the following: Nano-MEL (10 µM) > Nano-MEL (20 µM) > CS (0.1%) > MEL (100 µM) > Nano-MEL (5 µM) > untreated control > Nano-MEL (30 µM).

In comparison with the untreated control, treated with 10 µM Nano-MEL increased the content of soluble sugars, insoluble sugars, and total carbohydrates in basil leaves by 109.32%, 105.22%, and 108.34%, respectively. However, it increased by 107.48%, 103.63%, and 106.57% in response to 20 µM Nano-MEL treatment. These contents increased by 106.83%, 102.88%, and 105.89% in response to 10 µM Nano-MEL and by 105.03%, 101.33%, and 104.16% as the results 20 µM Nano-MEL treatment as compared with normal melatonin.

Results revealed that 10 µM Nano-MEL treatments increased the contents of FAA and TSP by 113.67 % and 127.39%, respectively as compared with untreated plants and by 111.84 % and 115.81 %, respectively as compared with conventional melatonin.

MEL plays an important role in improving carbohydrate content and alters its metabolite accumulation. However, the mechanism underlying these actions remains under research. Zhao *et al*. [43], [58] found that melatonin regulates carbohydrates metabolism. Moreover, Wang *et al*. [41] proposed the exogenous melatonin application may be evolved in the metabolism of carbohydrates signals. They found that the sorbitol, sucrose, and starch levels were significantly increased in apple leaves. In addition, the study done by Dou *et al.*[59] reported that the application of melatonin could significantly increase the activity of the sucrose synthase (SS), sucrose phosphate synthase (SPS), and acid convertase (AI) enzymes that evolved in the accumulation and conversion of sugar in tomato fruit. Our results are supported by a previous study showed that MEL treatment increased the accumulation of glucose, sucrose, soluble sugar, and starch in *Solanum lycopersicum* [60]. Moreover, related research conducted by Sardar *et al*. [9] found a foliar spray of melatonin increased TSS on broccoli head. Melatonin may influence cellular processes involved in protein synthesis [61]. Melatonin is also implicated in the regulation in the nitrogen metabolism pathway. The application of melatonin triggered the nitrate transport gene *OsNPF6.5*, the glutamine synthetase gene *OsGS2*, and the amino acid transporter gene *OsAAP14* evolved in nitrogen metabolism, and nitrate uptake was affected [62]. Nitrate reductase and S-nitrosoglutathione reductase, the key enzymes evolved in N uptake and metabolism, are regulated by melatonin [63] [64]. Furthermore, MEL promote certain amino acid biosynthesis like arginine and ornithine [65]. Also, the arginine and polyamine content that evolved in nitrogen distribution and recycling were increased in *Vitis vinifera* treated with melatonin is advantageous for nitrogen recycling and distribution as well [61].

These results are in harmony with those attained by Dou *et al*. [59], they demonstrated that the exogenous MEL markedly increased the level of aromatic amino acids, which include

tyrosine, tryptophan, glutamate, and phenylalanine. Exogenous application of MEL enhances the free amino acid in Corchorous olitorius and Corchorous capsularis [66]. Wenfei et al. [67] concluded that MEL application on blueberry significantly improved its photosynthetic capacity, and soluble protein. Also, Al-Shammari et al. [8] reported the foliar nano-silica and melatonin application, individually or in combination, enhanced the protein content in the stressed pea plants

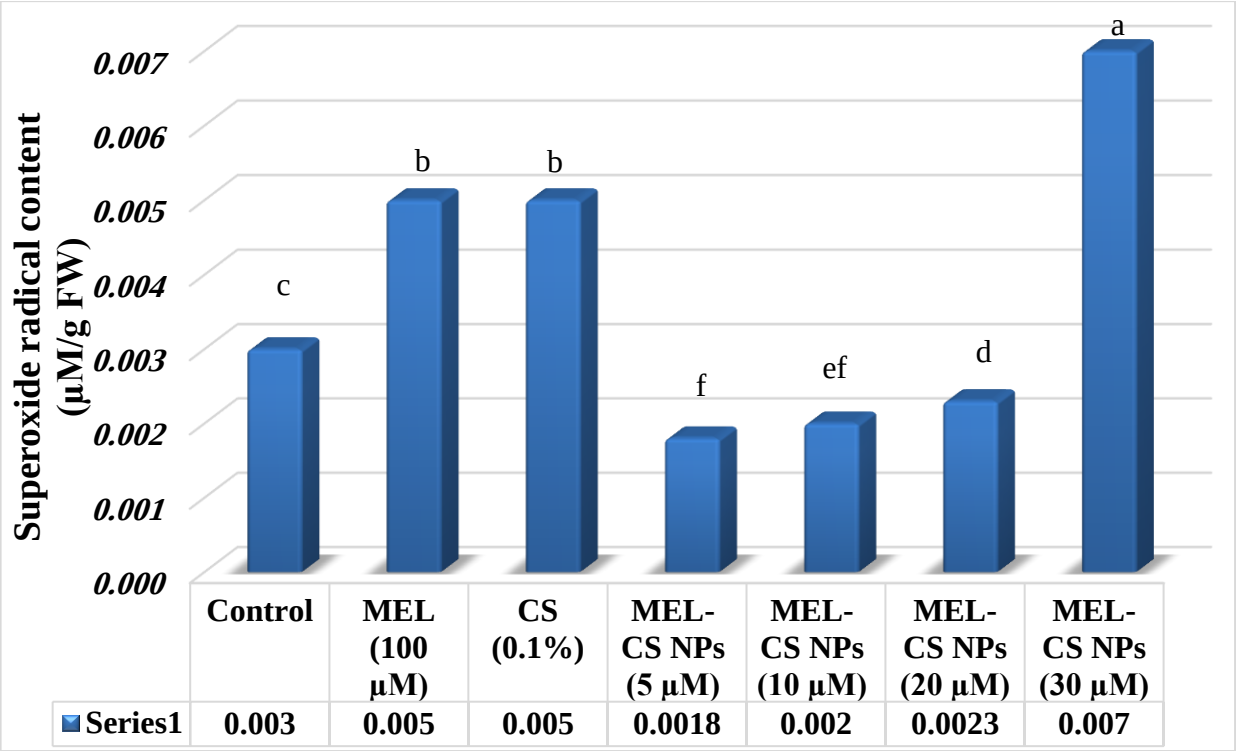
Table 2. Change in the contents of carbohydrate fractions (mg g^{-1} DW) in *Ocimum basilicum* L. leaves (after 30 days) treated with either conventional MEL, CS, or different concentrations of Nano-melatonin.

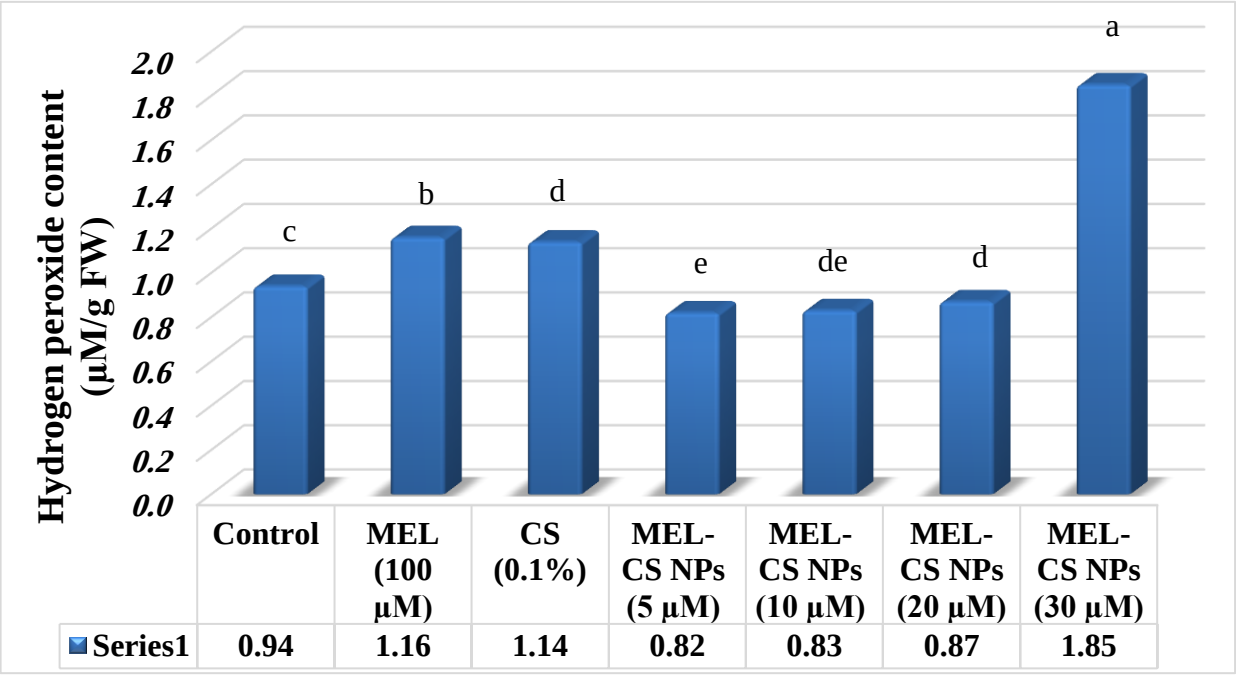
Treatments	Soluble Sugars	Insoluble sugars	Total carbohydrate	FAA	TSP
Control	1.417±0.002 ^{cd}	0.441±0.004 ^{b}	1.858±0.006 ^{e}	4.352±0.006 ^{e}	0.690±0.007 ^{e}
MEL (100 µM)	1.450±0.003 ^{c}	0.451±0.002 ^{a}	1.901±0.005 ^{c}	4.423±0.005 ^{c}	0.759±0.005 ^{c}
CS (0.1%)	1.452±0.006 ^{c}	0.454±0.010 ^{a}	1.906±0.016 ^{c}	4.434±0.017 ^{c}	0.769±0.018 ^{c}
MEL-CS NPs (5 µM)	1.470±0.006 ^{d}	0.456 ±0.009 ^{a}	1.926±0.003 ^{d}	4.404±0.003 ^{d}	0.740±0.003 ^{d}
MEL-CS NPs (10 µM)	1.549±0.002 ^{a}	0.464±0.010 ^{a}	2.013±0.008 ^{a}	4.947±0.009 ^{a}	0.879±0.008 ^{a}
MEL-CS NPs (20 µM)	1.523 ±0.021^{b}	0.457±0.027 ^{a}	1.980±0.006 ^{b}	4.909±0.006 ^{b}	0.842±0.006 ^{b}
MEL-CS NPs (30 µM)	1.345±0.006 ^{e}	0.351±0.003 ^{b}	1.696±0.003 ^{f}	4.308±0.003 ^{f}	0.647±0.003 ^{f}
F-value	99.412***	22.500*	306.857***	315.520***	3301.96***

Different letters indicate a significant difference at *: P<0.1, **: P<0.01, ***<0.001.

Data in Figure 2 showed that levels of $O_2^{\bullet-}$, H_2O_2, and MDA in leaves of basil significantly increased as a result of being treated with either conventional melatonin, chitosan, and 30 µM Nano-melatonin. In contrast, the basil leaves treated with 5, 10, and 20 µM of Nano-melatonin caused a significant decrease in this content. The highest increments of $O_2^{\bullet-}$, H_2O_2, and MDA were 0.007, 1.85, and 14.57 µmole g^{-1} FW, respectively found in plants treated with 30 µM Nano-melatonin. Results revealed that Nano-melatonin treatment at concentrations 5, 10, and 20 µM had a remarkable effect on reducing the accumulation of $O_2^{\bullet-}$, H_2O_2, and MDA. In a previous study, the foliar application of melatonin and nano-silica individually or in combination decreased the content of $O_2^{\bullet-}$, H_2O_2, and MDA significantly through promoting the upregulation of genes of antioxidant enzymes [8]. In Glycine max seedlings, foliar spraying of melatonin caused a significant boost in the activity of lipoxygenases, superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase. Also, H_2O_2 and $O_2^{\bullet-}$contents were decreased, which led to a reduction in MDA content [68]. Melatonin activates crucial enzymes of the lipid metabolic pathway (LPS, LOX, and PLD) [69]. Farooq *et al*. [20] evaluated the rule of MEL and selenium (Se) nanoparticles on *Brassica napus* growth and stress tolerance to arsenic. They found a significant reduction in H_2O_2, and MDA contents. The application of MEL and selenium (Se) nanoparticles enhanced CAT, SOD,POD, and APX enzyme activity.

Thus, the accomplished study by Wang *et al*. [64] noted that ~~the~~ melatonin reduced the reactive oxygen species ($O_2^{\bullet-}$, H_2O_2) levels and enhanced antioxidant enzymes activity (CAT, SOD, and POD) in pear fruits. Askari *et al*. [57] investigated the melatonin treatment lowered MDA production on white bean seedlings. Imran *et al*. [10] stated that melatonin could be attributed to decreasing the accumulation of reactive oxygen species and malonaldehyde by promoting the antioxidant enzyme activity. Similarly, Cherono *et al*. [52] conclusions showed the application of melatonin in both foliar and direct soil application reduced malonaldehyde content and oxidative damage in *Coffea arabica* seedlings. Moreover, Sardar *et al*. [9] results instituted that exogenous foliar application of MEL reduced oxidative damage in the broccoli by lowering the concentration of H_2O_2 and MDA and increasing antioxidant activity.


Superoxide radical content (µM/g FW)
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
c
b
b
f
ef
d
a
Control
MEL (100 µM)
CS (0.1%)
MEL-CS NPs (5 µM)
MEL-CS NPs (10 µM)
MEL-CS NPs (20 µM)
MEL-CS NPs (30 µM)
Series1
0.003
0.005
0.005
0.0018
0.002
0.0023
0.007


Hydrogen peroxide content (µM/g FW)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
c
b
d
e
de
d
a
Control
MEL (100 µM)
CS (0.1%)
MEL-CS NPs (5 µM)
MEL-CS NPs (10 µM)
MEL-CS NPs (20 µM)
MEL-CS NPs (30 µM)
Series1
0.94
1.16
1.14
0.82
0.83
0.87
1.85

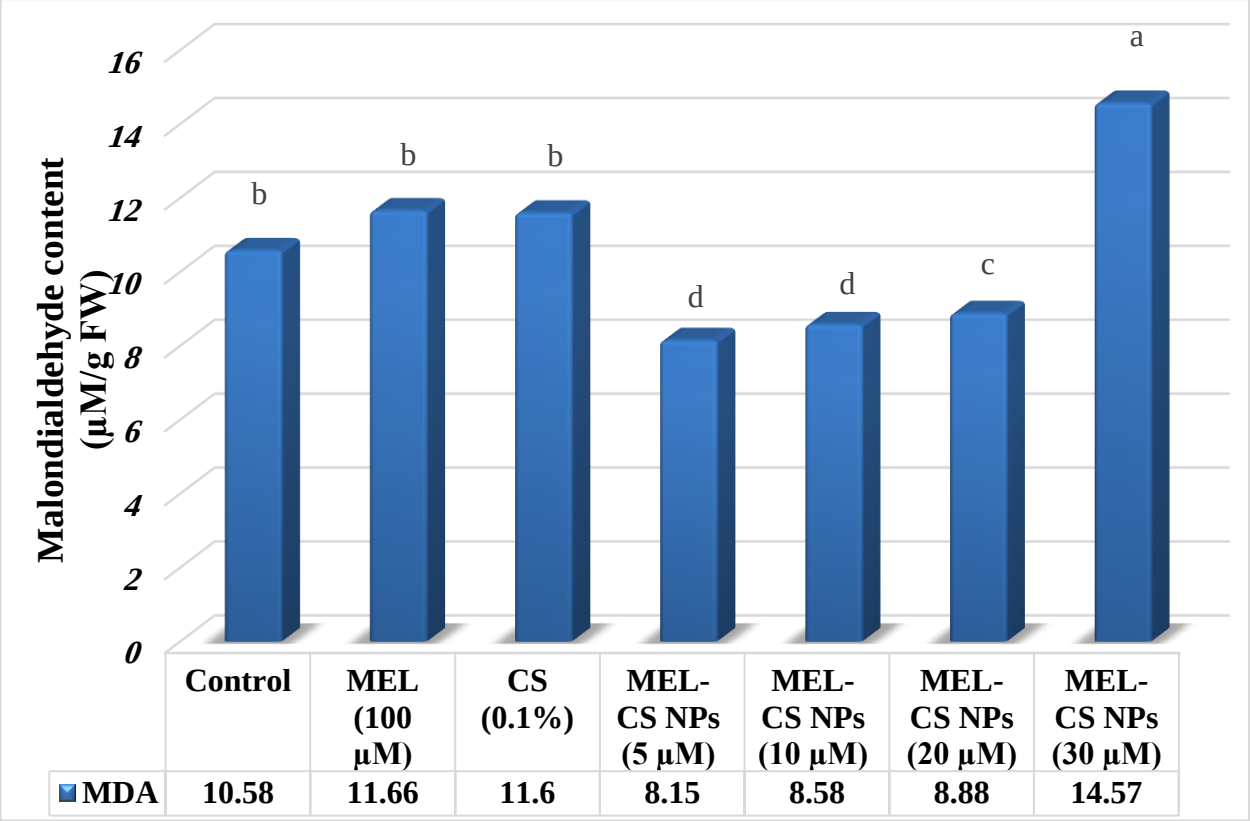


Figure 2. Levels of superoxide radical, hydrogen peroxide, and malonaldehyde (µmole g^{-1} FW) in *Ocimum basilicum* L. leaves (after 30 days) treated with either conventional MEL, CS, or different concentrations of Nano-melatonin. Different letters above the bars indicate significant differences using Duncan's multiple range tests.

Figure 3 showed the activity of catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), and polyphenol oxidase (PPO) in leaves of basil (after 30 days) treated with either conventional melatonin, chitosan, or Nano-melatonin. The activity of these antioxidant enzymes significantly increased in leaves treated with either conventional melatonin, chitosan, or Nano-melatonin at different concentrations, but the concentration of 30 µM Nano-MEL led to a significant reduction in antioxidant enzymes activity. The treatment with 10 µM Nano-MEL recorded the highest activity values of CAT, SOD, POX, and PPO in basil leaves, where they increased by about 170.95%, 137.165%, 177.085%, and 213.61% respectively as compared with untreated control. Increasing the antioxidant system is one of the best strategies to get rid of reactive oxygen species [70]. Also, melatonin significantly enhanced the expression of genes related to antioxidant enzymes as a nicotinamide adenine dinucleotide phosphate (*NADPH*) oxidase gene, and mitogen-activated protein kinase genes (*MAPK3, MAPK4, MAPK6*) [71].

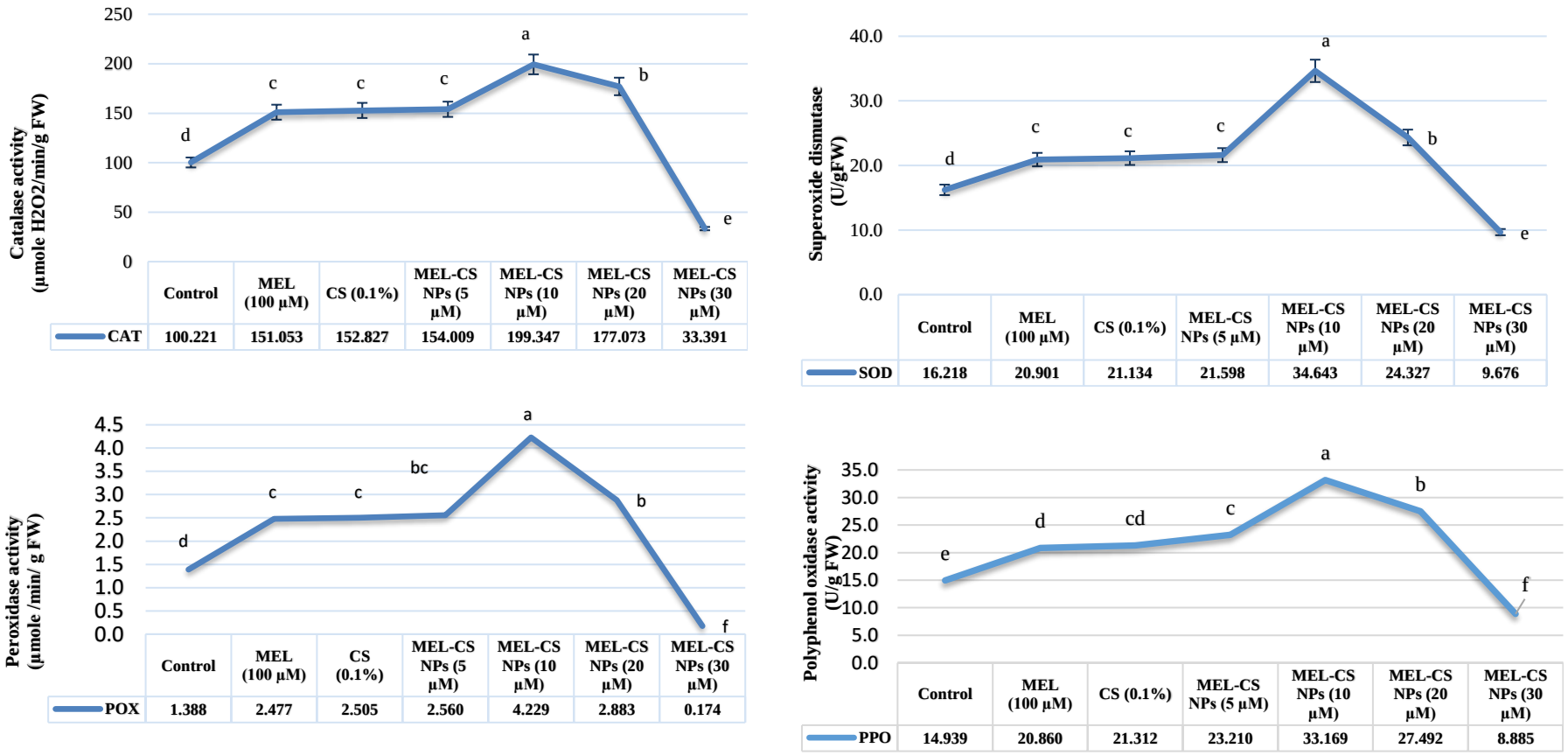


Figure 3. The activity of antioxidant enzymes in *Ocimum basilicum* L. leaves (after 30 days) treated with either conventional MEL, CS, or different concentrations of Nano-melatonin. Vertical bars represent the standard division (±SD). Different letters above the bars indicate significant differences using Duncan's multiple range tests.

The study conducted by Yin *et al*. [72], indicated that melatonin application reduced oxidative stress by regulating antioxidant enzyme activities. The Vougeleka *et al*. [73] study investigated that significant increases were observed in SOD and CAT content in *Phaseolus vulgaris* and *Nicotiana tabacum* sprayed with MEL. Chang *et al*. [48] showed that MEL treatment at 100 µM greatly enhanced in the activity of SOD and POD, as well as decreased hydrogen peroxide and monoaldehyde content in *Prunella vulgaris* leaves. Similarly, melatonin application increased the activity of antioxidant enzymes such as SOD, POD, and CAT, which reduced the oxidative damage in the broccoli and bean plants as reported by Sardar *et al*. [9] and Askari *et al*. [57] respectivelly.

4.Conclusion

The present study was designed to explore the uses of nanotechnology and its application in agriculture by exogenous application of MEL-CS NPs at a concentration of 5, 10, 20, and 30 µM for the enhancing plant growth and antioxidants system in *Ocimum basilicum* L. growing in half-strength MS media.

It was concluded that MEL-CS NPs at a concentration of 10 µM was the appropriate and most effective concentration compared to other treatments. Where it triggered a notable increase in the growth characteristics of *O. basilicum* plant growing on half-strength MS media. Additionally, there was an increase in the biochemical activity of *O. basilicum* leaves, which includes photosynthetic pigments, carbohydrates, TSP, FAA, and antioxidant enzyme activities concomitant with a reduction in the content of $O_2^{\bullet-}$, H_2O_2, and MAD in *O. basilicum* leaves. The results confirmed the effective role of 10 µM of MEL-CS nanoparticles for enhancing the *O. basilicum* growth and metabolism as well as enhancing plant antioxidant activity.

5.Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Majdi C, Pereira C, Dias MI, Calhelha RC, Alves MJ, Rhourri-Frih B. Phytochemical characterization and bioactive properties of cinnamon basil (*Ocimum basilicum cv.'Cinnamon*') and lemon basil (*Ocimum*× *citriodorum*). Antioxidants 2020;9:369.

[2] Sharma AD, Kaur I, Angish S, Thakur A, Sania S, Singh A. Comparative phytochemistry, antioxidant, antidiabetic, and anti-inflammatory activities of traditionally used *Ocimum*

basilicum L. *Ocimum gratissimum* L., *and Ocimum tenuiflorum* L. BioTechnologia 2022;103:131.

[3] Zhao J, Hu J. Melatonin: Current status and future perspectives in horticultural plants. Front Plant Sci 2023;14:1140803.

[4] Ahmad I, Song X, Hussein Ibrahim ME, Jamal Y, Younas MU, Zhu G. The role of melatonin in plant growth and metabolism, and its interplay with nitric oxide and auxin in plants under different types of abiotic stress. Front Plant Sci 2023;14:1108507.

[5] Wang K, Xing Q, Ahammed GJ, Zhou J. Functions and prospects of melatonin in plant growth, yield, and quality. J Exp Bot 2022;73:5928–46.

[6] Chen J, Qin H, Zhang B, Mao W, Lou L, Shen C. Development of melatonin nano-delivery systems to reduce cadmium accumulation in rice (*Oryza sativa* L.) seedlings: Insights from photosynthetic efficiency, antioxidative response and gene expression. Environ Exp Bot 2022;196:104822.

[7] Altaf MA, Shu H, Hao Y, Mumtaz MA, Lu X, Wang Z. Melatonin affects the photosynthetic performance of pepper (*Capsicum annuum* L.) seedlings under cold stress. Antioxidants 2022;11:2414.

[8] Al-Shammari WB, Altamimi HR, Abdelaal K. Improvement in physiobiochemical and yield characteristics of pea plants with nano silica and melatonin under salinity stress conditions. Horticulturae 2023;9:711.

[9] Sardar H, Ramzan MA, Naz S, Ali S, Ejaz S, Ahmad R. Exogenous application of melatonin improves the growth and productivity of two broccoli (*Brassica oleracea* L.) cultivars under salt stress. J Plant Growth Regul 2023;42:5152–66.

[10] Imran M, Latif Khan A, Shahzad R, Aaqil Khan M, Bilal S, Khan A. Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defence system of soybean plants. AoB Plants 2021;13:plab026.

[11] Esmaeili S, Sharifi M, Ghanati F, Soltani BM, Samari E, Sagharyan M. Exogenous melatonin induces phenolic compounds production in Linum album cells by altering nitric oxide and salicylic acid. Sci Rep 2023;13:4158.

[12] Hernández-Ruiz J, Ruiz-Cano D, Giraldo-Acosta M, Cano A, Arnao MB. Melatonin in Brassicaceae: role in postharvest and interesting phytochemicals. Molecules 2022;27:1523.

[13] Vafadar F, Amooaghaie R, Ehsanzadeh P, Ghanadian M, Talebi M, Ghanati F. Melatonin and calcium modulate the production of rosmarinic acid, luteolin, and apigenin in *Dracocephalum kotschyi* under salinity stress. Phytochemistry 2020;177:112422.

[14] Miao H, Zeng W, Zhao M, Wang J, Wang Q. Effect of melatonin treatment on visual quality and health-promoting properties of broccoli florets under room temperature. Food Chem 2020;319:126498.

[15] Turk H, Erdal S, Genisel M, Atici O, Demir Y, Yanmis D. The regulatory effect of melatonin on physiological, biochemical and molecular parameters in cold-stressed wheat seedlings. Plant Growth Regul 2014;74:139–52.

[16] Farooq MA, Islam F, Ayyaz A, Chen W, Noor Y, Hu W. Mitigation effects of exogenous melatonin-selenium nanoparticles on arsenic-induced stress in *Brassica napus*. Environmental Pollution 2022;292:118473.

[17] Chen J, Qin H, Zhang B, Mao W, Lou L, Shen C. Development of melatonin nano-delivery systems to reduce cadmium accumulation in rice (*Oryza sativa* L.) seedlings: Insights from photosynthetic efficiency, antioxidative response and gene expression. Environ Exp Bot 2022;196:104822.

[18] Zhao Z, Yun C, Gu L, Liu J, Yao L, Wang W. Melatonin enhances biomass, phenolic accumulation, and bioactivities of rosemary (*Rosmarinus officinalis*) in vitro shoots under UV-B stress. Physiol Plant 2023:e13956.

[19] Wei L, Liu C, Wang J, Younas S, Zheng H, Zheng L. Melatonin immersion affects the quality of fresh-cut broccoli (*Brassica oleracea* L.) during cold storage: Focus on the antioxidant system. J Food Process Preserv 2020;44:e14691.

[20] Farooq MA, Islam F, Ayyaz A, Chen W, Noor Y, Hu W. Mitigation effects of exogenous melatonin-selenium nanoparticles on arsenic-induced stress in *Brassica napus*. Environmental Pollution 2022;292:118473.

[21] Cheng G, Ma T, Deng Z, Gutiérrez-Gamboa G, Ge Q, Xu P. Plant-derived melatonin from food: A gift of nature. Food Funct 2021;12:2829–49.

[22] De Sousa Victor R, Marcelo da Cunha Santos A, Viana de Sousa B, de Araújo Neves G, Navarro de Lima Santana L, Rodrigues Menezes R. A review on Chitosan's uses as biomaterial: Tissue engineering, drug delivery systems and cancer treatment. Materials 2020;13:4995.

[23] An C, Sun C, Li N, Huang B, Jiang J, Shen Y. Nanomaterials and nanotechnology for the delivery of agrochemicals: strategies towards sustainable agriculture. J Nanobiotechnology 2022;20:1–19.

[24] Luz JMQ, Asmar SA, Morais TP, Araruna EC, Pasqual M. *In vitro* germination of basil seeds. Acta Hortic 2015:347–52.

[25] Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol 1949;24:1.

[26] Lichtenthaler HK, Babani F. Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. Chlorophyll a fluorescence: a signature of photosynthesis, Springer; 2004, p. 713–36.

[27] DuBois M, Gilles KA, Hamilton JK, Rebers PA t, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956;28:350–6.

[28] Mocready RM, Guggolz J, Silviera V, Owens HS. Determination of starch and amylose in vegetables. Application to peas. 1950.

[29] Li M, Xiong Z, Dai L, Huang Y. Effects of Copper on Nitrogen Assimilation in Copper-tolerant and Non-tolerant Populations of Elsholtzia haichowensis S. Water Air Soil Pollut 2007;184:323–33.

[30] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.

[31] Ramel F, Sulmon C, Bogard M, Couée I, Gouesbet G. Differential patterns of reactive oxygen species and antioxidative mechanisms during atrazine injury and sucrose-induced tolerance in Arabidopsis thaliana plantlets. BMC Plant Biol 2009;9:1–18.

[32] Alexieva V, Sergiev I, Mapelli S, Karanov E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 2001;24:1337–44.

[33] Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 1968;125:189–98.

[34] Grace SC, Logan BA. Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. Plant Physiol 1996;112:1631–40.

[35] Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952;195:133–40.

[36] Giannopolitis CN, Ries SK. Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol 1977;59:309–14.

[37] Chance B, Maehly AC. [136] Assay of catalases and peroxidases 1955.

[38] Soffan A, Alghamdi SS, Aldawood AS. Peroxidase and polyphenol oxidase activity in moderate resistant and susceptible *Vicia faba* induced by *Aphis craccivora* (Hemiptera: Aphididae) infestation. Journal of Insect Science 2014;14:285.

[39] Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. Applied linear statistical models. vol. 4. Irwin Chicago; 1996.

[40] Arnao MB, Hernández-Ruiz J. Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. J Pineal Res 2009;46:58–63.

[41] Wang P, Sun X, Li C, Wei Z, Liang D, Ma F. Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. J Pineal Res 2013;54:292–302.

[42] Simlat M, Ptak A, Skrzypek E, Warchoł M, Morańska E, Piórkowska E. Melatonin significantly influences seed germination and seedling growth of *Stevia rebaudiana Bertoni*. PeerJ 2018;6:e5009.

[43] Zhao H, Su T, Huo L, Wei H, Jiang Y, Xu L. Unveiling the mechanism of melatonin impacts on maize seedling growth: sugar metabolism as a case. J Pineal Res 2015;59:255–66.

[44] Posmyk MM, Kuran H, Marciniak K, Janas KM. Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. J Pineal Res 2008;45:24–31.

[45] Posmyk MM, Bałabusta M, Wieczorek M, Sliwinska E, Janas KM. Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress. J Pineal Res 2009;46:214–23.

[46] Chen Q, Qi W, Reiter RJ, Wei W, Wang B. Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of Brassica juncea. J Plant Physiol 2009;166:324–8.

[47] Mao J, Niu C, Li K, Chen S, Tahir MM, Han M. Melatonin promotes adventitious root formation in apple by promoting the function of MdWOX11. BMC Plant Biol 2020;20:1–11.

[48] Chang Q, Zhang L, Chen S, Gong M, Liu L, Hou X. Exogenous melatonin enhances the yield and secondary metabolite contents of *Prunella vulgaris* by modulating antioxidant system, root architecture and photosynthetic capacity. Plants 2023;12:1129.

[49] Jiang M, Dai S, Wang B, Xie Z, Li J, Wang L. Gold nanoparticles synthesized using melatonin suppress cadmium uptake and alleviate its toxicity in rice. Environ Sci Nano 2021;8:1042–56.

[50] Sarropoulou V, Dimassi-Theriou K, Therios I, Koukourikou-Petridou M. Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (*Prunus avium*× *Prunus cerasus*). Plant Physiology and Biochemistry 2012;61:162–8.

[51] Jahan MS, Guo S, Baloch AR, Sun J, Shu S, Wang Y. Melatonin alleviates nickel phytotoxicity by improving photosynthesis, secondary metabolism and oxidative stress tolerance in tomato seedlings. Ecotoxicol Environ Saf 2020;197:110593.

[52] Cherono S, Ntini C, Wassie M, Mollah MD, Belal MA, Ogutu C. Exogenous application of melatonin improves drought tolerance in coffee by regulating photosynthetic efficiency and oxidative damage. Journal of the American Society for Horticultural Science 2021;146:24–32.

[53] Zhu L, Gao H, Li L, Zhang Y, Zhao Y, Yu X. Promoting lutein production from the novel alga Acutodesmus sp. by melatonin induction. Bioresour Technol 2022;362:127818.

[54] Chen Y, Mao J, Sun L, Huang B, Ding C, Gu Y. Exogenous melatonin enhances salt stress tolerance in maize seedlings by improving antioxidant and photosynthetic capacity. Physiol Plant 2018;164:349–63.

[55] Verde A, Míguez JM, Gallardo M. Role of melatonin in apple fruit during growth and ripening: possible interaction with ethylene. Plants 2022;11:688.

[56] Karaca P, Cekic FÖ. Exogenous melatonin-stimulated defense responses in tomato plants treated with polyethylene glycol. International Journal of Vegetable Science 2019;25:601–9.

[57] Askari M, Hamid N, Abideen Z, Zulfiqar F, Moosa A, Nafees M. Exogenous melatonin application stimulates growth, photosynthetic pigments and antioxidant potential of white beans under salinity stress. South African Journal of Botany 2023;160:219–28.

[58] Zhao H, Xu L, Su T, Jiang Y, Hu L, Ma F. Melatonin regulates carbohydrate metabolism and defenses against *Pseudomonas syringae* pv. tomato DC 3000 infection in Arabidopsis thaliana. J Pineal Res 2015;59:109–19.

[59] Dou J, Wang J, Tang Z, Yu J, Wu Y, Liu Z. Application of exogenous melatonin improves tomato fruit quality by promoting the accumulation of primary and secondary metabolites. Foods 2022;11:4097.

[60] Khan HMT, Balal RM, Hussain Z, Javed SA, Jaffar MT, Alsahli AA. Exogenous application of melatonin mitigate the heat stress in different tomato (*Solanum lycopersicum* L.) cultivars. Journal of King Saud University-Science 2024;36:103086.

[61] Wang L, Yang M, Dong Y, Reiter RJ, Xu Y, Lin X. Melatonin confers enhanced polyamine metabolism and cell tolerance in *Vitis vinifera* against oxidative damage: Quantitative proteomic evidence. Postharvest Biol Technol 2022;184:111756.

[62] Yang G, Wei X, Fang Z. Melatonin mediates axillary bud outgrowth by improving nitrogen assimilation and transport in rice. Front Plant Sci 2022;13:900262.

[63] Wang K, Cai S, Xing Q, Qi Z, Fotopoulos V, Yu J. Melatonin delays dark-induced leaf senescence by inducing miR171b expression in tomato. J Pineal Res 2022;72:e12792.

[64] Wang Y, Wang G, Xu W, Zhang Z, Sun X, Zhang S. Exogenous melatonin improves pear resistance to Botryosphaeria dothidea by increasing autophagic activity and sugar/organic acid levels. Phytopathology 2022;112:1335–44.

[65] Pan Y, Huang H, Zhang J. Melatonin-mediated development and abiotic stress tolerance in plants. Front Plant Sci 2023;14:1100827.

[66] Dey S, Biswas A, Deng Y, Birhanie ZM, Wentao C, Li D. Exogenous melatonin enhances low-temperature stress of jute seedlings through modulation of photosynthesis and antioxidant potential. Heliyon 2023;9.

[67] Wenfei J, Xiaoqiong W, Jingheng M, Lixin W, Linyu L, Jinying L. Exogenous application of melatonin improves the growth and physiological properties of blueberry seedlings under salt stress. Biotechnology & Biotechnological Equipment 2023;37:2202781.

[68] Alharbi BM, Elhakem AH, Alnusairi GSH, Soliman MH, Hakeem KR, Hasan MM. Exogenous application of melatonin alleviates salt stress-induced decline in growth and photosynthesis in *Glycine max* (L.) seedlings by improving mineral uptake, antioxidant and glyoxalase system. 2021.

[69] Fan S, Xiong T, Lei Q, Tan Q, Cai J, Song Z. Melatonin treatment improves postharvest preservation and resistance of guava fruit (*Psidium guajav*a L.). Foods 2022;11:262.

[70] Zhang T, Wang J, Sun Y, Zhang L, Zheng S. Versatile roles of melatonin in growth and stress tolerance in plants. J Plant Growth Regul 2022:1–17.

[71] Zhang T, Shi Z, Zhang X, Zheng S, Wang J, Mo J. Alleviating effects of exogenous melatonin on salt stress in cucumber. Sci Hortic 2020;262:109070.

[72] Yin Y, Xu J, He X, Yang Z, Fang W, Tao J. Role of exogenous melatonin involved in phenolic acid metabolism of germinated hulless barley under NaCl stress. Plant Physiology and Biochemistry 2022;170:14–22.

[73] Vougeleka V, Risoli S, Saitanis C, Agathokleous E, Ntatsi G, Lorenzini G. Exogenous application of melatonin protects bean and tobacco plants against ozone damage by improving antioxidant enzyme activities, enhancing photosynthetic performance, and preventing membrane damage. Environmental Pollution 2024;343:123180.

الملخص العربي

النانوميلاتونين يحسن النمو والأنشطة البيوكيميائية لنبات الريحان معملياً

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الملخص العربى

يلعب الميلاتونين (MEL) أدوارًا مهمة في فسيولوجيا النبات المختلفة، وعلى وجه التحديد تنظيم نمو النبات وتطوره وايضه. أجريت الدراسة الحالية على نبات (*Ocimum basilicum* L.) الذي ينمو في بيئة موراشج وسكوج بتركيز ٥٠٪ مع إضافة ٥ و١٠ و٢٠ و٣٠ ميكرومول من جسيمات الميلاتونين المحملة على جسيمات الشيتوزان النانونية MEL-CS (NPs). وبعد أربعة أسابيع، تم اخذ نباتات الريحان النامية لقياس التغير في النمو وعمل الدراسات الفسيولوجية والكيميائية الحيوية. كشفت نتائجنا أن معاملة النبات بجسيمات الميلاتونين النانونية MEL-CS (NPs) أدت إلى زيادة كبيرة في النمو الخضري ووكذالك الجذور من حيث الطول ، والوزن الرطب والجاف، وأصباغ التمثيل الضوئي، والمحتوى الكربوهيدراتي، والبروتيني الكلي، والأحماض الأمينية الحرة، ورافق ذلك زيادة ملحوظة في نشاط الإنزيمات المضادة للأكسدة (سوبر أكسيد ديسميوتيز SOD، كاتاليز CAT، بيروكسيديز POX، بولي فينول أوكسيديز PPO. ومع ذلك، فإن تراكم فوق الأكسيد الفائق ($O_2^{\bullet-}$)، وبيروكسيد الهيدروجين (H_2O_2)، والمالونديالدهيد (MDA) انخفض في أوراق الريحان. كان المعاملة الأكثر فعالية هو ١٠ ميكرومول من جسيمات MEL-CS النانونية. في الوقت نفسه، كان تركيز ٣٠ ميكرومول هو الأقل فعالية مقارنة بالنبات الغير معامل ووكذالك المعامل ب MEL وCS العادي. أكدت النتائج الإيجابية لدراستنا الدور الفعال للجسيمات MEL-CS النانونية في تعزيز نمو النبات، وايضه المقاس وتقليل الإجهاد التأكسدي ($O_2^{\bullet-}$، H_2O_2، وMDA) من خلال تحسين نظام الدفاع المضاد للأكسدة.