Cadmium stress consolation in melatonin supplemented *Cucumis sativus* through modulation of antioxidative defense system

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Abstract

Current studies elucidate the metal stress attenuation potential of melatonin in *Cucumis sativus* seedlings growing in cadmium contaminated conditions. Melatonin is an indoleamine molecule, capable of ameliorating environmental stresses and regulate plant growth. Seeds of *C. sativus* were immersed in different levels of melatonin and grown under cadmium stress for 15 days. Cadmium stress reduced seed germination, growth, biomass production, gas exchange capacity, stomatal conductance and chlorophyll (Chl) content in *C. sativus* seedlings. The increased level of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) caused oxidative stress in *C. sativus* plants under cadmium stress. Melatonin application emphatically revamped germination, shoot, and plant biomass production. The melatonin pretreatment aggrandized plant length, root length, and expression of stress related genes (CsHA2, CsHA3, CsHA4, CsHA8 and CsHA9) along with amplification in activity of superoxide dismutase (SOD), nitrate reductase (NR), and ascorbate peroxidase (AP) helping in modulation of cadmium stress in *C. sativus* seedlings. The improved activity of antioxidant scavengers was ascribed to the reduced level of H_2O_2 and MDA in plants under stress. Furthermore, conjugated increase of photosynthetic activity, indole acetic acid contents, and glutathione contents was observed in melatonin treated seedlings in a dose-responsive manner. The present study elicits the metal stress attenuating potential of melatonin by regulating metabolic activities and growth of *C. sativus* under cadmium stress.

Keywords: antioxidant; cadmium; cucumber; H_2O_2; MDA; melatonin

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Introduction

The inappropriate disposal of industrial effluents in drains and subsequent application of this heavy metal contaminated water for irrigation purpose is deteriorating productivity of the agricultural lands. It is estimated that deaths of 4.9 million people are solely accredited to toxic effects of environmental hazards (Mamtani et al., 2011). The growth and yield of crops growing on metal polluted area is drastically low, owing to metal stress. Therefore, development of economical and eco-friendly metal stress alleviation techniques becomes compulsory to meet the quality food,

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clothing, and constructional needs of the human beings and animals.

Cucumis sativus L. is one of the most admired cucurbitaceous crop grown, cultivated and consumed worldwide (Amin et al., 2018). This crop is globally admired due to its enormous economical and nutritional values (Dingal et al., 2018). It is the 4th main vegetable crop of the Asian countries following *Lycopersicon esculentum*, *Brassica oleracea capitate*, and *Allium cepa* (Eifediyi and Remison, 2010). The growth and yield of this crop is severely affected by heavy metal stress (Shekari et al., 2019).

Melatonin (N-acetyl-5-methoxytryptamine), present in all living organisms, is an important bio-molecule involved in heightening of antioxidant enzymes and morpho-physiological attributes in plants exposed to environmental stress (Tan et al., 2010; Bajwa et al., 2014; Zhou et al., 2016). Melatonin role has been observed in numerous new plant aspects like improvement in storage life, fruit quality, nutrients uptake, and grafting (Nawaz et al., 2016). Melatonin acts as an anti-stress agent and helps to combat drought, salinity, toxic chemicals, ultra violet (UV) radiations, and harsh climatic factors. It was also observed that melatonin has a significant contribution in regulation of plant growth, rhizogenesis, and photosynthetic activities. Additionally, melatonin supplementation enhanced the tolerance and survival of under stress. Arnao and Hernandez-Ruiz (2015) have observed that apple saplings pretreated with melatonin showed improvement in physiological properties like shoot height, leaf number, and chlorophyll contents as compared with untreated saplings.

Van Tassel et al. (2001) have reported that this pleotropic biochemical has a pivotal role in improvement of plant cultivars. Numerous investigations encompass melatonin role in crop breeding through activation of tryptophan related metabolic pathways (Arnao and Hernández-Ruiz, 2014). The melatonin and some other polyamines reduce or inhibit the scavenging action of ROS, thereby decreasing the organismal senescence (Reiter et al., 2015). This polyamine can be applied either exogenously or endogenously for stress alleviation in plants facing environmental hazards (Li et al., 2012).

In certain cases, melatonin acts as a phytohormone and increases the concentration of polyamines in *Cucumis sativus*, *Daucus carota, Cynodon dactylon*, and *Prunus persica* under abiotic stresses (Zhao et al., 2017; Shi et al., 2014; Cao et al., 2006; Lei et al., 2004).

Exogenously applied melatonin helps in improvement of physiological attributes and growth of numerous agronomic and horticultural crops. Melatonin has a crucial role in regulation of antioxidant activity of plant defense. Seedlings raised from melatonin primed *C. sativus* seeds displayed significant enhancement in the activity of superoxide dismutase and detoxified ROS in saplings under chilling stress (Marta et al., 2016). Higher amounts of proline, sucrose, and polyamines in conjunction with up-regulation of chilling stress related genes were found in melatonin pre-treated tomato plants subjected to chilling conditions (Ding et al., 2017). Salt stress caused higher generation of ROS leading to pronounced leaf membrane damage in *Citrullus vulgaris* seedlings (Li et al., 2017). However, exogenous application of melatonin improved rate of photosynthesis and modulated the activity of antioxidant enzymes resulting in mitigation of *C. vulgaris* plants growing in saline conditions. Correspondingly, Turk et al. (2014) reported increased activity of antioxidant enzyme, higher amount of ascorbic acid, and glutathione contents in pre-treated wheat seedlings under cold stress. Park et al. (2013) also advocated that improved activity of catalase and superoxide dismutase assist in alleviation of herbicide toxicity in melatonin treated seedlings.

Melatonin primed *Vicia faba* L. seeds exhibited higher uptake of K^{+}, ca^{+2} together with improved biosynthesis of indole acetic acid and photosynthetic pigments in developed saplings (Dawood and El-Awadi, 2015).

Regardless of copious research on the advantageous effects of melatonin in moderating abiotic stresses in many plants, diminutive information is available about the effect of this phytohormone on alleviation of cadmium stress in cucumber. Cadmium concentration between 0.5-2.5 Mm have a noteworthy effect on growth attributes of cucumber seedlings (Munzuroglu and Geckil, 2002). In this investigation, the role of melatonin, a pleiotropic molecule to mitigate

cadmium stress in *C. sativus* seedlings was evaluated.

Materials and Methods

Plant materials and growth conditions

Cucumis sativus seeds were disinfected by applying 2% sodium hypochlorite (w/v) for 10 min and subsequently washed three times with deionized water. These seeds were sown in petri plates at equidistance over 3 layers of Whatman filter paper (no. 1) soaked with melatonin (0, 100, and 150 µM) and supplemented with 10 ml of 0 or 2.5 mM cadmium. The petri plates were divided into different groups. Seeds placed in petri plates, in the absence of melatonin and cadmium, were termed as un-contaminated control (C). The seeds grown in plates polluted with cadmium were regarded as contaminated control (Cd). Seeds present in plates supplemented with 50, 100, and 150 µM melatonin were termed as M1, M2, and M3, respectively.

The covered petri plates were transferred in a plant incubator having 14 h light/10 h dark period arranged by 500-550 mmol m^2 s^1 light, 65-75% relative humidity, at 25 ± 2° C for 15 d. Each petri plate was irrigated with 20 ml distilled water on every second day.

Analysis of seed germination

For germination test, 9 seeds were placed in each petri plate at equidistance. The cucumber seeds having ruptured testa and perceptible radicle were regarded as germinated. The germinated seeds, having at least 3 mm radicle were sampled after 3 d.

Estimation of seedling growth rate and health index

Leaves, stems, and roots of harvested seedlings were submerged in sterilized double distilled water and dried using blotting paper. Total plant weight, root and shoot weight, and lengths were also calculated. For estimation of biomass, shoot and root samples were oven-dried for 48h at 80° C.

Estimation of leaf relative water content

The fully expanded leaves were immersed in deionized H_2O at 10° C for 24 hrs. Turgid weight of the leaves was measured by wrapping leaves in blotting paper for two minutes. The leaves were oven dried and their leaf relative water content was estimated by methods of Smart and Bingham (1974) using the following equation:

LRWC (%) = FW-DW / TW-DW × 100

where FW= fresh weight of leaf, DW= dry weight of leaf, and TW= turgid weight of leaf.

Estimation of photochemical efficiency and photosynthetic pigments

For spectrophotometric determination of Chl a, Chl b, total chlorophyll, and carotenoids, 50 mg foliage sample was crushed and homogenized with 4 ml of acetone 80% (v/v) in an ice-chilled mortar. The solution was passed through a filter paper. The volume of filtrate was adjusted to 5 ml by adding cold acetone and absorbance was observed at 663.2, 646.8, and 470 nm and the levels of pigments involved in photosynthesis were measured as described by Lichtenthaler (1987).

The photochemical efficiency (*Fv*/*Fm*) of leaf was estimated after 0.5 h of dark adaptation with the help of chlorophyll fluorimeter according to Zhou et al. (2018).

Assessment of gas exchange parameters

The net photosynthetic rate (P_n) and other gas exchange parameters including intercellular CO_2 concentration, transpiration rate, and stomatal conductance from 2nd top fully expanded leaf were determined by an infrared gas analyzer (Li-6400, LICOR, United States of America). The air temperatures as well as relative humidity in the chamber were kept at 27° C and 84%, respectively. Whereas, the concentration of CO_2 and density of photosynthetic photon flux inside the analyzer were adjusted at 400 mmol mol^{-1}, 1000 mmol m^2 s^{-1}, respectively.

Estimation of hydrogen peroxide

Leaf or root sample (100 mg) was homogenized amid 2 ml 0.1% (w/v) TCA by crushing in an ice chilled mortar. After centrifugation of the mixture at 12,000 RCF for 15 min at 4 °C, the 1 ml supernatant was homogenized with an equivalent volume of 10 mM KH_2PO_4 buffer at pH 7 and 2 ml of 1 M KI. The H_2O_2 was quantified by taking absorbance at 390 nm according to Velikova et al. (2000).

Estimation of lipid peroxidation

The extent of MDA produced during lipid peroxidation was evaluated for evaluation of the level of lipid peroxidation according to Rubin et al. (1976). Foliage sample (0.1 g) was vortexed with 1.5 ml of phosphate buffer (50 mM) at pH 7.4. This was followed by 15 min centrifugation at 15,000 RCF. Then 2 ml supernatant aliquot assorted with 4.0 ml of 0.5% thiobarbituric acid (TBA) in TCA (20 %) was supplemented followed by 15 min centrifugation at 15,000 RCF. The reaction mixture was geared up by mixing 2ml supernatant in 8 ml of 20% TCA (W/V) including 0.5% 2 TBA (W/V). The mixture was placed on hot water bath at 97° C for 0.5 h. Ice cool mixture was centrifuged for 10 min at 10,000 RCF. The optical density of supernatant was observed at 600 nm and deducted from optical density obtained at 532 nm.

Estimation of electrolyte leakage (EL) in leaves

Fresh sample (0.2 g) obtained from fully expanded top leaves was subdivided into 1 cm segments. After rinsing of segments three times with deionized water, they were immersed in 15 ml deionized water present in sterile glass tubes. The tubes were placed over a shaker at 25 °C for 24 h. The initial conductance (Ci) of segments was measured followed by autoclaving of the segment-containing tubes at 120° C for 0.5 h. The mixture containing leaf segments was cooled at 25 °C prior to calculation of maximum conductance (Cmax). The value of relative EL was deliberated as described by Li et al., (2018) by following formula:

Relative EL= (Ci/Cmax) 100

Activities of antioxidative enzymes

For assessment of nitrate reductase (NR) activity, the small sections of freshly harvested leaf sample was placed in phosphate buffer (pH 7.5) present in a plastic vial. Afterwards, isopropanol and potassium nitrate solutions were added and the vial was placed at 30 °C. After 1 h sulphanilamide and N-1-naphthylethylenediamine dihydrochloride were added in the mixture and spectrophotometry was performed at 540 nm (Jaworski, 1971).

Pro evaluation of CAT activity, 0.5 g foliage sections were homogenized with 1.5 ml of 100 mM KH_2PO_4 buffer (pH = 7) and centrifuged at 12,000 RCF for 20 min at 4 °C. The supernatant (35 µL) was mixed with 750 µL 50 mM KH_2PO_4 buffer and 465 µL 15 mM H_2O_2. The absorbance of solution was evaluated at 240 nm (Aebi, 1984).

For estimation of dehydroascorbate reductase (DHAR) activity, 0.5 g foliage sample was ground with 1.5 ml of 100 mM KH2PO4 buffer at pH = 7.0. Afterwards, 200 µL supernatant obtained through centrifugation of homogenate at 12,000 RCF for 20 min at 4 °C was mixed with 150 µL dehydroascorbate and 0.75 ml of phosphate buffer. The absorbance of reaction mixture was determined at 265 nm (Dalton et al., 1986). Foliage sample (0.5 g) was homogenized with 1.5 ml solution (0.3 ml H_2O, 1.18 ml CH_3OH, and 0.015 ml HCl) and centrifuged at 1500 RCF for 20 min at 4 °C. The spectrophotometric values of the supernatant were assessed at 651 and 530 nm (Mancinelli, 1984).

For the analysis of POD activity, 0.5 g foliage sections were crushed with 1.5 ml KH_2PO_4 buffer 100 mM (pH = 7). The homogenized samples were centrifuged at 12,000 RCF for 20 min at 4 °C. The resulting supernatant (50 µL) was mixed with 25 µL guaiacol solution, 1.5 ml of KH_2PO_4 buffer plus 15 µL H_2O_2. The optical density of solution was observed at 436 nm (Putter, 1974).

For evaluation of superoxide dismutase (SOD) activity, 0.5 g foliage sample was homogenized with 1.5 ml sodium carbonate buffer. After centrifugation of solution at 12,000 rpm for 20 min at 4 °C, the resulting supernatant was used to prepare reaction mixture. For this purpose, 35 µL centrifuged sample was mixed with

250 µL 24 µM NBT, 50 µL 0.1 mM EDTA, 50 µL 1 mM $HONH_2$·HCl, 50 µL 0.03% Triton-X-100, and 815 µL sodium carbonate buffer (pH = 10.2). The solution absorbance was observed at 560 nm Kono (1978).

filtrate. The mixture was placed for at 37 °C for 3 h. The solution was cooled at 25 °C, followed by supplementation of 3.1 ml H_2SO_4 (65%) and incubation for 0.5 h. The spectrophotometric values of solution were calculated at 520 nm (Roe

Table 1
Primers utilized for q PT-PCR

Gene	Accession Number	Primers Sequence (5'-3')
CsHA2	EU735752	F: ACCCGAGTCGACAAACATCT
		R: CTTGGCACAGCAAAGTGAAA
CsHA3	EF375892	F: AAGTTTCTGGGGTTCATGTGGAAT
		R: GTAACAGGAAGTGACTCTCCAGTC
CsHA4	HO054960	F: CTACAGCTTGGTAACATACATTC
		R: GTTGTAGTCCATGTAATGTCCTC
CsHA8	HO054964	F: CTCATGCGCAAAGAACATTAC
		R: CTGAATTGTGTCAATGTCAAGTC
CsHA9	HO054965	F: AAACCAGAAGTGCTGGAG
		R: CTCAGCACCCTCACTAGTAA
CsHA10	HO054966	F: GACATAATCAAGTTTGCAATCAGATA
		R: TTCTGTATAAGTTGTGCGGT

Estimation of non-enzymatic antioxidants

For estimation of proline contents, freshly harvested leaves were homogenized with sulphosalicylic acid. This homogenate was mixed with equal amount of ninhydrin and glacial acetic acid (v/v) and placed at 100 °C. Then toluene (5 ml) was added and spectrophotometric values were observed at 528 nm (Bates et al., 1973).

For estimation of glutathione, foliage sample (2 g) was mixed with 6 ml of 50 mM Tris buffer at pH 10 and 1 mM EDTA. The supernatant (50 µL) of the mixture obtained through centrifugation at 12,000 RCF for 15 min was mixed with 2 ml absolute methanol, 25 µl Ellman's reagent and 0.5 ml of Tris buffer. The solution was centrifuged at 3,000 RCF for 15 min and optical density was observed at 412 nm according to Sedlak and Lindsay (1968).

For assessment of ascorbate, 2 g fresh plant sample was mixed with 6 ml of 50 mM tris-buffer (pH 10) and 1 mM EDTA. The solution was subjected to centrifugation at 12,000 RCF for 15 min at 4 °C. The resulting 1 ml supernatant was blended with 0.2 g activated charcoal, 1 ml TCA (50%) and 4 ml double distilled water. This mixture was passed through Whatman No. 1 filter paper and 2,4-dinitrophenylhydrazine was added in 2 ml and Kuether, 1943).

For determination of tocopherol, the plant tissue was homogenized with 3 ml of 50 mM tris-buffer (pH 10.0) along with 1 mM EDTA. The homogenate was centrifuged at 12,000 RCF for 15 min at 4 °C. The vortex solution of distilled water, supernatant, ethanol, and xylene (1:1:1) was centrifuged at 12,000 RCF for 10 min at 4° C. The supernatant was then merged with 2,4,6-tripyridyl-s-triazine and absorbance of solution was measured at 600 nm Martinek (1964).

For estimation of total polyphenols, foliage sample (50 mg) was ground with 80% ethanol and 0.25 ml extract was dissolved in 0.25 ml 10% Na_2CO_3 and 0.25 ml Folin–Ciocalteau reagent and placed in dark for 60 min at 25 °C. Afterwards, the absorbance of reaction mixture was observed at 760 nm (Kumazawa et al., 2004).

Metal tolerance index

The metal tolerance index (MTI) will be measured as described by Shetty et al. (1995) according to the following formula:

$$\mathrm{MTI} = \frac{\mathrm{DWPS\ or\ DWPT}}{\mathrm{DWP - N}} \times 100$$

where DWPS = dry weight of plant under metal stress, DWPT = dry weight of antioxidant compounds supplemented plant, and DWP-N = dry weight of non-stressed/non-supplemented plants.

Auxins estimation

Leaf sample (1 mg) ground by ice chilled mortar and pestle was dissolved in 10 ml extraction solution (conc. HCl/, water/2-propanol, 0.002: 1:2, v/v/v) followed by addition of 1 ml dichloromethane. The solution was kept over a platform shaker operating at 100 rpm at 4 °C. After 0.5 h, lower phase of sample obtained through 5 min centrifugation at 13,000 RCF and 4 °C was dried with the help of nitrogen gas. The dried sample was mixed with methanol (0.1 ml) for determination of IAA (Pan et al., 2008; Ke et al., 2015). The IAA contents in the sample were calculated with the help of auxin ELISA kit (Sunlong Biotech Co., Ltd., Zhejiang, China). The values of optical density obtained at 405 nm were measured with the help of software Gen 5 Data Analysis (BioTek Instruments, Inc., USA) and compared with standard curve of known IAA concentrations.

Assessment of leaf ethylene production rate

The level of ethylene biosynthesis from top 3rd expanded leaf of seedlings was estimated according to Wilkinson and Davies (2009) with slight alteration. Leaf samples (1 g) collected during 30 min and 1, 5, and 10 days of treatments, were placed in 25 ml sealed glass flask and kept under 5000 Lx illumination at 25 °C. After 5h 1 ml gas sample obtained from flask head space by using disposable plastic syringe was injected in a gas chamber (Model: 5890 C, Agilent Technologies, Ltd. UK) for gas chromatography according to Zhang et al. (2019). The amount of ethylene emission was determined with reference to incubation period and fresh weight of the plant sample as described by Wilkinson and Davies, (2009).

Evaluation of gene expression

An identical experiment was performed using best stress mitigating concentrations of antioxidants to demonstrate their role in

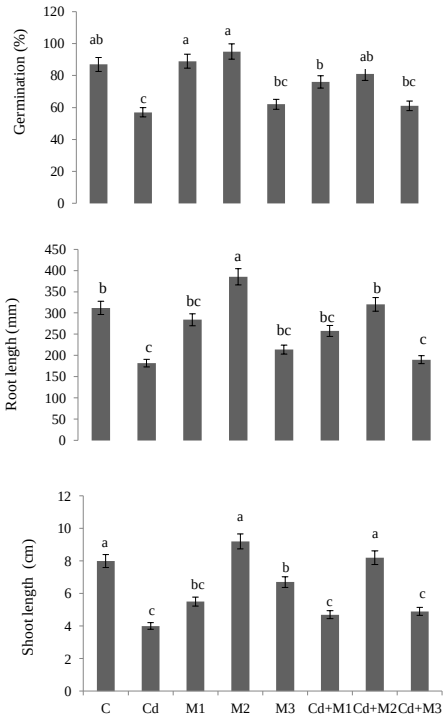

Fig. I. The effect of melatonin on seed germination rate, root length, and shoot length of *Cucumis sativus* seedlings under cadmium stress; values demonstrate means ± SD (n=3). Different letters above the bars indicate significant difference among the treatments (p≤0.05). C= un-contaminated control, Cd= contaminated control, M1= 50 µM melatonin, M2= 100 µM melatonin, M3= 150 µM melatonin, Cd + M1= 2.5 mM cadmium + 50 µM melatonin, Cd + M2= 2.5 mM cadmium + 100 µM melatonin, Cd + M3= 2.5 mM cadmium + 150 µM melatonin.

expression levels of some stress responsive genes in *C. sativus* seedlings grown in contaminated media. Total RNA from leaf samples obtained from 15 d old seedlings was extracted using a Tri reagent (Enzynomics, Korea) RNA Extraction Kit as per instructions of the manufacturer. The quantitative real-time PCR examination was executed with the help of Bio-Rad Real-Time PCR system (Bio-Rad, USA). The SYBR Green-based one step RT-PCR kit (Enzynomics, Korea) was used for PCR replications according to Guo et al., (2012). The genes including CsHA2, CsHA3, CsHA4, CsHA8, and CsHA9 are involved in encoding of activity of H^+-ATPase embedded in plasma membrane. The expression level of aforesaid genes from root tissues was evaluated using the primers enlisted in Table 1. The primer of a particular gene was

premeditated on the basis of the peculiar gene sequences by applying Primer 5 program. The gene expression was normalized by using gene encoding TIP41-like protein as a house keeping gene.

Results

Determination of growth attributes

During current the study, germination percentage of *C. sativus* was significantly decreased in seeds grown under metal stress. Cucumber seeds displayed sensitivity to cadmium and least germination percentage (57%) was demonstrated by those present in the contaminated media without melatonin supplementation. However, the melatonin primed seeds exhibited improved germination percentage in normal and metal contaminated regimes. Root length of plants grown in M2 was more pronounced in contrast to the seedlings supplemented with 50 µM melatonin. The lowest root length was also recorded in plants growing under cadmium stress without supplementation of melatonin. A similar trend was found in data showing root and shoot fresh and dry weight. (Fig. I). Nevertheless, higher values of root and shoot biomass were noted in treatments receiving M2 and M1, respectively. Significantly higher values for number of leaves, root diameter, and number of first lateral roots were recorded in case of cucumber seeds supplemented with M2 treatment in all cases (Table 2).

Determination of leaf water content, photosynthetic rate, and gas exchange parameters

During current study, leaf water content improved in both M2 (86%) and M3 (72%) treated seedlings as compared to seedlings subjected to Cd (49%). Melatonin application improved photosynthetic rate in both un-contaminated and contaminated plants (Fig. II). The M2 treatment showed 45% increase in photosynthetic rate as compared to the plants grown under Cd treatment. Furthermore, stress caused by Cd altered gas exchange attributes including stomatal conductance, intercellular CO_2 concentration, and transpiration rate. Melatonin application improved these gas exchange attributes in both normal and stress conditions (Fig. III). In addition, the leaf photochemical efficiency also improved in melatonin supplemented seedlings. The highest value of M2 treatment was shown by M2 treatment without supplementation of Cd.

Table 2
Effect of melatonin on number of leaves, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, root diameter and number of first lateral roots of *Cucumis sativus* seedlings under Cd stress.

Treatments	Growth traits						
	No. of leaves	Root FW (g plant^{-1})	Shoot FW (g plant^{-1})	Root DW (g plant^{-1})	Shoot DW (g plant^{-1})	Root diameter (mm)	Number of first lateral roots
C	6 ±0.41a	06 ± 0.35a	18 ± 0.58b	1.8 ± 0.01a	4.2± 0.02a	112± 0.02ab	15 ± 0.58a
Cd	3± 0.56c	3.2 ± 0.45ab	13 ± 0.63c	0.3 ± 0.21c	0.4± 0.06c	32 ± 0.02c	7± 0.31c
M1	6± 0.28a	6.4 ± 0.73a	20± 0.98a	1.9 ± 0.04a	4.3± 0.21a	134± 0.02a	16± 0.42a
M2	8± 0.34a	7.3± 0.52a	24 ± 0.72a	2.1± 0.31a	5.8± 0.51a	144± 0.02a	21± 0.56a
M3	4± 0.58b	4.4± 0.02b	18 ± 0.68b	0.7± 0.32b	2.7± 0.39b	78± 0.02c	15± 0.36a
Cd+M1	4± 0.45b	4.2± 0.14b	17 ± 0.58ab	0.4± 0.12bc	1.9± 0.78bc	112± 0.02ab	14±0.43ab
Cd+M2	7± 0.76a	6.3± 0.17a	18 ± 0.43b	1.6± 0.22a	4.7± 0.91a	124± 0.02b	13±0.24ab
Cd+M3	6± 0.34a	3.9± 0.28b	14 ± 0.21c	0.45± 0.22bc	1.2± 0.32bc	72± 0.02c	11± 0.32b

Values demonstrate means ± SD (n=3). Different letters indicate significant difference among the treatments (p≤0.05). C= uncontaminated control, Cd= contaminated control (2.5 mM cadmium), M1= 50µM melatonin, M2= 100µM melatonin, M3= 150µM melatonin, Cd + M1= 2.5 mM cadmium + 50µM melatonin, Cd + M2= 2.5 mM cadmium + 100µM melatonin, Cd + M3= 2.5 mM cadmium + 150 µM melatonin.

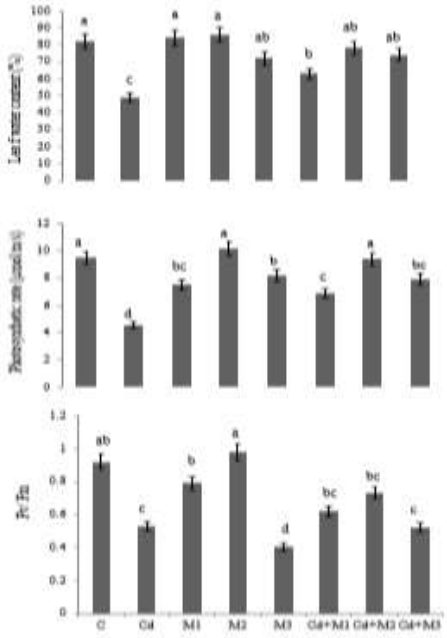

Fig. II. The effects of melatonin on leaf water content, photosynthetic rate, and photochemical efficiency of *Cucumis sativus* seedlings under cadmium stress; values demonstrate means ± SD (n=3). Different letters above the bars indicate significant difference among the treatments (p≤0.05). C= un-contaminated control, Cd= contaminated control, M1= 50 µM melatonin, M2= 100 µM melatonin, M3= 150 µM melatonin, Cd + M1= 2.5 mM cadmium + 50 µM melatonin, Cd + M2=2.5 mM cadmium + 100 µM melatonin, Cd + M3= 2.5 mM cadmium + 150 µM melatonin.

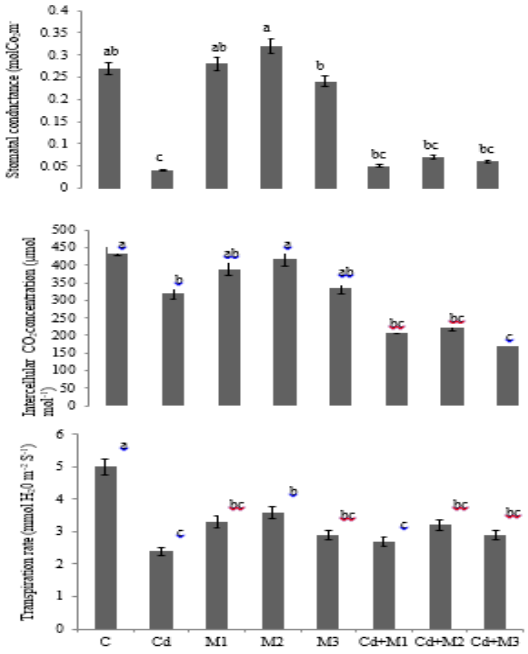

Fig. III. The effects of melatonin on the stomatal conductance, intercellular CO_2 concentration, and transpiration rate of *Cucumis sativus* seedlings under cadmium stress; values demonstrate means ± SD (n=3). Different letters above the bars indicate significant difference among the treatments (*P*≤0.05). C= un-contaminated control, Cd= contaminated control, M1= 50 µM melatonin, M2= 100 µM melatonin, M3= 150 µM melatonin, Cd + M1= 2.5 mM cadmium + 50 µM melatonin, Cd + M2=2.5 mM cadmium + 100 µM melatonin, Cd + M3= 2.5 mM cadmium + 150 µM melatonin.

Estimation of photosynthetic pigments and proteins estimation

A conspicuous decrease in the amount of photosynthetic pigments was revealed by seedlings under metal stress. M2 primed seeds expressed reasonably higher amount of chl. contents in cases of contaminated and non-contaminated situations. The protease activity and total soluble proteins were increased in plants subjected to cadmium stress. However, melatonin application further elevated these parameters in contaminated and non-contaminated cases (Table 5).

Estimation of H2O2, MDA content and electrolyte leakage

The present study revealed 49% increase in H_2O_2 content in Cd treated plants as compared to control. Melatonin supplementation significantly lowered H_2O_2 content in all *C. sativus* seedlings. A significant decrease of 47%, 43%, and 42% was observed in M2, M3, and Cd+M3 treatments, respectively as compared to Cd treated plants. Furthermore, melatonin treated plants decreased MDA content in all treatments as compared to Cd exposed plants. Both M2 and M3 un-contaminated concentrations significantly lowered MDA content as compared to Cd. A similar trend was observed while calculating electrolyte leakage in plants. Both M2 and M3 treatments decreased electrolyte leakage by 63% as compared to plants subjected to Cd (Fig. IV). Least value of electrolyte leakage (18%) was observed in M3 un-contaminated medium.

Activities of antioxidative Enzymes

Melatonin application significantly enhanced the activity of NR, CAT, APX, and POD. The activity of these antioxidative enzymes was

Table 3
Effect of melatonin on cadmium (Cd) uptake (µg g^{-1} DW) in root and shoot, translocation factor (TF), and metal tolerance index (MTI) in *Cucumis sativus* under cadmium stress

Treatments	Cd uptake			MTI
	Root (µg g^{-1} DW)	Shoot (µg g^{-1} DW)	TF	
C	ND	ND	ND	-
Cd	13546± 46a	639± 28a	0.02± 0.01c	11.67±1.26c
M1	0.31± 0.02e	0.21± 0.03c	0.67± 0.02b	103.4±9.73a
M2	0.41± 0.03e	0.37± 0.01c	0.90± 0.04a	132±11.28a
M3	0.19± 0.04e	0.12± 0.03c	0.63± 0.02b	57±4.92b
Cd+M1	10289± 32b	612± 40a	0.05± 0.01bc	38±2.86b
Cd+M2	09237± 24c	525± 23b	0.05± 0.01bc	105±8.74a
Cd+M3	08176± 12d	515± 12b	0.06± 0.02b	27±1.69c

Values demonstrate means ± SD (n=3). Different letters indicate significant difference among the treatments (p≤0.05). C= uncontaminated control, Cd= contaminated control (2.5 mM cadmium), M1=50µM melatonin, M2=100µM melatonin, M3=150µM melatonin, Cd + M1=2.5 mM cadmium + 50µM melatonin, Cd + M2=2.5 mM cadmium + 100µM melatonin, Cd + M3=2.5 mM cadmium + 150 µM melatonin

Table 4
Effect of melatonin on proline, glutathione, ascorbate, tocopherol, and total polyphenols of *Cucumis sativus* seedlings under Cd stress

Treatments	Non-enzymatic Antioxidants				
	Proline (µmol g^{-1} FW)	Glutathione (µmol g^{-1} FW)	Ascorbate (µmol g^{-1} FW)	Tocopherol (µmol g^{-1} FW)	Total polyphenols (µmol g^{-1} FW)
C	6 ± 0.41a	06 ± 0.35a	18 ± 0.58b	1.67 ± 0.01a	4.1± 0.02a
Cd	3± 0.56c	3.2 ± 0.45ab	13 ± 0.63c	0.29 ± 0.21c	0.39± 0.06c
M1	6± 0.28a	6.4 ± 0.73a	20± 0.98a	1.82 ± 0.04a	4.24± 0.21a
M2	8± 0.34a	7.3± 0.52a	24 ± 0.72a	2.14± 0.31a	5.79± 0.51a
M3	4± 0.58b	4.4± 0.02b	18 ± 0.68b	0.67± 0.32b	2.69± 0.39b
Cd+M1	4± 0.45b	4.2± 0.14b	17 ± 0.58ab	0.39± 0.12bc	1.68± 0.78bc
Cd+M2	7± 0.76a	6.3± 0.17a	18 ± 0.43b	1.58± 0.22a	4.65± 0.91a
Cd+M3	6± 0.34a	3.9± 0.28b	14 ± 0.21c	0.48± 0.22bc	1.13± 0.32bc

Values demonstrate means ± SD (n=3). Different letters indicate significant difference among the treatments (p≤0.05). C= uncontaminated control, Cd= contaminated control (2.5 mM cadmium), M1=50µM melatonin, M2=100µM melatonin, M3=150µM melatonin, Cd + M1=2.5 mM cadmium + 50µM melatonin, Cd + M2=2.5 mM cadmium + 100µM melatonin, Cd + M3=2.5 mM cadmium + 150 µM melatonin

significantly higher in M2 and M3 treatments as compared with that of M1 and in stress conditions. The activity of these antioxidant enzymes was less in contaminated control as compared with melatonin supplemented control. The activity of NR, CAT, POD, and APX significantly increased in seedlings growing under M2 and M3 treatments. The enhanced POD activity correlated with the enhanced melatonin concentrations. A significant higher CAT activity was observed in plants growing in M2 treatment contaminated with cadmium (Fig. V). Melatonin increased the antioxidant activity of CAT, POD, and APX in both contaminated and un-contaminated media. The contaminated medium supplemented with melatonin showed the highest activity of antioxidant enzymes. Least activity of CAT, POD, and APX was observed in un-contaminated control.

Estimation of non-enzymatic antioxidants

Another imperative observation of current study was disparity in the non-enzymatic antioxidants of seedlings subjected to metal stress under influence of melatonin. Seedlings treated with M2 hoarded utmost non-enzymatic antioxidants under stress. Furthermore, melatonin application enhanced the concentration of proline, glutathione, ascorbate, tocopherol, and total polyphenols in non-contaminated and contaminated seedlings (Table 4).

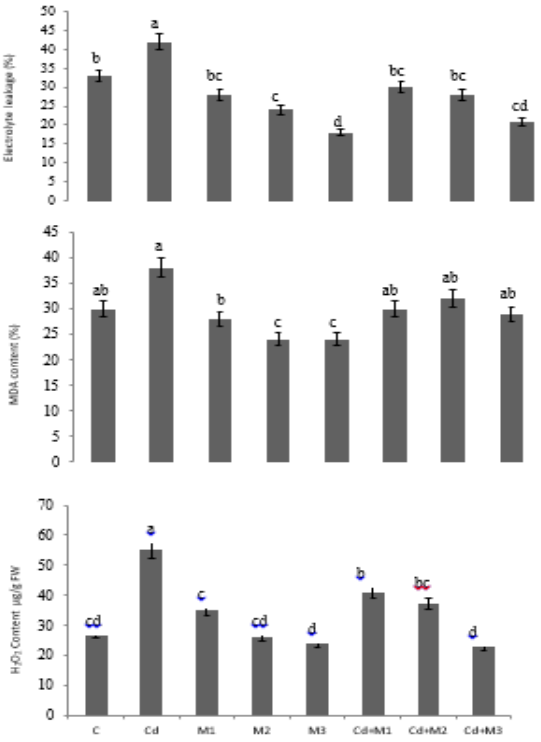

Fig. IV. The effects of melatonin on electrolyte leakage, MDA contents, and H_2O_2 contents of *Cucumis sativus* seedlings under cadmium stress; values demonstrate means ± SD (n=3). Different letters above the bars indicate significant difference among the treatments (p≤0.05). C= un-contaminated control, Cd= contaminated control, M1= 50 µM melatonin, M2= 100 µM melatonin, M3= 150 µM melatonin, Cd + M1= 2.5 mM cadmium + 50 µM melatonin, Cd + M2=2.5 mM cadmium + 100 µM melatonin, Cd + M3= 2.5 mM cadmium + 150 µM melatonin.

Estimation of cadmium uptake

All concentrations of melatonin reduced cadmium uptake and were effective in mitigating cadmium induced toxicity to some extent. However, M2 treatment exaggerated rest concentrations of melatonin. Shoot displayed less cadmium uptake as compared to root in all treatments. Maximum translocation factor was observed in M2 treatment followed by M1 and M3. The value of metal tolerance index (MTI) was higher in M2 treatment followed by M1 and Cd+M2 treatment (Table 3).

Estimation of auxin and leaf ethylene production

Indole acetic acid contents significantly reduced in plants subjected to cadmium stress.

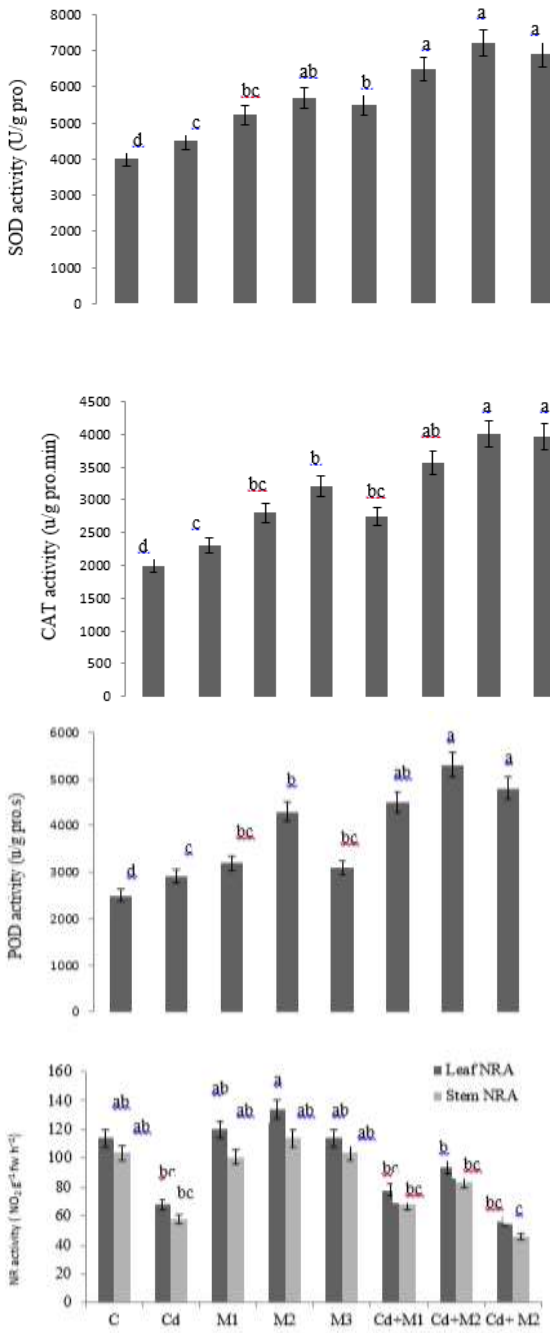

Fig V. The effects of melatonin on the superoxide dismutase activity, catalase activity, ascorbate peroxidase activity and nitrate reductase activity of *Cucumis sativus* seedlings under cadmium stress. Values demonstrate means ± SD (n=3). Different letters above the bars indicate significant difference among the treatments (*P*≤0.05). C= un-contaminated control, Cd= contaminated control, M1= 50 µM melatonin, M2= 100 µM melatonin, M3= 150 µM melatonin, Cd + M1= 2.5 mM cadmium + 50 µM melatonin, Cd + M2=2.5 mM cadmium + 100 µM melatonin, Cd + M3= 2.5 mM cadmium + 150 µM melatonin.

However, melatonin treatments in M1 and M2 improved IAA content by 53% and 34% in *C.*

sativus seedlings, respectively as compared to plants subjected to Cd. The minimum value of IAA production was shown by Cd. Nevertheless, the highest value of leaf ethylene production was observed in plants under Cd. Melatonin application lowered leaf ethylene production rate in all treatments as compared to the plants treated with Cd (Fig. VII).

Evaluation of gene expression

Different isoforms of the plasmic membranous H^+-ATPase genes including CsHA2, CsHA3, CsHA4, CsHA8, and CsHA9 exhibited higher expression level in seedlings subjected to Cd as compared with C. An increase in the expression of all stress responsive genes was observed in seedlings under M2 and M3 treatments. The least value of gene expression was observed in seedlings treated with Cd (Fig. VI). M2 and M3 raised the expression of all genes in both contaminated and un-contaminated media. The highest value of gene expression was observed in M2 treated without the supplementation of cadmium.

Discussion

Melatonin is a universal biochemical, present in almost all living organisms. The growth of cucumber seedlings subjected to cadmium stress was remarkably reduced. Latest research studies have implicated that melatonin acts as a growth stimulator which improves plants growth

Table 5
Effect of melatonin on chlorophyll a, chlorophyll b, total chlorophyll (mg g^{-1}), carotenoids, and protease activity (mg g^{-1} FW) activity

Treatments	Photosynthetic pigments and Proteins estimation					
	Chl a	Chl b	Total chlorophyll	Carotenoids	Protease enzyme	Total soluble proteins
C	0.73 ± 0.41ab	0.55 ± 0.35ab	1.28 ± 0.58ab	4.1± 0.02a	4.1± 0.02a	4.1± 0.02a
Cd	0.23± 0.56c	0.34 ± 0.45c	0.57 ± 0.63c	0.39± 0.06c	0.39± 0.06c	0.39± 0.06c
M1	0.82± 0.28a	0.62 ± 0.73a	1.44± 0.98a	4.24± 0.21a	4.24± 0.21a	4.24± 0.21a
M2	0.89± 0.34a	0.65± 0.52a	1.54 ± 0.72a	5.79± 0.51a	5.79± 0.51a	5.79± 0.51a
M3	0.79± 0.58a	0.52± 0.02ab	1.31 ± 0.68ab	2.69± 0.39b	2.69± 0.39b	2.69± 0.39b
Cd+M1	0.62± 0.45b	0.49±0.14b	1.11 ± 0.58b	1.68± 0.78bc	1.68± 0.78bc	1.68± 0.78bc
Cd+M2	0.69± 0.76b	0.42± 0.17b	1.11± 0.43b	4.65± 0.91a	4.65± 0.91a	4.65± 0.91a
Cd+M3	0.63± 0.34b	0.39± 0.28c	1.02 ± 0.21bc	1.13± 0.32bc	1.13± 0.32bc	1.13± 0.32bc

Values demonstrate means ± SD (n=3). Different letters indicate significant difference among the treatments (p≤0.05). C= uncontaminated control, Cd= contaminated control (2.5 mM cadmium), M1=50µM melatonin, M2=100µM melatonin, M3= 150µM melatonin, Cd + M1= 2.5 mM cadmium + 50µM melatonin, Cd + M2= 2.5 mM cadmium + 100µM melatonin, Cd + M3=2.5 mM cadmium + 150 µM melatonin.

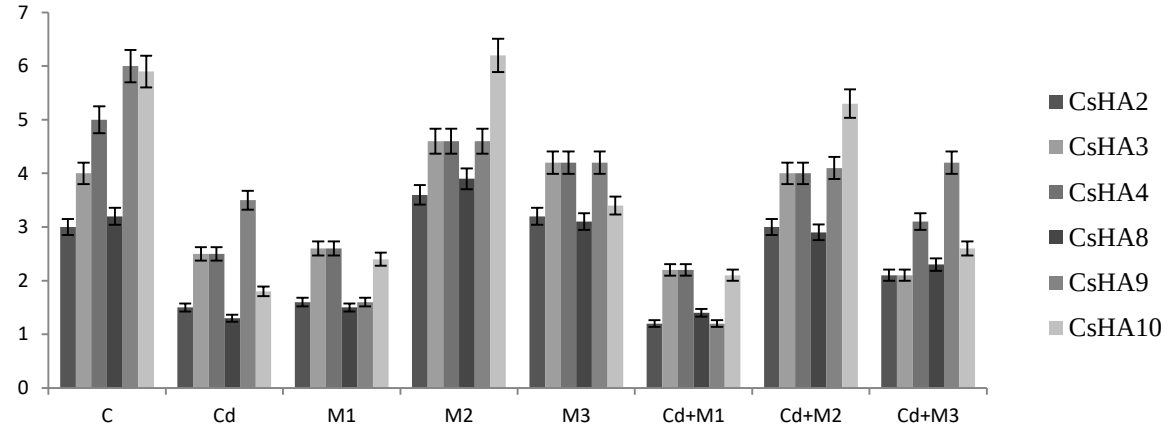

Fig. VI. The effect of melatonin on the expression level of CsHA2, CsHA3, CsHA4, CsHA8, CsHA9, and CsHA10 genes of *Cucumis sativus* seedlings under cadmium stress; values demonstrate means ± SD (n=3). C= un-contaminated control, Cd= contaminated control, M1= 50 µM melatonin, M2= 100 µM melatonin, M3= 150 µM melatonin, Cd + M1= 2.5 mM cadmium + 50 µM melatonin, Cd + M2=2.5 mM cadmium + 100 µM melatonin, Cd + M3= 2.5 mM cadmium + 150 µM melatonin.

and enables them to adopt various environmental calamities by alleviating temperature, drought, and salinity stress (Zhang et al., 2017; Ye et al. 2016; Li et al., 2012). During the current study, melatonin treated seedlings presented higher level of growth promoting hormones. Similarly, reduced amount of stress related biochemical such as MDA and ROS was observed in seedlings under influence of melatonin. Therefore, melatonin mitigated cadmium toxicity and improved markers encompassing growth of *C. sativus* seedlings.

Zhang et al. (2013) revealed that melatonin pre-treated germplasm stabilized chlorophyll architecture and enhanced root elongation and seed germination percentage in *C. sativus* under drought stress. Our results also indicated augmentation in photosynthetic rate, root length, shoot length, and biomass production in *C. sativus* exposed to melatonin levels under cadmium stress.

The reduced leaf relative water contents and higher values of osmotic regulators (proline and soluble sugars), observed during the current research in melatonin treated seedlings under stress are analogous to the findings of other researchers (Urano et al., 2009; Jiang et al., 2016). The improvement in leaf water contents by melatonin application confirms the role of this biomolecule in increasing water use efficiency, perhaps by regulation of ABA biosynthesis and reduction in the uptake and translocation of sodium ions (Jiang et al., 2016).

Melatonin is a crucial antioxidant that helps the plants to enhance their photosynthetic activity under abiotic stress circumstances (Li et al., 2019). Our results with reference to melatonin mediated amplification of photosynthesis through elevated level of chlorophyll contents; intercellular CO_2 concentration and stomatal conductivity are harmonious with Zhao et al. (2015). However, this improvement in photosynthetic activity of *C. sativus* by melatonin was in a dose dependent manner under identical stress conditions. Our findings are a signpost that M2 concentrations significantly enhanced the net photosynthetic rate compared to M1 under cadmium stress.

Heavy metal stress damages the membrane properties, occasioning the

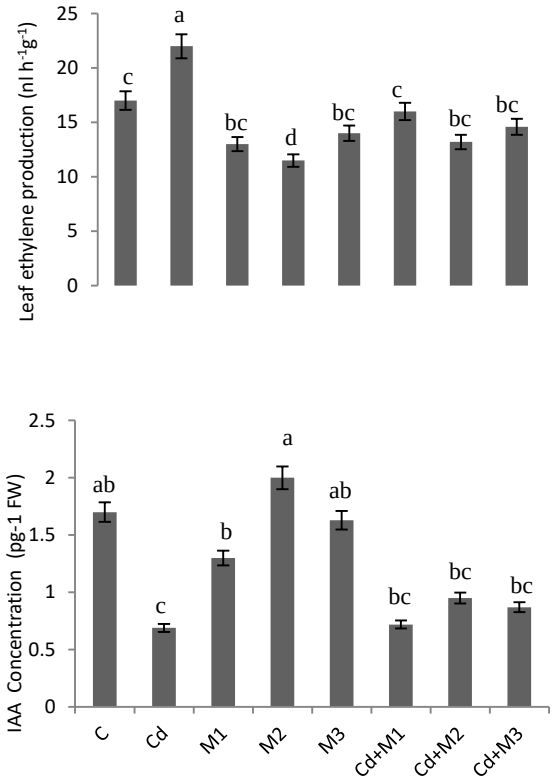

Fig. VII. Effect of melatonin on ethylene and IAA production in *Cucumis sativus* seedlings under cadmium stress; values demonstrate means ± SD (n=3). Different letters above the bars indicate significant difference among the treatments (p≤0.05). C= un-contaminated control, Cd= contaminated control, M1= 50 µM melatonin, M2= 100 µM melatonin, M3= 150 µM melatonin, Cd + M1= 2.5 mM cadmium + 50 µM melatonin, Cd + M2= 2.5 mM cadmium + 100 µM melatonin, Cd + M3= 2.5 mM cadmium + 150 µM melatonin.

delocalization of membrane protons associated with ATPase activity and affecting membrane potential, thereby plummeting Mg^{2+} uptake which leads to suppression of chlorophyll synthesis. Preceding studies designated that melatonin priming enhanced photosynthetic rate and gas exchange efficiency under drought conditions (Ye et al., 2016). Melatonin application enhanced chlorophyll stabilization in melon seedlings similar to our findings under heavy metal stress (Fan et al., 2015). Melatonin application had a strong potential to stabilize chlorophyll membranous structure under cold stress. Furthermore, it improved the net photosynthetic rate, biomass production, light capturing potential and ultimately reduced the impact of cold stress in melon seedlings (Zhang et al., 2017).

A number of researchers have reported that PSI and PSII in plants are the dominant locations for biosynthesis of ROS under harsh environmental conditions (Gill and Tuteja, 2010). D1 protein of PS II is regarded as a crucial bio-marker for alleviation of environmental stress in plants (Chen et al., 2016; Su et al., 2014). Similarly, our results indicate that enhanced production of D1 and other stress-tolerance related proteins might be involved in the stability of photosynthetic apparatus. Moreover, melatonin application abridged the breakdown of photosynthetic membranes under abiotic stress conditions (Shi et al., 2015). Likewise, current results indicated that melatonin enhanced photosynthetic activity in contrast to untreated plants.

Numerous plants have developed remarkable strategies to reduce stress-induced bioaccumulation of ROS. Melatonin is a multi-dimensional antioxidant which has accomplished an ability to scavenge ROS (Shi et al., 2015). The enhanced production of ROS under stress conditions led to the malfunctioning of cell membrane and ultimately caused plants death. The production of hydrogen peroxide and $^{\cdot}OH$ is effectively reduced by the exogenous application of melatonin (Li et al., 2012). Nevertheless, exogenous melatonin reduced ROS accumulation in maize seedlings resulting in the stress reduction (Smirnoff, 1993). The higher melatonin concentration during current study proved more effectual in reducing the biosynthesis of ROS. Consequently, melatonin application has a scavenging effect in reducing ROS by increasing the activity and up-regulation of various antioxidative enzymes. The up-regulation of antioxidant enzymes activities in melatonin treated seedlings during current study scavenged ROS and enhanced tolerance to cadmium stress.

The higher peroxide level is the key rationale for growth retardation in plants facing environmental toxicants. Hydrogen peroxide is the principal peroxide that reduces the net photosynthetic rate and restrains the activity of antioxidant enzymes (Hancock et al., 2005). Consistently, these oxygen free radicals are highly damaging to thylakoid membrane stability and suppress protein formation through Haber Weiss mechanism (Gill and Tuteja, 2010). Hence, it is necessary to keep these hazardous chemicals within a tolerance range in plants.

Malondialdehyde content and electrolyte leakage indicate the degree of susceptibility to oxidative stress (Zhang et al., 2014). A lower quantity of EL and MDA was found in seedlings treated with melatonin under abiotic stress conditions, in conjunction with smaller quantity of ROS production. This decrease in ROS proposes the positive part of melatonin in continuation of structural stability of cell membrane against damage caused by oxidative stress (Jiang et al., 2016). In our studies, melatonin treated *C. sativus* seedlings maintained health index by reducing ROS under cadmium stress.

Melatonin enhances the production and activity of antioxidant enzymes in plants subjected to various environmental regimes. The lower concentration of melatonin resulted in minor modification in the activity of antioxidant enzymes of cucumber seedlings although the higher concentration of exogenously applied melatonin displayed a pronounced impact on regulation and improvement of the activity of some antioxidative enzymes including APX and CAT. Some other researchers have also observed matching response in case of various plants supplemented with different concentrations melatonin (Shi et al., 2015).

Higher activity of superoxide dismutase, ascorbate peroxidase, and peroxidase has been demonstrated in plants by exogenous application of Melatonin (Li et al., 2012; Zhang et al., 2014; Jiang *et al.,* 2016). It is well-documented that glutathione ascorbate cycle is a crucial defensive strategy regarding stress tolerance in plants (Zhang et al., 2015). The melatonin supplemented tomato seedlings showed higher amount of soluble solids, lycopene, ascorbate, phosphorous, and quality yield (Wen et al., 2016).

Nitrate reductase is a molybdoenzyme pyranopterin derivative involved in nitric oxide (NO) production (Mur et al., 2013). Nitric oxide manages a number of physiological functions including seed emergence, stomatal movements, apoptosis, root/shoot length, and mitigation of plant stress (Farnese et al., 2016; Gupta et al., 2011).

The preserved structural core of this molybdopterin is absent in nitrogenase (Fischer et

al., 2005). The ordinary site for cadmium binding is OsNR- cadmium. However, cadmium makes complex with OsNR-nitrate-cadmium and severely affects the activity of NR (Seenivasagan et al., 2016). The current study exhibited that melatonin enhanced NR activity under cadmium stress. Our results in this regard are congruent with findings of Sing et al. (2018).

Melatonin treatment stabilized the membrane structure, augmented bioactivity of peroxidase, superoxide dismutase, and peroxidase, and decreased malondialdehyde contents in *Cucurbita melo* (Arnao and Hernández-Ruiz, 2015; Zhang et al., 2015).

The regulation of hydrogen peroxide level by glutathione swimming pool activity is a crucial factor for alleviation of plant stress. Turk et al. (2014) found that melatonin treated wheat seedlings enhanced activity of glutathione swimming pool, superoxide dismutase and ameliorated the level of ascorbate. Likewise, Marta et al. (2016) found that melatonin application in cucumber seeds augmented bioactivity of superoxide dismutase. Based on our findings, melatonin enhanced the bioactivity of ascorbate peroxidase and superoxide dismutase in *C. sativus* under cadmium stress. The current data revealed that melatonin counterbalanced H_2O_2 overproduction by increasing the activity of ascorbate peroxidase, superoxide dismutase, glutathione peroxidase, and glutathione reductase in *C. sativus* seedlings under cadmium impact. Some other researchers have also reported higher quantities of glutathione and ascorbate in melatonin treated plants (Shi et al., 2015; Wang et al., 2013).

This self-triggering mechanism for higher melatonin bioaccumulation is an expeditious mechanism of enhancing resistance to environmental hazards. Tan et al. (2011) reported that most of the plant varieties including corn, rice, wheat, barley, and oat have diverse amount of melatonin in their tissues. Tan et al. (2007) observed that melatonin supports plants in scavenging uncharged molecules and regulating many enzymes involved in senescence.

Cadmium phytotoxicity can be decreased by the application of melatonin. The mechanism involves chelation of metal ion with metal ligands and other organic compounds I.e., organic acids, proteins, and polysaccharides. Those organic compounds that are not chelated are transferred to other crucial organelles resulting in non-functioning of cellular structures (Li et al., 2016). Melatonin-iron complex prevents the catalysis of injurious reactions such as regulation in the level of oxygen free radicals in plants under abiotic stress (Maharaj et al., 2007). During the current study, melatonin reduced the uptake of cadmium in roots and shoots.

Melatonin assists in metabolism of plant growth promoting hormones such as auxins, cytokinins, and gibberellins. Melatonin and auxins have a synergistic effect on physiological functions and metabolic pathways of a number of plants (Hernández Ruiz et al., 2005). Recent studies have acknowledged the pivotal role of this compound in mitigation of abiotic stresses (Li et al., 2017). Furthermore, it monitors biosynthesis of Abscisic acid, jasmonic acid, ethylene, and ethylene related proteins such as NR and ETR4.

Melatonin regulates ethylene biosynthesis in plant organs (Sun et al., 2014). This biomolecule up-regulates the expression of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase resulting in small increase in ethylene synthesis along with up-regulation of ethylene transducing and receptor genes including EIL1, EIL3, ERF2, NR, and ETR4, respectively. Furthermore, melatonin was capable of reducing 65% ethylene generation in lupine plants under stress (Arnao and Hern-Andez-Riiz, 2007). Melatonin modulates IAA synthesis which in return up-regulates the expression of ACC synthase resulting in reduced expression of ACC oxidase in mung bean and *Arabidopsis* (Weeda et al., 2014; Kim et al., 2001).

Melatonin may modulate expression level of about 320 genes involved in root formation of *C. sativus*. Ethylene transcription factors may alter the expression level of these root related genes causing poor root growth in plants. However, melatonin application administers the expression of these genes and promotes root growth in *C. sativus* (Zhang et al., 2014).

Rice seedlings struggle to alleviate cadmium toxicity by over-expression of methytransferase, hydroxylase, and tryptophan decarboxylase genes involved in biosynthesis of melatonin (Byeon et al., 2015). Wei et al. (2014)

found that melatonin regulated genes involved in ascorbic acid metabolism, photosynthesis, carbohydrate metabolism, and cell division. These molecular and biochemical changes significantly stemmed higher aggregation of seed pods and fatty acid contents improving leaf size and plant weight in melatonin applied *Glycine max* plants. Our data revealed intonation in expression level of genes involved in stress resistance of *C. sativus* plants under different conditions. The expression of genes including CmCAT, Cm POD, and CmSOD enhanced at 100 µM. Previous finding also advocated that melatonin application enhances the expression of stress responsive genes resulting in enhanced activities of antioxidants in plants (Mayo et al., 2002).

Additionally, it is supposed that metal tolerance proteins family (MTP) involved in cation diffusion facilitation, assist in regulation of osmotic movement of metal ions in plants. During current study, up-regulation in the level of *MTP8* was found in plants under cadmium stress. Therefore, it is assumed that MTP8 may have an authoritarian role in cytosolic Cd-efflux. Talke et al. (2006) has also reported up-regulated level of MTP8 in *Arabidopsis halleri* plants under cadmium stress. Cadmium contamination causes malfunctioning of cytoplasmic membrane and enhance deposition of cadmium ions in plant tissues. The proton gradient through H^+-ATPase supports active efflux of cadmium and other biochemicals from cytoplasmic membrane, helping in alleviation of cadmium stress. The higher expression level of CsHA genes during current study may lead to enhancement of membranous proton pump in plants under stress.

Conclusion

The present study revealed that melatonin ameliorated cadmium toxicity in *C. sativus* seedlings by intonating the activity of *antioxidative scavenging enzymes*. Furthermore, melatonin enhanced the production of non-enzymatic antioxidants involved in mitigation of oxidative stress. The regulatory role of melatonin in biosynthesis of stress markers such as photosynthates, indole acetic acid, glutathione, and malondialdehyde conferred metal stress alleviation potential of this phytohormone in *C. sativus* seedlings. Current findings advocate the application of melatonin in cucumber crop growing in cadmium contaminated area. Similarly, use of melatonin in some other crops subjected to various stress factors may also be exploited to improve crop yield and quality. The identification and gene pyramiding of desirable stress related genes will assist in development of abiotic stress tolerant crop varieties. Correspondingly, auxiliary acquaintance with epistasis and interaction of genes with other biomolecules is essential in administering the development and management of abiotic stress in plants.

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