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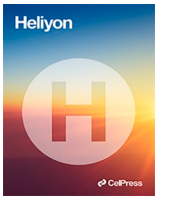

Exogenous melatonin enhances low-temperature stress of jute seedlings through modulation of photosynthesis and antioxidant potential

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ABSTRACT

Developing new varieties of natural fibers that can grow throughout the year is very crucial to replace and avoid the bad effect of synthetic fiber. As a result of its beneficial role in protecting plants from abiotic stressors, melatonin (N-acetyl-5-methoxytryptamine) has gained recognition as a novel plant growth regulator. This study aimed to investigates the role of exogenous melatonin (200 μM) on two varieties of *Corchorous olitorius* and *Corchorous capsularis* in response to low-temperature stress (8 °C) for different periods of treatment (0, 24, 36, and 48 h) based on biochemical properties, and antioxidant system. The results demonstrated that exogenous melatonin had inhibitory effects of low-temperature stress on seedlings at different period of treatment when compared to non-melatonin treated seedlings, potentially improved photosynthetic apparatus (total chlorophyll up to 29.93 and 33.37%; total carotenoid up to 29.93 and 19.05%; anthocyanin up to 40.47 and 31.94% in M33 and Y49, respectively), reduced oxidative damage (MDA up to 53.59 and 44.28%; H_2O_2 up to 41.04 and 16.88% in M33 and Y49, respectively) by boosting the antioxidant enzymes (SOD up to 12.75 and 4.65%; POD up to 39.08 and 81.39%; total phenolic up to 43.38 and 56.48% in M33 and Y49, respectively) reduced electrolyte leakage (EL) up to 15.37 and 13.64% in M33 and Y49, respectively) and increased osmoregulation (soluble sugars up to 25.86 and 25.86%; proline up to 105.19 and 172.07%; FAA up to 48.50 and 30.06% in M33 and Y49, respectively) content. Thus, this study showed that exogenous melatonin effectively mitigated the low-temperature-induced oxidative in *C. olitorius* and *C. capsularis* seedlings by regulating the antioxidant system and improving the low-temperature resistance.

1. Introduction

Plants are exposed to multiple biotic and abiotic stresses during their life cycle. Low-temperature stress is regarded as one of the greatest environmental threats to plant distribution, crop growth, survival, and production in agriculture because of its detrimental effects on plant growth and development [1–3], notably in temperate regions and high-altitude environments [4]. It inhibits a wide

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range of physiological processes in plants by directly influencing various metabolic reactions such as membrane fluidity, enzyme activities, and metabolism homeostasis, while osmotic and oxidative stresses indirectly [2,5]. Photosynthetic apparatus is directly affected by low-temperature stress, primarily by inducing photoinhibition at both photosystem I (PSI) and photosystem II (PSII) [6]. Reactive oxygen species inhibits plant growth by causing lipid peroxidation and protein oxidation within cells [7,8]. Therefore, plants have adopted a robust antioxidant defense system consisting of both enzymatic and non-enzymatic antioxidants to protect cells from the adverse effects of ROS [9–12]. Plants' low-temperature tolerance molecular mechanisms are reasonably well recognized, and recent research has predominantly focused on strategies for enhancing plant resistance to low-temperature stress [13,14].

Since melatonin was first detected in plants in 1995, and it is found in practically all plant organs, including leaves, flowers, fruits, seeds, stems, and roots [15,16] and regulator which also performs various physiological functions in plants and enhances plant tolerance to abiotic stress factors [17–19]. Melatonin enhances the plant's resistance against various environmental stresses like oxidative stress, salt, cold, drought, leaf senescence, heavy metals and drought [20]. Under different abiotic stresses, melatonin enhances the detoxification of a variety of free radicals and reactive oxygen species (ROS) including hydroxyl radical ($OH^{\bullet}$), peroxynitrite anion (NO_3^-), singlet oxygen ($^1O_2^{\bullet-}$) and nitric oxide ($NO^{\bullet}$) through metabolites [21] and modulates the different enzymatic/non-enzymatic antioxidant systems including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GSH-PX), phenolics and flavonoids [22–24] and redox status [25]. In recent years, melatonin's significance in abiotic stress responses in many species has been well-studied, but little is known about the effects of melatonin on jute plants. Tossa jute (*Corchorus olitorius* L.) is a major lingo-cellulosic natural, annual, herbaceous bast fiber crop that produces biodegradable, eco-friendly long and shiny fibers next to cotton [26]. Like other crops, jute plants experience a profoundly suppressive influence on their physiology and biochemistry when exposed to low temperatures [27]. It has been reported that different biological reactions are influenced by temperature in jute, and it has been found that early planting results in premature blooming and stunts plant development and fiber yield due to thermal sensitivity. It was reported that some varieties can be planted early with the absence of premature flowering in appropriate sowing time [28]. In the subtropical regions of China, where low-temperature weather is unpredictable, extending the cultivation season (early planting and late harvesting) is important. In jute-producing countries, extending the growing season would be more profitable into late March and early April [29,30]. There was little research on low temperature research on jute. In a previous research carried out to distinguish cold-tolerant and cold-sensitive jute variety at molecular level using DNA fingerprinting [30]. In another study, it was screened out the low temperature tolerant and low temperature sensitive varieties based on physiological and biochemical traits [27]. There is currently no research on the effect of melatonin treatment on cultivated *C. olitorius* and *C. capsularis* at low temperature stress. Considering the significance of natural fiber obtained from jute and extending the growing areas, this study was carried out to determine the effect of exogenously applied melatonin on *C. olitorius* and *C. capsularis* at low-temperature stress. The main focus of this study was on physiological and biochemical changes in the plants when leaves were pre-treated with melatonin prior to low-temperature stress, where ROS production, lipid peroxidation, photosynthesis activity, different enzymatic and non-enzymatic antioxidant activities were evaluated. The results could be helpful in understanding the physiological functions of melatonin in plants under low temperature stress.

2. Plant materials and experimental design

Seeds of M33 variety of *C. olitorius* and Y49 variety of *C. capsularis* were germinated in a growth medium containing three layers of sterile filter paper in the germination chamber. Sprouted seedlings for 3 days were transferred into plastic culture pots (10 × 10 cm) containing a mixture of peat and vermiculite (3:1,v/v) that also added quarter-strength Hoagland nutrient solution which was prepared by the previously described method for subsequent growth [31]. The seedlings were nurtured in a controlled environment with a temperature range of 28/16 °C, photoperiod of 16 h/8 h (light/dark), relative humidity of 60%, and 250 μmol $m^{-2}s^{-1}$ of light intensity. The water was continuously applied every other day. The melatonin solution were made by dissolving the substance in ethanol and then diluting it with phosphate-buffered saline (PBS) to concentrations of 200 μM, as described by Li et al. [32]. At the 3–4 leaf stage, seedlings were sprayed once daily with 200 μM melatonin solution and distilled water as a control for 5 days. After that, plants were grown under low-temperature stress while others were grown normally as a control. This procedure developed four distinct plant experimental groups: (i) Control + Water: raised at normal temperatures, sprayed with distilled water; (ii) Control + Melatonin: raised at normal temperatures, sprayed with 200 μM melatonin solution; (iii) Low-temperature stress: seedling were kept in the chamber by maintain 8 °C temperature for 48 h; (iv) Low-temperature stress + Melatonin: seedling kept in the chamber by maintain 8 °C temperature for 48 h, sprayed with 200 μM melatonin solution. Leaf samples were collected at 0, 12, 24, 36, and 48 h for MDA, H_2O_2, electrolyte leakage, and antioxidant enzymes analyses. Each leaf sample contained top 3 leaves a minimum of three individual plants of similar variety that were mixed to form one sample. Three biological replicate of each treatment of both species was performed at every harvest for further analysis.

2.1. Chemicals and equipments

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), polyvinylpyrrolidone, sulfosalicylic acid, ninhydrin, glacial acetic acid, and toluene were provided from Tianjin Section Co., Ltd (Tianjin, China). L-proline and HCl (HPLC grade) were obtained from Shanghai Macklin, China. Melatonin obtained from Sigma-Aldrich, St. Louis, USA. All solvents and reagents were analytical grade, and all aqueous solutions were developed using double distilled water.

The water-bath sonicator (KQ5200DE, 40 kHz) and Microplate Reader (Tianjin, China) were obtained from Kemio Chemical Reagent Co., Ltd. Whereas, spectrophotometer 2700 made by Shimadzu, Japan was used in this study. A Millipore system collected from

ULTRA Scientific (North Kingstown, USA) was used to obtain ultrapure water.

2.2. Determination of photosynthesis activities

Total chlorophyll content and carotenoid content were measured by assessing the absorbance at 645, 663, and 470 nm in a spectrophotometer according to the earlier reported formulae [33]:

Total chlorophyll (mg/g leaf fresh weight) = [20.2 (OD645) + 8.02 (OD663)] × V/1000 × W

Carotenoid (mg/g leaf fresh weight) = [OD470 + (0.114 * OD663) – (0.638 * OD645)] × V/1000 × W

According to previously described procedure [34], the chlorophyll stability index (CSI) was estimated and calculated as follows: CSI = (Total Chl under stress/Total Chl under control) × 100.

2.3. Determination of H_2O_2, MDA, electrolyte leakage, SOD and POD activities

The activities of Malondialdehyde (MDA), Hydrogen peroxide (H_2O_2), superoxide dismutase (SOD), and peroxidase (POD) were calculated to determine the extent of plasma membrane degeneration and lipid peroxidation [35]. In short, 0.2 g of fresh leaf samples were extracted by ground in 5 mL of 0.5% thiobarbituric acid (TBA) dissolving 5% trichloroacetic acid (TCA) for MDA and H_2O_2 content determination; and for the determination of SOD and POD activities homogenized in 5 mL ice-cold 0.2 M phosphate buffer (pH 7.0–7.5) containing 0.1 mM EDTA and 2% polyvinylpyrrolidone (w/v), respectively. MDA, H_2O_2, SOD, and POD assay kits were collected from Nanjing Jian Cheng Bioengineering Institute, Nanjing, China to determine the activities. According to the earlier approach, protein concentration was calculated using bovine serum albumin (BSA) as a reference protein [36].

Following the procedure outlined by the researcher [37], calculated electrolyte leakage (EL) indicates cell membrane damage. The EL was calculated using the following equation: EL (%) = $EC_1/EC_2 \times 100$.

2.4. Determination of non-enzymatic antioxidant compounds

Total phenolic concentration was calculated using a modified Folin-Ciocalteu colorimetric method [38]. A spectrophotometer measured the solution's absorbance at 765 nm against a reagent blank. Total phenolic content was expressed in milligrams of gallic acid equivalent mg of GAE/g FW).

Anthocyanin concentrations were determined by extracting about 0.03 g of fresh leaf samples in 2 mL of methanol-HCl (1% HCl, v/v) and shaking the vials at random intervals for the next 48 h at 4 °C temperature. The supernatant was subjected to a vortex, filtered, and spectrophotometric analysis at 530 nm and 657 nm (UV-1280, Shimadzu, Kyoto, Japan). The anthocyanin content was assessed according to Mancinelli et al. [39].

2.5. Determination of proline, soluble sugars, and free amino acid

Proline content was measured using a previously observed method [40]. Afterwards homogenization of fresh leaf samples with 3% aqueous sulfosalicylic acid, the mixture was extracted with acid ninhydrin, glacial acetic acid, and toluene. Then, at 520 nm, absorbance was measured with a spectrophotometer, while toluene served as the standard. A standard curve was drawn using an L-proline reference solution, and the proline content was determined from the slope of the line.

The soluble sugar content was measured following the previously published anthrone method [41]. Soluble sugar concentrations were measured by comparing the absorbance at 630 nm to a reference glucose solution.

According to Lee & Takahashi. [42], the ninhydrin reagent method was utilized to determine the total free amino acid content, and the absolute absorbance was measured at 570 nm.

2.6. Statistical analysis

Three replications were used to estimate all determinations. The statistical tool SPSS 22.0 (SPSS, Chicago, USA) was utilized to perform a one-way analysis of variance (ANOVA). The LSD test was utilized to compare treatment means and find a statistically significant difference ($p > 0.05$). Pearson's correlation was utilized to analyze the correlation coefficient.

3. Results

3.1. Effect of exogenous melatonin on photosynthesis under low-temperature stress

To determine the function of exogenous melatonin under low-temperature stress, the authors initially focused on phenotypic changes of *C. olitorius* and *C. capsularis* seedlings. Under standard conditions for both varieties, no significant changes or effect was observed in the total chlorophyll and carotenoid content after melatonin pretreatment for 5 days. Low temperature significantly reduced total chlorophyll content by 20.48–33.50% in M33 variety and 9.91 to 30.0 % in Y49 variety compared to control (Figs. 1a and 2a). Carotenoid content reduced by 13.42–34.05% in M33 and 6.51–21.71% in Y49 variety relative to control (Figs. 1b and 2b). But,

foliar application of 200 μM exogenous melatonin in low-temperature stress-treated plants significantly increased the total chlorophyll increased by approximately 4.36/10.08%, 22.90/27.06%, 29.93/33.72%, 22.45/1.36%, and carotenoid content was increased by 3.54/0%, 22.49/10.97%, 34.13/19.05%, 34.13/7.34%, respectively, for M33/Y49 at 12 h, 24 h, 36 h, 48 h in relative to stress. Interestingly, in *C. olitorius,* the reduction and exogenous elevated total chlorophyll and carotenoid content were higher compared to *C. capsularis*. In 24 and 36 h of treatment the melatonin alleviated total chlorophyll and carotenoid content was higher compared to other treatments.

3.2. Impacts of melatonin on malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and electrolyte leakage (EL) under low-temperature stress

The histochemical examination revealed that ROS concentration remained nearly constant in both varieties, even when exogenous melatonin was administered under control situations. On the other hand, the low-temperature treatment led to dramatic elevations in ROS (H_2O_2 and MDA) accumulation. At different levels of low-temperature stress, the MDA concentrations increased from 40.92 to 225.55% in M33 and 201.18–731.01% in the Y49 variety compared to control (Figs. 3a and 4a). It has been demonstrated that melatonin treatment caused the greatest reduction of low temperature-induced ROS accumulation resulting in approximately 39.92/11.82%, 53.59/44.28%, 41.35/37.65%, and 49.05/38.79% for M33/Y49, respectively at 12 h, 24 h, 36 h, and 48 h in relative to stress. Like MDA, H_2O_2 content significantly increased by low-temperature stress from 62.29 to 276.81% in M33 variety and 52.19–74.79% in Y49 variety (Figs. 3b and 4b). It has been recorded that melatonin treatment reduced the low temperature-induced H_2O_2 accumulation by approximately 15.20/16.33%, 41.04/12.73%, 32.03/15.08%, and 35.33/16.87% respectively, for M33/Y49 at 12 h, 24 h, 36 h, and 48 h in relative to stress. EL increased from 7.68 to 41.18% in M33 and 20.08–52.74% at a different level of low-temperature stress

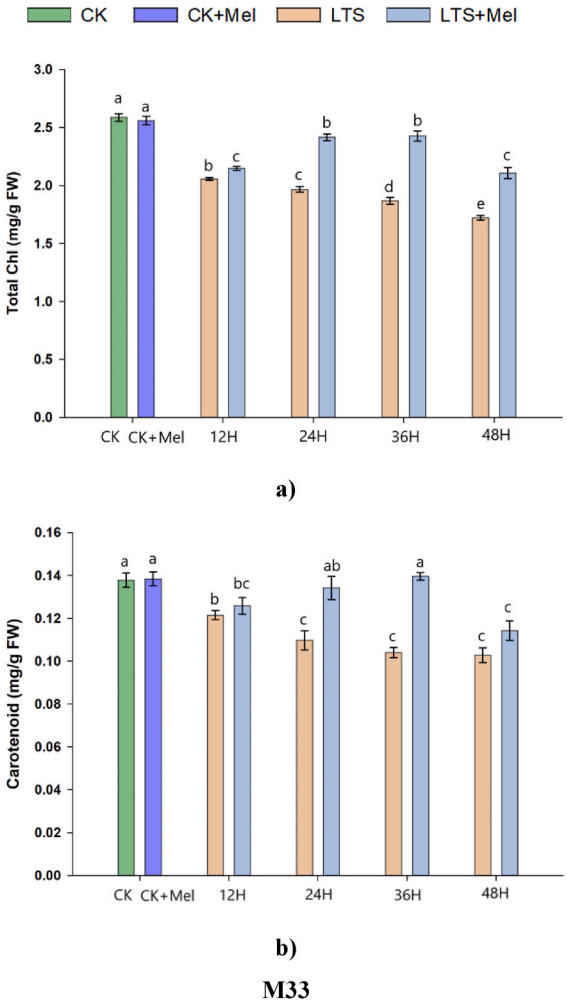

Fig. 1. Effect of low-temperature stress and exogenous melatonin treatments osmolyte content in total chlorophyll and carotenoid content in M33 variety. The values presented are means ± SD (n = 3). Significant difference is exhibited by different letters above the bars at $p < 0.05$ among treatments. (CK:Control, Mel:Melatonin, LTS:Low-Temperature Stress).

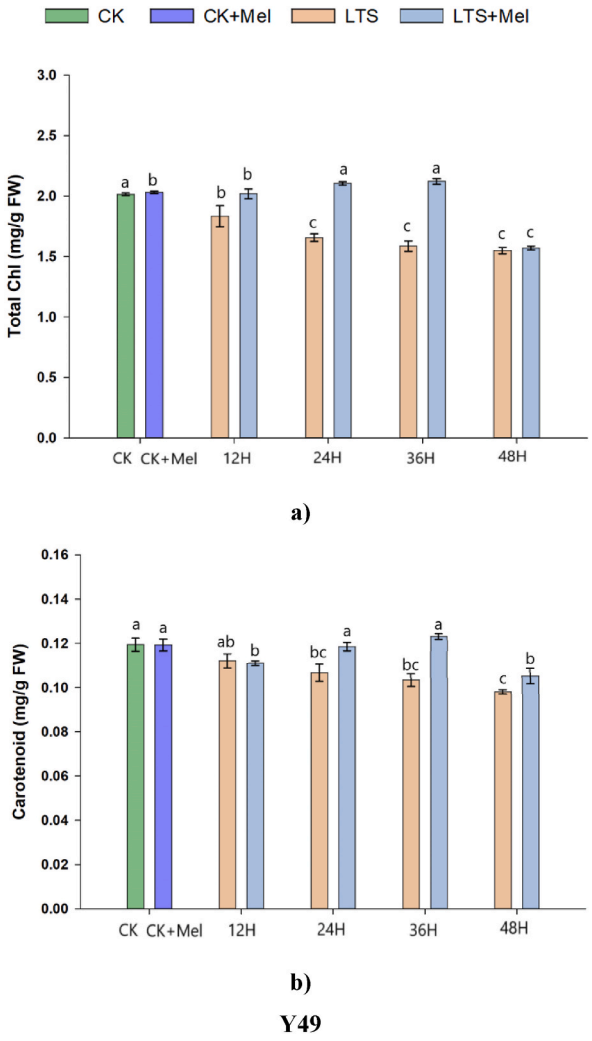

Fig. 2. Effect of low-temperature stress and exogenous melatonin treatments osmolyte content in total chlorophyll and carotenoid content in Y49 variety. The values presented are means ± SD (n = 3). Significant difference is exhibited by different letters above the bars at $p < 0.05$ among treatments. (CK:Control, Mel:Melatonin, LTS:Low-Temperature Stress).

compared to control (Figs. 3c and 4c). But, in the treatment of melatonin, the EL level increased approximately 5.10–15.37%, for M33 and 10.32–13.64% at a different level of treatment relative to stress.

3.3. Effects of exogenous melatonin on activities of enzymatic antioxidants at low-temperature stress

To investigate the melatonin's role in regulating plants' antioxidative system during low-temperature stress, the SOD and POD activities were analyzed after low-temperature stress with or without melatonin pre-treatment. As shown in Figs. 5 and 6, increased the activities of SOD (approximately 0.53–14.09% for M33, 5.28–28.68% for Y49 with respect to control) and POD (approximately 118.68–373.28% for M33, 16.64–136.96% for Y49 respect to control) were after low-temperature stress. Interestingly, the application of 200 μM exogenous melatonin induced further increases in the activities of these enzymes to different extents. The melatonin treatment increased the SOD activities by approximately 8.34/3.85%, 12.75/4.65%, 6.39/1.95%, 7.28/3.03% (Figs. 5a and 6a), and POD was increased by 39.08/81.39%, 13.71/37.09%, 9.49/21.91%, 16.37/3.63%, respectively, for M33/Y49 at 12 h, 24 h, 36 h, 48 h in relative to stress (Figs. 5b and 6b). Antioxidant enzymes are crucial in preventing damage to plants caused by oxidative stress. Whereas melatonin foliar treatment with 200 μM caused gradual increases in SOD and POD in the two varieties of seedlings as compared with their corresponding controls. Surprisingly, when compared to low-temperature stress alone, exogenous melatonin increased the activity of those enzymes in most cases.

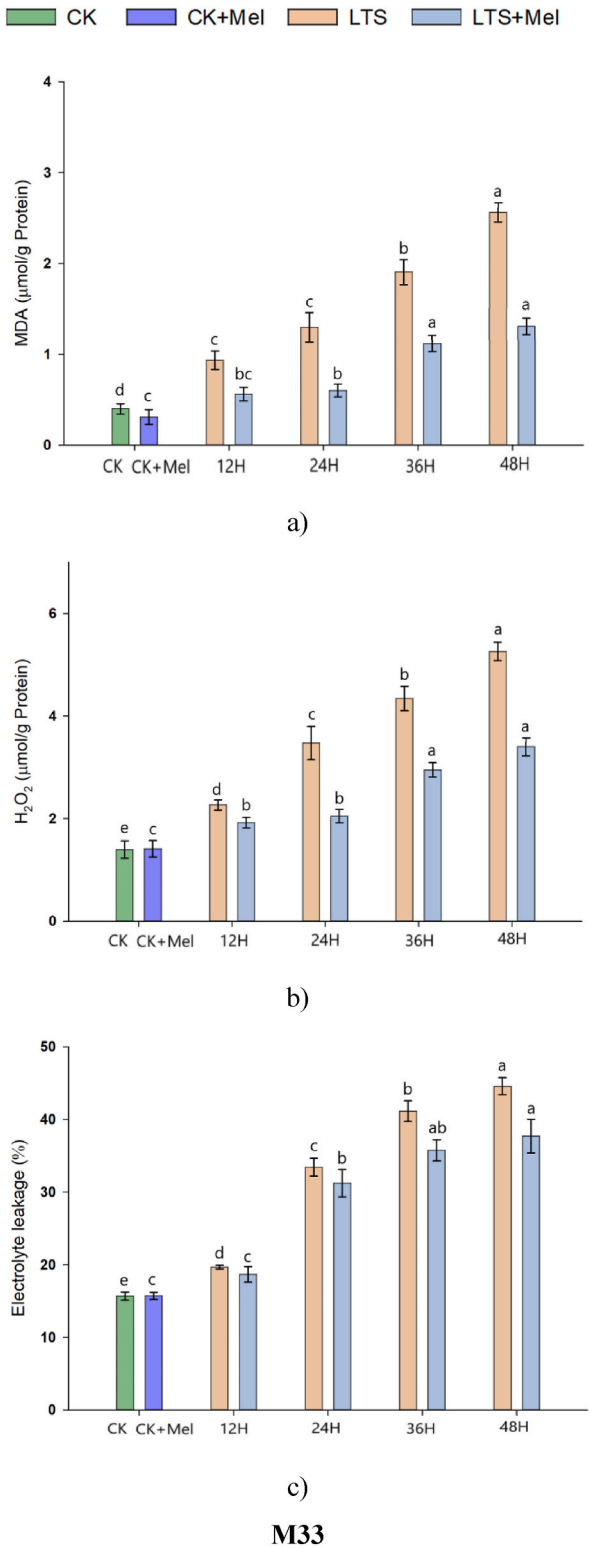

Fig. 3. Effect of low-temperature stress and exogenous melatonin on Malondialdehyde (MDA), Hydrogen Peroxide (H_2O_2) and Electrolyte leakage (EL) in M33 variety. The values presented are means ± SD (n = 3). Significant difference is exhibited by different letters above the bars at $p < 0.05$ among treatments.

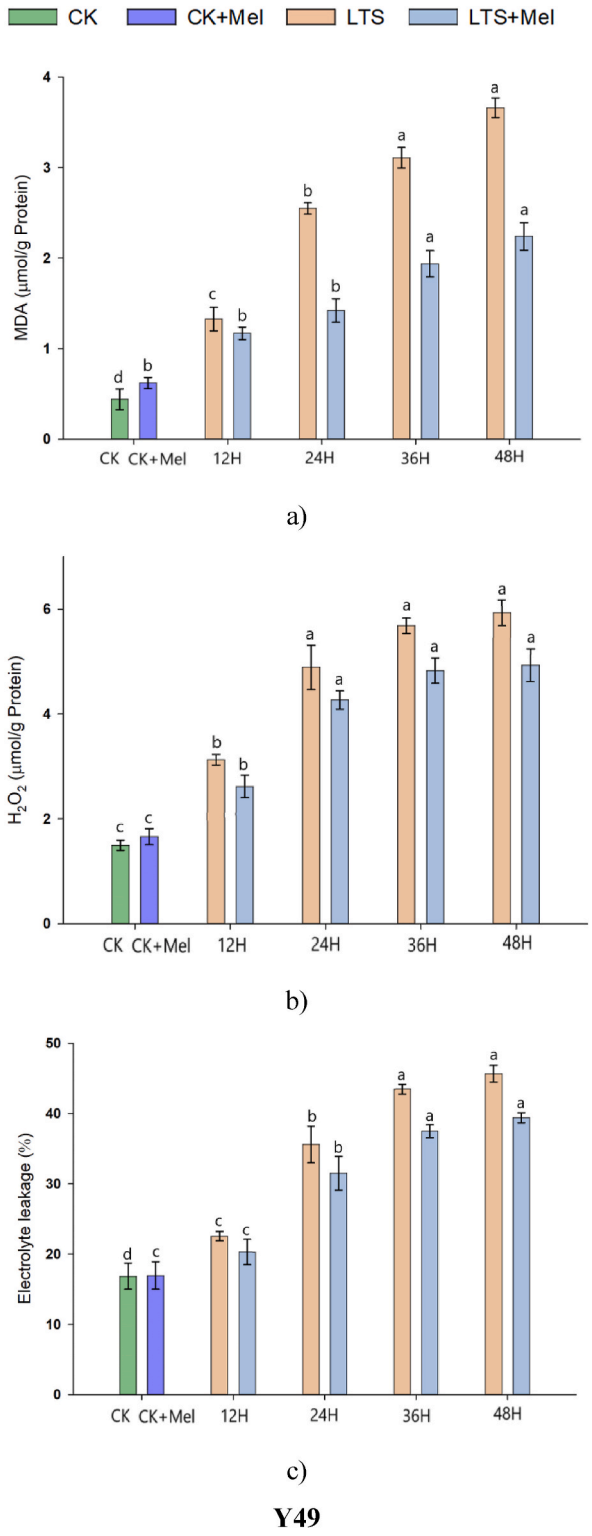

Fig. 4. Effect of low-temperature stress and exogenous melatonin on Malondialdehyde (MDA), Hydrogen Peroxide (H_2O_2) and Electrolyte leakage (EL) in Y49 variety. The values presented are means ± SD (n = 3). Significant difference is exhibited by different letters above the bars at $p < 0.05$ among treatments. (CK:Control, Mel:Melatonin, LTS:Low-Temperature Stress).

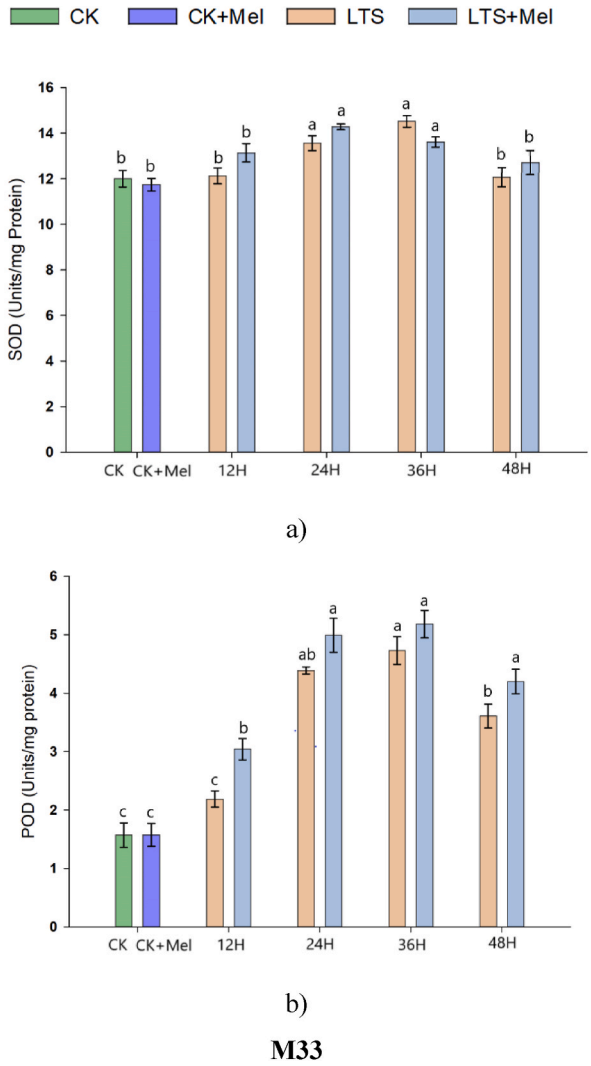

Fig. 5. Effect of low temperature stress and exogenous melatonin on enzymatic and non-enzymatic antioxidants superoxidase dismutase (SOD) activity and peroxidase (POD) activity in M33 variety. The values presented are means ± SD (n = 3). Significant difference is exhibited by different letters above the bars at $p < 0.05$ among treatments. (CK:Control, Mel:Melatonin, LTS:Low-Temperature Stress).

3.4. Effects of exogenous melatonin on non-enzymatic and total antioxidant activity at low-temperature stress

In almost all treatments, soluble sugar, proline, phenolic content, anthocyanin, and free amino acids contents increased compared with control seedlings after melatonin application. After low temperature stress the content of anthocyanin in both of variety was high at 36 h, and 48 h with the value of 82.60/61.36% and 61.62/36.47%, respectively, for M33/Y49 relative to stress (Figs. 7a and 8a). In case of proline content increased from 12.97 to 105.19% in M33 and 54.43–172.07% in the Y49 variety compared to control (Figs. 7b and 8b). Pre-treatment of leaves with melatonin increased the proline content by 15.68/15.68%, 23.92/7.98%, 30.90/10.42%, 38.27/29.0%, respectively, for M33/Y49 at 12 h, 24 h, 36 h, 48 h in relative to stress. In M33, proline content increased at a higher level than Y49 variety after melatonin treatment relative to stress (Figs. 7b and 8b). After temperature stress, free amino acid increased from 14.54 to 54.40% for M33, and 19.51–45.28% for Y49 regarding control. The pre-treatment with melatonin increased the free amino acid by approximately 17.21/10.36%, 48.50/30.06%, 55.68/18.77%, 19.45/19.05%, respectively, for M33/Y49 at 12 h, 24 h, 36 h, and 48 h in relative to stress (Figs. 7c and 8c). In the case of total phenolic increased from 14.66 to 43.38% for M33, and 10.04–56.48% for Y49 (Figs. 7d and 8d); soluble sugar increased from 1.35 to 22.87% for M33, and 1.75–27.49% for Y49 respect to control (Figs. 7e and 8e). Total phenolic levels further increased by approximately 8.26/6.59%, 6.83/1.34%, 7.23/3.09%, 2.09/6.14% and soluble sugar increased by 6.19/25.86%, 11.52/19.19%, 10.95/10.09%, 10.85/0%, respectively, for M33/Y49 at 12 h, 24 h, 36 h, 48 h in relative to stress in pre-treatment with exogenous melatonin.

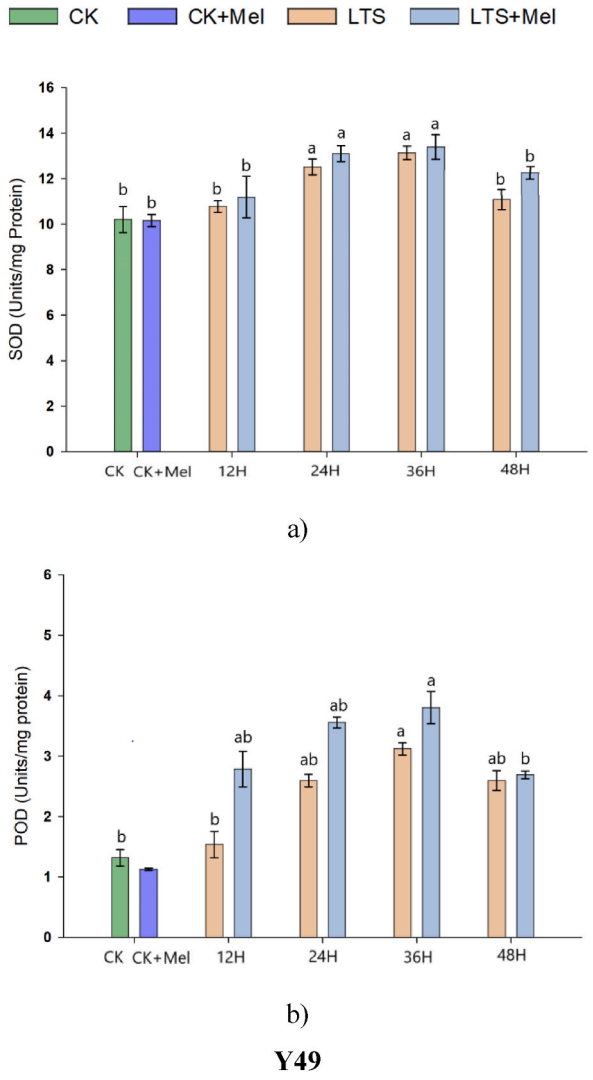

Fig. 6. Effect of low temperature stress and exogenous melatonin on enzymatic and non-enzymatic antioxidants superoxidase dismutase (SOD) activity and peroxidase (POD) activity in Y49 variety. The values presented are means ± SD (n = 3). Significant difference is exhibited by different letters above the bars at $p < 0.05$ among treatments. (CK:Control, Mel:Melatonin, LTS:Low-Temperature Stress).

4. Discussion

Recent research indicates that melatonin protects plants from various abiotic stresses, including low temperature, heat, drought, salinity, and heavy metal toxicity [5,23,43]. Low temperature causes some alterations in cell structure, cell membranes, and cell wall compounds. The study aimed to explore the protective roles of melatonin in minimizing the low-temperature effects in *C. olitorius* and *C. capsularis* plants. There are scarce studies on the interaction of melatonin with low temperature stress in both of *Corchorous* spp. Our results revealed that the exogenous melatonin administration reduced the negative effects of low-temperature stress.

It is widely known that low temperatures can disrupt photosynthesis by causing the reduction of photosynthetic pigments, the loss of chloroplast structure, the closing of stomata, and can induce the production of reactive oxygen species (ROS) and the accumulation of malondialdehyde (MDA) and proline in plants [16,44,45]. Under biotic or abiotic stress, seedlings have reduced chlorophyll levels due to impaired chlorophyll biosynthesis or increased chlorophyll decomposition [46]. The findings of this study showed that low-temperature stress increased total chlorophyll and carotene levels and light-harvesting ability in *C. olitorius* and *C. capsularis* seedlings when exogenous melatonin was applied. Melatonin possibly repairs the impairments in protein synthesis and improves the chlorophyll synthesis that enhances the plant growth attributes [47]. Recent reports also indicated that melatonin effectively suppresses the low-temperature induced photosynthetic inhibition in gardenia [48]. Possibly, exogenous melatonin significantly minimized photo-inhibition by boosting photosynthetic efficiency via bio-stimulatory pathways, improving photochemical performance [49]. These findings corroborate earlier observations that melatonin treatment can increase cold tolerance in bermuda grass [2], wheat

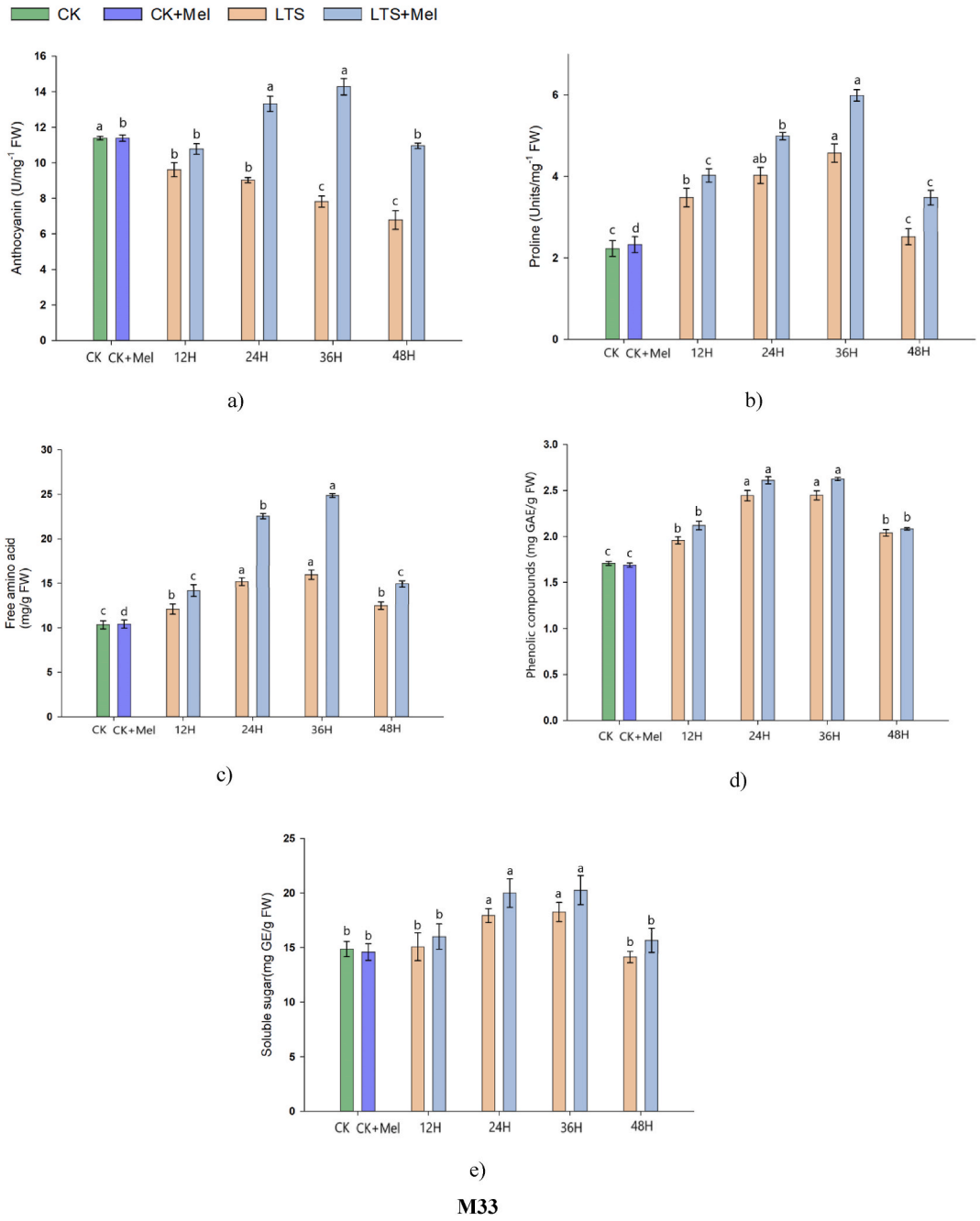

Fig. 7. Effect of low-temperature stress and exogenous melatonin on anthocyanin, proline, free amino acid, phenolic compounds and soluble sugar in M33 variety. The values presented are means ± SD (n = 3). Significant difference is exhibited by different letters above the bars at $p < 0.05$ among treatments. (CK:Control, Mel:Melatonin, LTS:Low-Temperature Stress).

[50] and *Arabidopsis* [5] by enhancing the synthesis and slower decomposition of chlorophyll. It has been linked to exogenous melatonin causes a delay in chlorophyll breakdown during abiotic stress by raising total chlorophyll concentration [6,51,52].

Membrane damage and electrolyte leakage are good indicators for measuring the level of lipid degradation and plasma membrane permeability in response to various environmental stresses [53,54]. However, excessive ROS accumulation can cause membrane lipid peroxidation, which can lead to cell membrane damage, loss of cellular integrity, and cell fatalities [54]. In our research, a steady rise in ROS (H_2O_2), MDA and EL was observed throughout the entire treatment suggests that the level of oxidative damages and loss of integrity of the plasma membrane is affected by the length of stress, which is consistent with previous results in maize, soybeans, and cucumbers [51]. Melatonin and several of its metabolites are known free radical scavengers and broad-spectrum antioxidants, and it

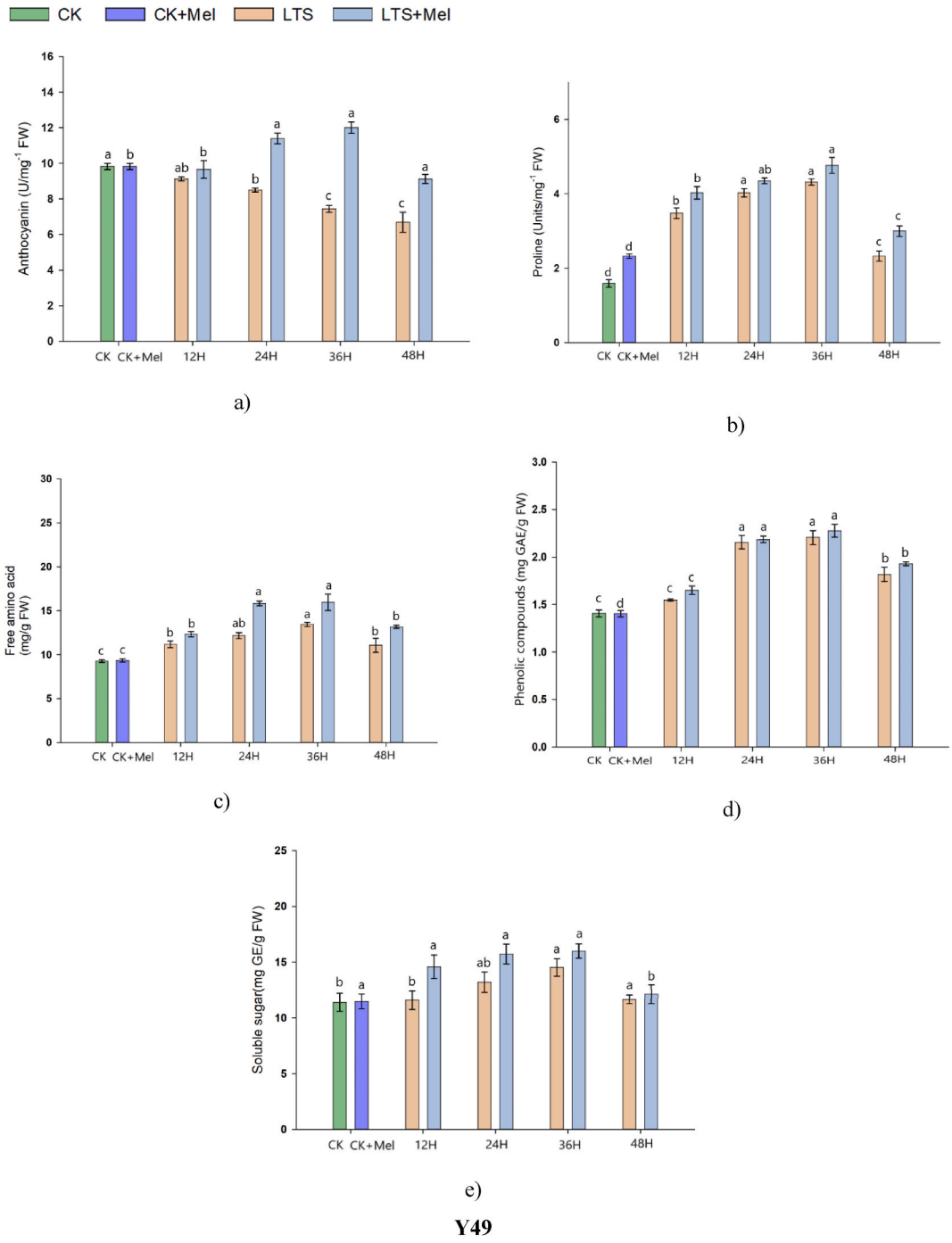

Fig. 8. Effect of low-temperature stress and exogenous melatonin on anthocyanin, proline, free amino acid, phenolic compounds and soluble sugar in Y49 variety. The values presented are means ± SD (n = 3). Significant difference is exhibited by different letters above the bars at $p < 0.05$ among treatments. (CK:Control, Mel:Melatonin, LTS:Low-Temperature Stress).

has been proposed that they directly scavenge ROS [5,55]. It was revealed that, exogenous melatonin minimizing the low-temperature mediated oxidative damages as revealed by the reduced MDA and ROS levels, authors speculated that the ROS levels were lower compared with non-melatonin treated plants under low temperature stress. Hydrogen peroxide (H_2O_2) is toxic, destructive ROS and more stable than other that can be used as an indicator of ROS scavenging activity. In contrast, malondialdehyde concentration can be used indicate membrane damage [56]. It was reported that H_2O_2 is subsequently removed by APX, POD, CAT and GPX through different pathways [57]. Our results showed that during low-temperature stress, exogenous melatonin suppressed H_2O_2 and $O_2\bullet$-

formation, leading to a fall in ROS production; this effect was more pronounced in the *C. olitorius* compared to the *C. capsularis* which might be due to direct enhancement antioxidant activities under low temperature stress. It has reported that reported that concentration 150 μM melatonin improved low temperature tolerance by reducing the ROS burst by alleviated the damaging effect of hydrogen peroxide, balancing photosynthetic efficiency, lowering EL and MDA level and elevating antioxidant activity. In this study, melatonin-mediated the proline accumulation under low temperature stress showed the synergetic capacity of melatonin to cope the oxidative damages. Previous findings showed that exogenous melatonin enhanced the proline content in under different stresses [58,59], which are support the findings of current study and indicating antioxidant potential of melatonin in regulating proline metabolism during stressful conditions. Thus, it supported the report that exogenous melatonin has a strong effect on endogenous melatonin accumulation under low-temperature stress to mitigate low temperature-induced oxidative stress. These finding indicate that melatonin could protect *C. olitorius* and *C. capsularis* seedlings from the negative effects of low temperature stress by inhibiting the accumulation of H_2O_2, electrolyte leakage and MDA concentration; and enhances low temperature stress tolerance.

Different antioxidant enzymes and non-enzyme activities are responsible for eliminating reactive oxygen species (ROS) when plants are stressed [60]. Authors found that under low-temperature stress increased the activities of the antioxidant enzymes (SOD, POD) compared to non-stressed plants. Whereas plants treated with melatonin demonstrated further considerable increased of SOD, POD activity. The increased in SOD, CAT and APX activities may be linked to the increased levels of ROS [61], indicating the activation of the plant defense system against oxidative stress [61]. SOD and POD act as important antioxidant enzymes that can eliminate redundant ROS from plant tissues, protecting the plasma membrane from peroxidation [62]. In plant cells, SOD converts O_2^- to O_2 and H_2O_2, while H_2O_2 can be further scavenged by POD and other antioxidant enzymes [2,32]. Our findings agree with other results [52,63], indicating activation and elevation of different enzymatic antioxidant such as SOD and POD protect ROS induced damage by low-temperature stress [64].

It has been reported that exogenous melatonin application might activate the enzymatic or non-enzymatic antioxidant system to maintain the redox balance [2,50]. Exogenous melatonin progressively increased the accumulation of soluble sugar, proline, total phenolic, anthocyanin, and free amino acids, suggesting the potential function of melatonin in increasing crop yield compared with control plants. It also reported that compatible solutes (proline, sugar, and amino acids) protect plants from environmental stress by osmoregulation, scavenging ROS accumulation and maintaining membrane integrity [65]. Melatonin-treated plants had even higher proline and phenolic compound, which could result from melatonin's antioxidant activity preventing proline breakdown [66]. Our finding reported that plants' phenolic compounds markedly increased in the low-temperature stressed plants compared to the control, and the melatonin-treated plants were able to retain significantly greater phenolic substance content under low-temperature stress, which are supported by others [50,67], and indicating this have a role in stress tolerance at low temperatures via increased phenolic levels contribute to ROS detoxification and increase cell wall thickness [68]. In *C. olitorius* and *C. capsularis*, melatonin treatment was found to accumulation of anthocyanin in both of species, which was also reported in other crops [69]. These consequences provided additional evidence that melatonin promoted redox homeostasis by activating the enzymatic and non-enzymatic antioxidant mechanisms in defending plants against abiotic stress and recovering from oxidative stress-induced damage at the cellular level, as previously demonstrated and reported [50,70].

5. Conclusion

Melatonin, a well-known chemical, plays many crucial roles in plants. The role of melatonin in plant physiology in response to low-temperature stress was investigated. The current study demonstrated that effect of melatonin on *C. olitorius* and *C. capsularis* seedlings under low-temperature stress and result revealed that it had protective effects on growth. During low-temperature stress, plants treated with 200 μM melatonin, recovered more rapidly than untreated plants by enhancing photosynthetic efficiency and enzymatic as well as non-enzymatic antioxidant activities, limiting ROS accumulation, mitigating oxidative damage. Therefore, melatonin increases low temperature tolerance in *C. olitorius* and *C. capsularis* seedlings. Thus, our findings suggest that as a signal molecule melatonin had a strong role in low-temperature stress tolerance and provided new insights into potential application of melatonin to increase the low-temperature tolerance and production of *C. olitorius* and *C. capsularis* throughout the year.

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Author contribution statement

Defang Li, Susmita Dey and Ashok Biswas: Conceived and designed the experiments. Susmita Dey and Ashok Biswas: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Yong Deng, Ziggiju Mesenbet Birhanie, and Chen Wentao: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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