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Melatonin improves lead (Pb) stress tolerance through physico-chemical modulation in Brinjal (Solanum melongena L.)

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Abstract

Lead (Pb) contamination poses a serious threat to crop productivity and food safety, particularly in widely consumed vegetables such as brinjal (*Solanum melongena* L.). This study investigated the role of exogenous melatonin in mitigating Pb-induced phytotoxicity through integrated physiological, biochemical, and metal accumulation analyses. Pb stress significantly impaired plant growth, reduced chlorophyll content and net photosynthesis, and induced oxidative damage, as evidenced by increased H_2O_2, malondialdehyde (MDA), and electrolyte leakage. It also disrupted nutrient homeostasis and enhanced Pb accumulation in roots, shoots, and fruits. In contrast, melatonin application (100 µM) markedly alleviated these adverse effects by enhancing antioxidant capacity, increasing polyphenol content, and reducing ROS accumulation and lipid peroxidation. Melatonin improved photosynthetic performance by up to 41.3%, thereby restoring biomass accumulation, and significantly enhanced fruit yield up to 2.3-fold relative to Pb only treatment. Importantly, melatonin reduced Pb accumulation and translocation, lowering Pb content in edible tissues while improving essential nutrient status. Multivariate analyses (PCA and heatmap) confirmed that melatonin modulated physiological and biochemical responses, contributing to improved metabolic and physiological performance under Pb stress. Taken together, these findings indicated that melatonin acts as an effective regulator of redox homeostasis, reduced Pb accumulation, and promoted growth, highlight its potential as an promising and eco-friendly strategy to enhance crop resilience and ensure food safety in heavy metal-contaminated environments.

***Keywords**:* Melatonin; Lead toxicity; Brinjal (*Solanum melongena* L.); Oxidative stress; Bioaccumulation and translocation

Introduction

Brinjal (*Solanum melongena* L.), commonly known as eggplant, is one of the most widely cultivated and economically significant vegetable crops throughout the world. It ranks fifth among solanaceous crops after potato, tomato, pepper, and tobacco, with an annual production of approximately 50 million tons and a global market value exceeding US$10 billion (Taher et al., 2017). The widespread cultivation of brinjal is attributed to its high nutritional value, adaptability to diverse agro-climatic conditions, and year-round production potential. Brinjal fruits are rich in essential vitamins (A, C, and B-complex), minerals (potassium, calcium, magnesium, and iron), dietary fiber, and diverse bioactive compounds, particularly phenolics and flavonoids (Sharma and Kaushik, 2021). These phytochemicals confer strong antioxidant, anti-inflammatory, and anti-carcinogenic properties, making brinjal an important component of human diets (Quamruzzaman et al., 2020). In many countries like Bangladesh, brinjal is not only a dietary staple but also a major source of income for smallholder farmers. Its cultivation contributes substantially to national vegetable production and rural livelihoods. However, despite its agronomic adaptability, brinjal production is increasingly constrained by environmental stresses, among which heavy metal contamination has emerged as a critical concern due to rapid urbanization, industrialization, improper waste disposal, and irrigation with polluted wastewater (Bushra et al., 2022; Riaz et al., 2026).

Among toxic heavy metals, lead (Pb) is particularly problematic because it is non-essential for plant metabolism and highly persistent in soils. Pb contamination disrupts multiple physiological and biochemical processes in plants, ultimately impairing growth and productivity (Rahman et al., 2024). Exposure to Pb inhibits seed germination, restricts root and shoot elongation, reduces biomass accumulation, and induces visible symptoms such as chlorosis and leaf necrosis. One of the primary targets of Pb toxicity is the photosynthetic apparatus; Pb interferes with chlorophyll biosynthesis, damages chloroplast ultrastructure, and reduces carbon assimilation efficiency (Rani et al., 2024; Zhou et al., 2017). Furthermore, Pb competes with essential nutrients such as calcium, magnesium, and iron, thereby disturbing nutrient uptake and ionic balance. At the cellular level, Pb toxicity is closely associated with excessive generation of reactive oxygen species (ROS). Even though ROS function as signaling molecules at low concentrations, their overproduction under Pb stress leads to oxidative stress, resulting in lipid peroxidation, protein oxidation, DNA damage, and membrane destabilization (Lopes et al., 2016). This oxidative damage disrupts metabolic homeostasis and accelerates cellular senescence, ultimately limiting plant productivity. Specific concern is the ability of brinjal to accumulate heavy metals in edible tissues, posing significant risks to food safety and human health (Rahman et al., 2024). In developing countries like Bangladesh, soil contamination is progressively increasing, the accumulation of Pb in vegetables represents a serious challenge to sustainable agriculture and nutritional security (Bushra et al., 2022; Quamruzzaman et al., 2020). Therefore, developing effective and environmentally sustainable strategies to mitigate Pb toxicity and reduce its accumulation in brinjal is of paramount importance.

In recent years, melatonin (N-acetyl-5-methoxytryptamine) has emerged as a promising regulator of plant stress tolerance (Hasan et al., 2025a). Though, initially discovered in animals as a

hormone regulating circadian rhythms, melatonin is now recognized as a ubiquitous and multifunctional molecule in plants (Khan et al., 2024). It plays critical roles in growth regulation, photosynthesis, root development, and stress signaling. Importantly, melatonin functions as a potent antioxidant, directly scavenging ROS and indirectly enhancing antioxidant defense systems (Shinde and Pareek, 2026). Numerous studies have demonstrated that exogenous application of melatonin can alleviate abiotic stresses such as salinity, drought, temperature extremes, and heavy metal toxicity. Under heavy metal stress, melatonin enhances plant tolerance through multiple coordinated mechanisms. It stimulates the activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), thereby reducing ROS accumulation and preventing oxidative damage (Lu et al., 2026). Additionally, melatonin modulates the expression of stress-responsive genes, maintains chlorophyll stability, preserves photosynthetic efficiency, and improves nutrient uptake and assimilation (Hasan et al., 2023). Comparable improvements in growth performance, physiological stability, photosynthetic efficiency, and stress adaptation have also been reported in horticultural crops exposed to zinc toxicity, drought, and heat stress following grafting-based approaches or exogenous applications of growth-promoting compounds and nanomaterials (Coşkun et al., 2025; Toprak and Coşkun, 2025a, b, 2026). Melatonin may influence metal chelation, sequestration, and compartmentalization processes through modulating at genetics level, thereby limiting metal translocation to aerial and edible tissues (Hasan et al., 2015; Xing et al., 2023). Recently, exogenous melatonin has been reported to mitigate Pb stress in rice by downregulating the metal transporter gene *OsMTP1* while upregulating the phytochelatin biosynthesis gene *OsPCS1* (Khan et al., 2025a). These integrative mechanisms, contributes to the maintenance of physiological homeostasis under adverse environmental conditions (Karpagam and Godwin, 2025; Xing et al., 2023).

Despite increasing evidence of melatonin-mediated stress mitigation in various crops, comprehensive investigations focusing on its role in alleviating Pb toxicity in brinjal remain limited. In particular, studies integrating plant growth, photosynthetic performance, oxidative stress responses, nutrient homeostasis, and Pb translocation to edible fruits are scarce, leaving important knowledge gaps regarding melatonin's potential to improve crop performance and food safety under Pb stress (Hoque et al., 2021). Given the economic and nutritional importance of brinjal, understanding the protective mechanisms of melatonin against Pb stress is both scientifically and practically significant. Based on the aforementioned evidence, we hypothesize that exogenous melatonin application mitigates Pb-induced phytotoxicity in brinjal by enhancing antioxidant defense systems, preserving physiological and metabolic homeostasis, and reducing Pb bioaccumulation and translocation, as reflected by Pb concentrations in roots, shoots, and fruits, together with bioaccumulation factor (BAF) and translocation factor (TF)(Wang et al., 2026). By integrating physiological, biochemical, and metal accumulation analyses, this research aims to elucidate the mechanistic basis of melatonin-mediated stress alleviation. Ultimately, the findings are expected to contribute to the development of sustainable agronomic strategies for enhancing crop resilience, safeguarding food quality, and promoting agricultural sustainability in heavy metal-contaminated agroecosystems.

2. Material and Methods

2.1 Experimental site, soil characteristics and fertilization

The experiment was conducted on the net house of the faculty of agriculture at Sylhet Agricultural University, Sylhet-3100, Bangladesh, from March to June 2023. The site is geographically located between 23°57′ and 25°13′ North latitude and 90°56′ and 92°21′ East longitude and belongs to the Piedmont plains of northeastern Bangladesh, characterized by subtropical climatic conditions. Floodplain soil with a sandy loam texture and dark brown color was used for the experiment. The soil was acidic, with a pH ranging from 3.9 to 5.9, and belongs to the Surma-Kushiara Floodplain under Agro-Ecological Zone (AEZ) 20 and the Khadimnagar Soil Series (Hasan et al., 2025c). Soil samples were collected from a depth of 0-15 cm and analyzed at the Key Laboratory of Integrated Research in food and Agriculture (IRFA), Department of Agricultural Chemistry, Sylhet Agricultural University. Soil pH was determined using a 1:2.5 soil-water suspension and a digital pH meter. Manures and fertilizers were applied following the Fertilizer Recommendation Guide (FRG), BARC, 2018. Nitrogen, phosphorus, and potassium were supplied in the form of urea, triple superphosphate (TSP), and muriate of potash (MoP), respectively, during soil preparation to ensure adequate nutrient availability and support optimal plant growth under pot culture conditions.

2.2 Collection of seedlings and treatment design

Uniform and healthy seedlings of Bankim Purple Long brinjal (*Solanum melongena* L.) were collected from a local nursery in Sylhet Sadar Upazila, Sylhet at third leaf stage. The experiment consisted of four treatment groups including Control, Pb, melatonin, and Pb + melatonin application. Lead stress was imposed by applying lead nitrate [$Pb(NO_3)_2$] at a concentration of 30 mg kg^{-1} soil during soil preparation to induce metal stress. The required amount of $Pb(NO_3)_2$ was dissolved in distilled water, thoroughly mixed with the soil to ensure uniform distribution, and incubated for 7 days before transplanting. The experiment was conducted in medium-sized plastic pots (30 cm diameter) each filled with 18 kg of air-dried prepared soil and arranged in a completely randomized design (CRD) with three biological replicates per treatment. Additionally, experiment was conducted using pot soil with a pH of 4.8-5.2, where Pb is relatively more bioavailable, and the selected Pb level was sufficient to induce measurable stress without causing complete growth inhibition (Hettiarachchi and Pierzynski, 2004). Recommended fertilizers were applied as basal doses during soil preparation in accordance with the Bangladesh Agricultural Research Council (BARC) Fertilizer Recommendation Guide (Guide, 2018). Melatonin (CAS No: 73-31-4; Shanghai Macklin Biochemical Co., Ltd.; molecular weight: 232.28 g mol^{-1}) was prepared as a fresh 100 µM solution, with the concentration selected based on our previously published study (Hasan et al., 2025a). Melatonin (100 µM) was applied as a foliar spray at 15, 25, 35, and 45 days after transplanting, with each plant receiving approximately 10-15 mL of spray solution per application, ensuring uniform wetting of the foliage without runoff. The foliar application ensured uniform exposure and effective absorption of melatonin to evaluate its effectiveness in mitigating Pb-induced stress in brinjal plants.

2.3 Harvest and storage of plant samples

Plant samples were collected one week after the final foliar application of melatonin. Samples from each replicate were harvested and composited. The fresh weights of roots and shoots were measured using an electric balance (Model: PA213, Ohaus Corp., Pine Brook, NJ, USA). Leaves, shoots, and roots were thoroughly washed with tap water followed by distilled water to remove soil and debris, and then oven-dried at 65 °C for 72 hours (Model: XUO58, France). The dried samples were ground into fine powder using a mortar and pestle. The powdered samples were transferred into ziplock bags and stored at 4 °C in a refrigerator (Model: Biobase, BPR-5V260/360, Shandong, China) for further biochemical and elemental analyses. Brinjal fruits (*Solanum melongena* L.) were harvested at full maturity over a period of up to 75 days after transplanting. The collected fruits were chopped into small pieces and oven-dried at 65 °C for 48 hours until constant weight was achieved. The dried fruit samples were then ground into powder using a mortar and pestle and stored in airtight ziplock bags at 4 °C for subsequent analysis.

2.4 Measurement of photosynthetic pigments and net photosynthesis

Fresh, fully expanded brinjal (*Solanum melongena* L.) leaves were collected from each treatment to determine chlorophyll and carotenoid contents. Approximately 0.3 g of fresh leaf tissue was homogenized in 8 mL of 80% (v/v) acetone using a chilled mortar and pestle under dim light conditions to prevent pigment degradation. The homogenate was centrifuged, and the clear supernatant was used for spectrophotometric analysis. The absorbance was recorded at 663.6 nm, 646.6 nm, and 450 nm using a UV-visible spectrophotometer (Model: T80+, PG Instruments Ltd., Leicestershire, UK). Chlorophyll a, chlorophyll b and total chlorophyll contents were calculated according to the method previously described (He et al., 2020). Net photosynthetic rate (Pn) was measured on fully expanded upper leaves between 11:30 and 13:30 h under clear sky conditions to ensure maximum and stable photosynthetic activity. Measurements were performed using a portable infrared gas analyzer (LI-6400, LI-COR Biosciences, Lincoln, NE, USA), following the procedure previously described (Hasan et al., 2024). During measurement, environmental conditions such as light intensity, ambient CO_2 concentration, and leaf temperature were maintained close to natural conditions to ensure accuracy and consistency.

2.5 Determination of Malondialdehyde (MDA) Content and Electrolyte Leakage (EL)

Malondialdehyde (MDA) content, an indicator of lipid peroxidation and oxidative membrane damage, was determined following the method as previously discussed (Mohi-Ud-Din et al., 2021). Fresh leaf samples (0.3 g) were homogenized in trichloroacetic acid (TCA) and centrifuged to obtain a clear extract. The supernatant was mixed with thiobarbituric acid (TBA) reagent and heated at 95 °C for 30 minutes, followed by rapid cooling in an ice bath. Under acidic and high-temperature conditions, MDA reacts with TBA to form a thiobarbituric acid-malondialdehyde (MDA-TBA) complex, producing a pink-colored solution. The absorbance was measured at 532 nm using a UV–visible spectrophotometer, and nonspecific absorbance was corrected at 600 nm. The MDA concentration was calculated using the appropriate extinction coefficient and expressed on a fresh weight basis. This method reflects the extent of lipid peroxidation and cellular oxidative damage in plant tissues.

Electrolyte leakage (EL), an indicator of cell membrane stability, was determined according to the previously described method (Li et al., 2016). Three uniform leaf discs (2 cm diameter) were collected from each treatment and placed in test tubes containing 10 mL of 0.4 M mannitol

solution prepared in deionized water. The samples were incubated in a water bath at 24 °C for 1 hour to allow electrolyte diffusion from damaged cells. The initial electrical conductivity (EC_1) of the solution was measured using a conductivity meter (Model: DsPH-3; Presto-Tek, Los Angeles, CA, USA). The samples were then frozen at -20 °C for 24 hours to completely disrupt the cell membranes, thawed, and the final electrical conductivity (EC_2) was measured. Electrolyte leakage was expressed as a percentage using the formula: EL (%) = (EC_1 / EC_2) ×100.

2.6 Histochemical staining of ROS and measurement of H_2O_2

Hydrogen peroxide (H_2O_2) in brinjal leaves was visualized using 3,3′-diaminobenzidine (DAB) staining. Leaves were immersed in 1 mg/mL DAB solution prepared in 10 mM phosphate buffer (pH 7.4) and incubated in the dark for several hours. To enhance infiltration of the staining solution, vacuum infiltration was applied for 15-30 minutes. After incubation, leaves were cleared with ethanol to remove chlorophyll, and brown precipitates indicated H_2O_2 accumulation (Hajam and Bashri, 2026). Superoxide radicals (O_2^-) were detected using nitroblue tetrazolium (NBT) staining. Leaves were immersed in 0.1 mg/mL NBT solution prepared in 10 mM potassium phosphate buffer (pH 7.8) and kept in the dark to prevent light-induced reactions. Vacuum infiltration ensured thorough penetration of the NBT solution. After several hours of incubation, leaves were cleared with ethanol, and blue formazan deposits indicated the presence of superoxide radicals (Hasan et al., 2024).

For quantitative determination of H_2O_2, 0.3 g of fresh leaf tissue was homogenized in 3 mL of ice-cold 1.0 M $HClO_4$ following previously described spectrophotometric method (Hasan et al., 2015). The homogenate was centrifuged at 6,000 × g for 5 min at 4°C (Model: HERMLE Z 326 K, Wehingen, Germany). The pH of the collected supernatant was adjusted to 6.5 using KOH, followed by the addition of 0.05 g activated charcoal to remove interfering compounds. After vortexing, the mixture was centrifuged at 12,000 × g for 5 min, and the supernatant was passed through a pre-packed AG 1-X8 anion-exchange column. An aliquot of the purified extract was mixed (1:1, v/v) with a reaction buffer containing 100 mM potassium acetate (pH 4.4), 1 mM ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt], and 0.25 U horseradish peroxidase (HRP). The reaction mixture was incubated at room temperature for 5 min, and the absorbance was recorded at 412 nm using a UV-Vis spectrophotometer (Model T80+, PG Instruments Ltd., UK). H_2O_2 concentration was quantified from a standard curve prepared using known concentrations of H_2O_2 and expressed as μmol g^{-1} fresh weight.

2.7 Determination of Total Polyphenol Content (TPC)

The total polyphenol content (TPC) in brinjal leaves was determined using the Folin-Ciocalteu method. Approximately 1 g of fresh leaves was finely ground in 100% ethanol, and the homogenate was filtered to obtain a clear extract. A 50 μL portion of the filtrate was further mixed with ethanol for analysis. For the assay, 1.0 mL of the leaf extract, blank, or a series of gallic acid standard solutions was combined with 2.0 mL of 1 M Folin-Ciocalteu reagent, followed by the addition of 2.0 mL of 7.5% (w/v) sodium carbonate solution. The mixture was allowed to stand for 5 minutes, then thoroughly mixed and incubated at room temperature for 30 minutes. After incubation, the samples were centrifuged at 2,000 rpm for 10 minutes using a Gyrozen benchtop centrifuge (Model-416G, Korea). The absorbance of the resulting blue

colored solution was measured at 765 nm using a UV-visible spectrophotometer (Model T60U, PG Instruments Ltd., UK). Total polyphenol content was calculated from a standard calibration curve prepared with gallic acid and expressed as milligrams of gallic acid equivalent per 100 g of fresh leaf tissue (mg GAE/100 g) as previously described (Jahan et al., 2022).

2.8 Determination of Total Antioxidant Activity by DPPH Radical Scavenging Assay

The total antioxidant activity of brinjal leaf extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Brief, 100 µL of leaf extract was mixed with 3 mL of DPPH solution, prepared by dissolving 4 mg of DPPH in a mixture of 10 mL acetonitrile and 90 mL methanol. The reaction mixture was incubated in the dark at room temperature for 30 minutes to prevent light-induced degradation of DPPH radicals. After incubation, the absorbance of the sample was measured at 515 nm using UV–Visible spectrophotometer (Model: T80+, PG Instruments Ltd., UK), with methanol as a blank reference. which was used as an indicator of the total antioxidant capacity of the leaf extracts, was calculated using the following equation: antioxidant activity (%)=$Abs_{control}$ -Abs_{sample}/$Abs_{control}$ ×100, where Abs_{sample} is the absorbance of the DPPH solution without the leaf extract (control), and Abs_{sample} is the absorbance of the reaction mixture containing the leaf extract after 30 min of incubation (Dekdouk et al., 2015).

2.9 Analyses of nutritional quality, bioaccumulation and translocation of lead in Brinjal

For nutritional analysis plant samples were harvested, washed thoroughly with distilled water, and separated into roots, shoots, and leaf tissues. The plant materials were oven-dried at 65-70 °C to constant weight and ground into fine powder. For nutrient analysis, standard procedures were followed to determine macronutrients (N, P, K, Ca, Mg, S), micronutrients (Fe, Zn, Cu, Mn) and Pb after acid digestion as discussed (Hasan et al., 2025b). Bioaccumulation factor (BAF) and translocation factor (TF) were calculated to evaluate Pb uptake and movement within the plant (Pachura et al., 2016). BAF was determined as the ratio of Pb concentration in plant tissues to that in the soil, while TF was calculated as the ratio of Pb concentration in shoots or fruits to that in roots. These indices were used to assess the extent of Pb accumulation and its internal distribution in brinjal plants.

2.10 Statistical Analysis

The experimental data were subjected to statistical analysis by first calculating the mean values from replicated observations, followed by estimation of the standard deviation. Analysis of variance (ANOVA) was performed to assess significant differences among treatments. When significant effects were detected, mean comparisons were conducted using the Least Significant Difference (LSD) test at an appropriate significance level. All statistical analyses were carried out using the R statistical software environment. Principal component analysis (PCA) was performed using treatment mean values of all measured growth, physiological, biochemical, yield, nutrient, and Pb accumulation parameters in Origin 2018 (OriginLab Corporation, Northampton, MA, USA). Prior to analysis, the data were standardized (mean-centered and

scaled to unit variance). Heatmap visualization was generated using ClustVis 2.0 (https://biit.cs.ut.ee/clustvis), as described (Jahan et al., 2022).

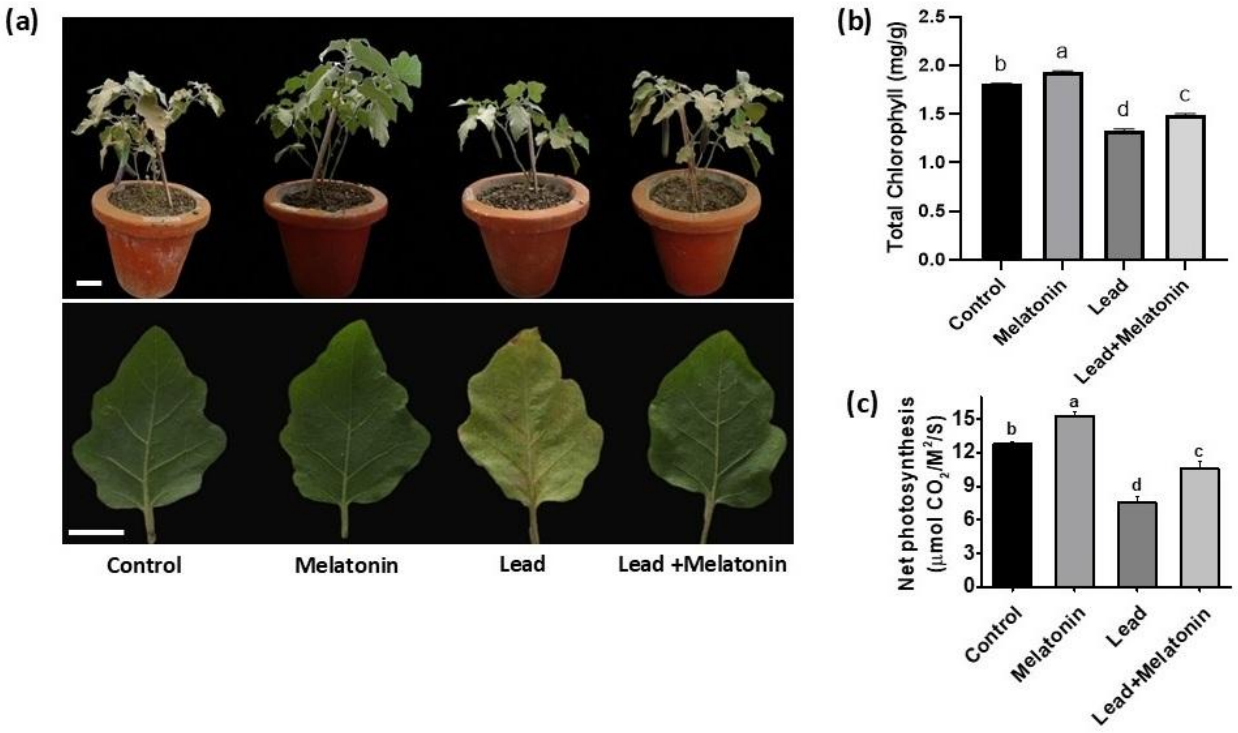


Figure 1: Effect of melatonin on growth and photosynthetic performance of brinjal under lead (Pb) stress: (a) phenotypic changes in plants and leaves, (b) total chlorophyll content, and (c) net photosynthetic rate. Values represent the mean of three replicates, and vertical bars indicate standard deviation (SD). Means followed by the same letter are not significantly different at $p < 0.05$ according to the least significant difference (LSD) test.

3. Results

3.1 Role of Melatonin on phenotype, chlorophyll content, and net-photosynthesis in Brinjal under lead (Pb) stress

Distinct phenotypic variations were observed in brinjal plants under different treatments. Control plants exhibited healthy growth with well-expanded green leaves, whereas melatonin treated plants showed comparable or slightly improved vigor, indicating that melatonin had no negative effect on plant morphology and may enhance overall plant performance. In contrast, Pb-treated plants displayed clear toxicity symptoms, including reduced plant height, leaf chlorosis, and overall growth inhibition (**Figure 1a**). However, the exogenous melatonin supplementation significantly alleviated these visible stress symptoms, with plants showing improved leaf coloration and growth recovery compared to Pb alone, demonstrating the protective role of melatonin against Pb-induced phytotoxicity (**Figure 1a**).

Chlorophyll analysis further supported these phenotypic observations. Pb exposure resulted in a substantial decline about 23.46% in total chlorophyll content compared to the control, indicating disruption of chloroplast structure and pigment biosynthesis under metal stress (**Figure 1b**). Melatonin treatment alone either maintained or slightly enhanced chlorophyll levels relative to the control. Importantly, melatonin application under Pb stress significantly improved total chlorophyll about 20.78% compared to Pb-only plants and restoring pigment levels close to values of control plant. This suggests that Melatonin helps preserve chlorophyll stability and biosynthetic activity under heavy metal stress conditions. A similar trend was observed in net photosynthetic rate (**Figure 1c**). Pb stress markedly reduced photosynthetic performance, reflecting impaired stomatal function and chloroplast efficiency. In contrast, melatonin treated plants maintained higher photosynthetic rates (15.25 µmol $CO_2/m^2/S$), comparable to or slightly above control levels. Notably, melatonin treatment significantly improved net photosynthesis about 41.3% under Pb stress compared to Pb alone treatment, indicating partial restoration of photosynthetic machinery and functional efficiency.

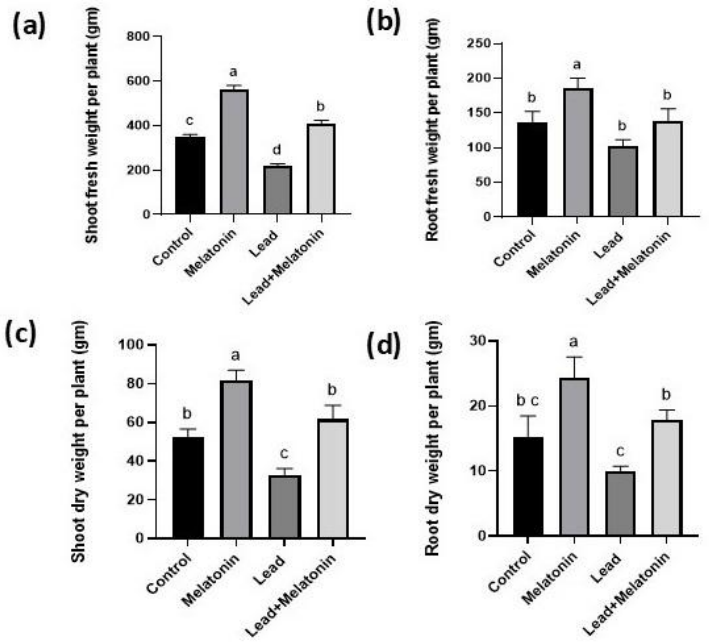


Figure 2: Effect of melatonin on biomass accumulation of brinjal under lead (Pb) stress: (a) shoot fresh weight per plant (g), (b) root fresh weight per plant (g), (c) shoot dry weight per plant (g), (d) root dry weight per plant (g), and (e) total dry weight per plant (g). Values represent the mean of three replicates, and vertical bars indicate standard deviation (SD). Means followed by the same letter are not significantly different at $p < 0.05$ according to the least significant difference (LSD) test.

3.2 Effect of Melatonin on Fresh and Dry Biomass Accumulation in Brinjal under Pb Stress

Brinjal plants exhibited significant variation in biomass accumulation under control, melatonin, lead (Pb), and Pb with melatonin treatments. Melatonin alone markedly enhanced growth

performance, showing the highest biomass accumulation across all parameters **(Figure 2)**. Shoot fresh weight increased by 60.1% over control plants, while root fresh weight rises 35.2%, resulting in a 53.1% increase in total fresh weight. In contrast, Pb exposure severely inhibited growth, reducing shoot and root fresh weight by 59.1% and 34.9%, respectively, and causing a 51.5% decline in total fresh biomass relative to control plants. In contrast, melatonin treatment significantly alleviated Pb-induced growth inhibition. Compared to Pb alone, melatonin increased shoot and root fresh weights by 86.5% and 36.5%, respectively, leading to a 70.7% recovery in total fresh weight **(Figure 2a-b)**. However, biomass levels remained lower than melatonin alone treatment, indicating partial mitigation of stress effects.

A similar pattern was observed in dry biomass accumulation. Melatonin treatment significantly enhanced shoot dry weight by 55.5% and root dry weight by 58.9% compared to control groups, resulting in a 53.5% increase in total dry weight. In contrast, Pb exposure reduced shoot and root dry weights by 59.5% and 53.4%, respectively, leading to an overall 53.8% reduction in total dry biomass relative to control. While, melatonin supplementation increased shoot dry weight by 65.6% and root dry weight by 46.6% compared to Pb alone, with a corresponding improvement in total dry weight **(Figure 2c-d)**. Overall, the results demonstrate that melatonin substantially promotes biomass accumulation in brinjal and effectively mitigates the inhibitory effects of Pb stress.

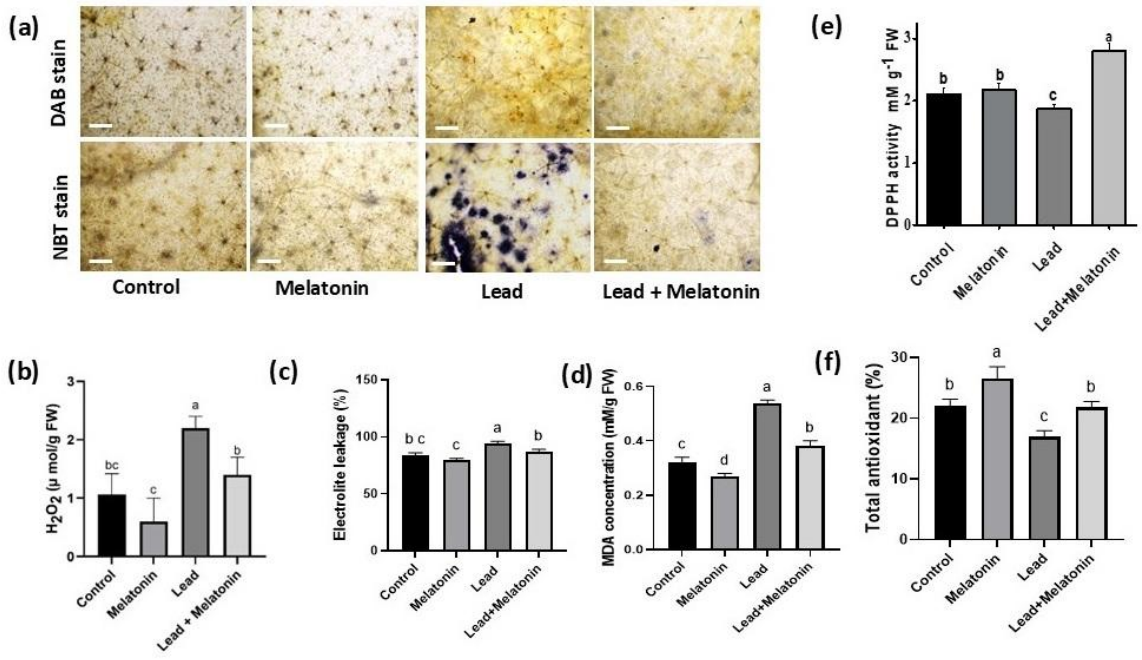


Figure 3: Effect of melatonin on minimizing ROS accumulation and enhancing antioxidant defense in brinjal under Pb stress: (a) DAB and NBT staining of leaves, (b) H_2O_2 accumulation (µmol g^{-1} FW), (c) electrolyte leakage (%), (d) MDA concentration (mM g^{-1} FW), (e) DPPH activity (mg g^{-1} FW), and (f) total antioxidant activity (%). Values represent the mean of three replicates, and vertical bars indicate standard deviation (SD). Means followed by the same letter are not significantly different at $p < 0.05$ according to the least significant difference (LSD) test.

3.3 Effect of Melatonin on Minimizing ROS Accumulation and Enhancing Antioxidant Defense in Brinjal under Pb Stress

Leaf histochemical staining using DAB and NBT assays revealed clear differences in reactive oxygen species (ROS) accumulation among treatments. Control and melatonin-treated leaves showed minimal staining, indicating low levels of H_2O_2 and superoxide radicals and overall healthy physiological status. In contrast, Pb-treated plants exhibited intense staining in both assays, reflecting severe ROS overproduction and oxidative damage **(Figure 3a)**. However, melatonin treatment markedly reduced staining intensity compared to Pb alone, demonstrating that melatonin effectively suppresses ROS accumulation and alleviates oxidative stress. In accordance with the visual staining the biochemical assay shows that the H_2O_2 accumulation under Pb stress condition increased about 1.9-fold compare to control. While exogenous melatonin supplementation to the stress plant showed 1.6-fold reduced H_2O_2 accumulation compared to PB only treatment **(Figure 3b)**.

Membrane stability, assessed through electrolyte leakage, further supported these findings. Control plants exhibited baseline leakage, while melatonin alone slightly reduced electrolyte leakage by 6.5% relative to control, indicating no harmful effect on membrane integrity. In contrast, Pb exposure sharply increased electrolyte leakage by 17.2% relative to control plants, reflecting severe membrane damage. However, exogenous melatonin application under Pb stress significantly electrolyte leakage by 12.9%, compared with the Pb only treatment, confirming its protective role in preserving membrane integrity under Pb stress. (**Figure 3c**). Malondialdehyde (MDA) content, a marker of lipid peroxidation, followed a similar trend. Control plants showed low MDA levels (0.34 mM g^{-1} FW), while melatonin slightly reduced it (0.25 mM g^{-1} FW), suggesting improved antioxidant status. Pb stress significantly elevated MDA (0.52 mM g^{-1} FW), indicating strong lipid peroxidation and oxidative injury. However, melatonin treatment reduced MDA to 0.37 mM g^{-1} FW, reflecting a 40.5% decrease compared to Pb alone and confirming reduced oxidative membrane damage (**Figure 3d**).

To unveil the mechanisms, we have also assed the total antioxidant capacity and DPPH activity. Results showed that total antioxidant capacity and DPPH activity significantly enhanced by melatonin application. For example, melatonin supplementation in control plant increased the antioxidant capacity about 14.2%. However, Pb exposure reduced antioxidant capacity to 45.3% compare to control plants, indicating suppression of defense mechanisms. Similarly, the DPPH activity reduced by 12.3% at Pb stress condition compare to control. Importantly, melatonin supplementation restored percent of antioxidant capacity and DPPH activity about 36.5% and 48.1% respectively, compare Pb only treatment (**Figure 3e-f**). This highlights melatonin's role in strengthening the antioxidant defense system under heavy metal stress. Overall, both histochemical and biochemical analyses confirm that melatonin effectively mitigates Pb-induced oxidative stress in brinjal by reducing ROS accumulation, stabilizing cell membranes, lowering lipid peroxidation, and enhancing antioxidant defense capacity.

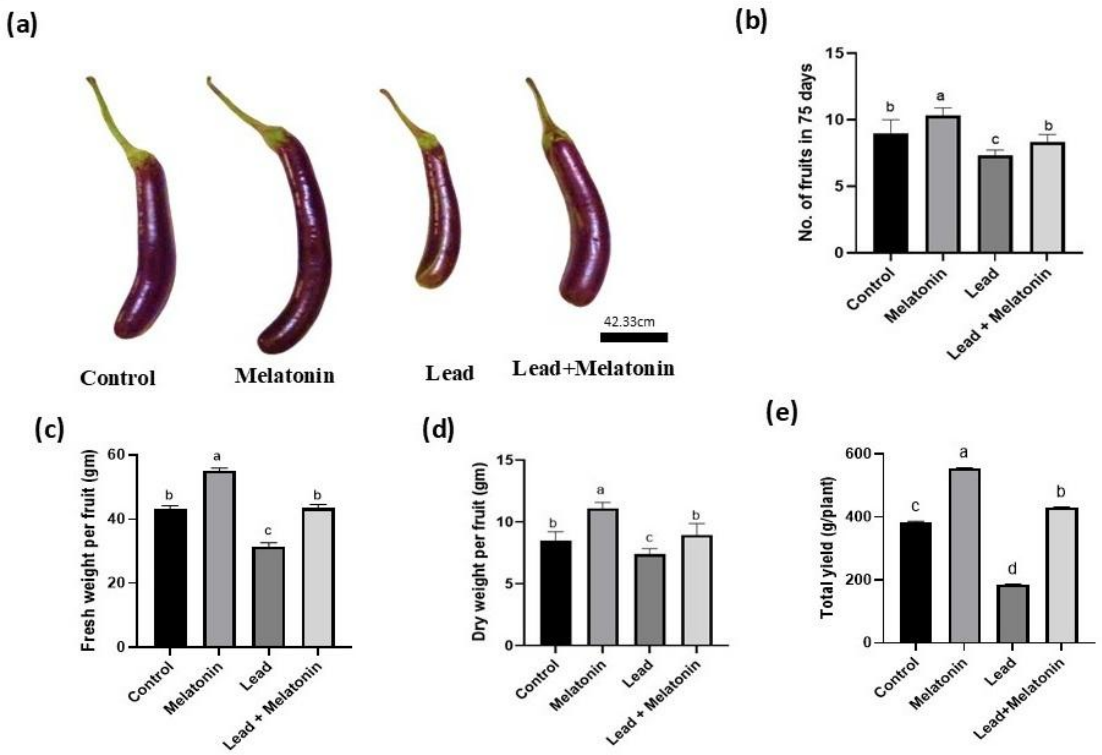


Figure 4: Effect of melatonin on yield attributes of brinjal under Pb stress: (a) fruit phenotypic traits, (b) total number of fruits per plant at 75 days after harvest, (c) fresh weight per fruit (g), (d) dry weight per fruit (g), and (e) total yield (g plant^{-1}). Values represent the mean of three replicates, and vertical bars indicate standard deviation (SD). Means followed by the same letter are not significantly different at $p < 0.05$ according to the least significant difference (LSD) test.

3.4 Effect of Melatonin on Fruit Phenotypic Traits and Yield of Brinjal under Pb Stress

Fruit phenotypic traits of brinjal varied markedly across treatments. Control plants produced fruits of normal size, shape, and pigmentation, indicating optimal development. Melatonin-treated plants exhibited visibly enhanced fruit size and improved coloration, suggesting stimulation of growth and developmental processes. In contrast, Pb exposure severely impaired fruit phenotype, resulting in smaller fruits with abnormal pigmentation, indicative of pronounced phytotoxicity (**Figure 4a**). However, melatonin supplementation under Pb stress partially restored fruit morphology, with noticeable improvements in size and appearance compared to Pb treatment alone. A comparable trend was observed in the number of fruit sets per plant. Melatonin application significantly increased fruit production to 13 fruits per plant, representing a 30% enhancement over the control. Conversely, Pb stress reduced fruit set to 7 fruits per plant, reflecting a 30% decline relative to the control plants. Notably, the combined treatment (Pb with melatonin) restored fruit number to 11 per plant, corresponding to a 57% improvement over Pb stress alone, demonstrating the protective role of melatonin in sustaining reproductive capacity under metal stress (**Figure 4b**).

Individual fruit weight was also significantly influenced by treatments. Melatonin application resulted in the highest fresh weight (60 g per fruit), a 20% increase over the control plants (50 g). In contrast, Pb stress reduced fresh weight to 35 g per fruit (30% decrease), whereas the combined treatment improved it to 45 g per fruit, reflecting a 28.5% recovery compared to Pb alone (**Figure 4c**). Similarly, dry weight per fruit was highest under melatonin (12 g), compared to control (9 g), while Pb exposure reduced it to 6 g. The combined treatment increased dry weight to 10 g per fruit, representing a 67% improvement over Pb treatment (**Figure 4d**). Consequently, fruit yield per plant was maximized under melatonin treatment (555 g per plant), indicating a 44.2% increase over the control (385 g). Pb stress drastically reduced yield to 185 g per plant, approximately 50% lower than the control plants. Importantly, exogenous melatonin significantly enhanced yield under Pb stress to 430 g per plant, corresponding to a 2.3-fold increase over Pb alone and an 11.6% improvement over the control (**Figure 4e**). Overall, these findings demonstrate that melatonin not only promotes fruit development and yield in brinjal but also effectively alleviates Pb-induced reproductive impairment, thereby sustaining productivity under heavy metal stress conditions.

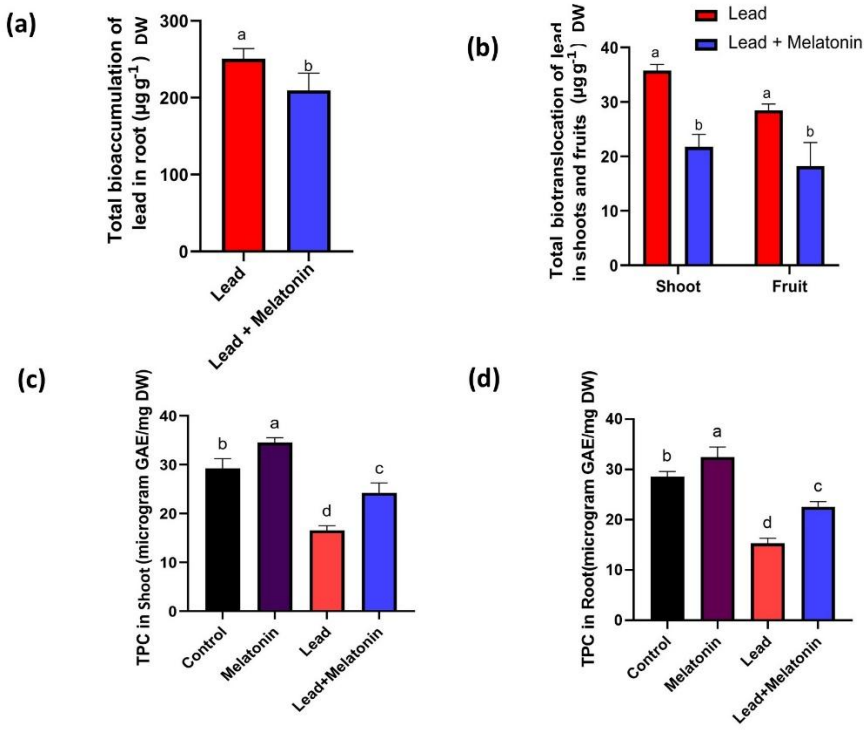


Figure 5: Effect of melatonin on regulation of Pb bioaccumulation and translocation in brinjal: (a) total Pb accumulation in roots (µg g^{-1} DW), (b) total Pb translocation to shoots and fruits (µg g^{-1} DW), (c) total phenolic compound (TPC) content (µg GAE/mg DW) in shoots, and (d) total phenolic compound (TPC) content (µg GAE/mg DW) in roots. Values represent the mean of three replicates, and vertical bars indicate standard deviation (SD). Means followed by the same letter are not significantly different at $p < 0.05$ according to the least significant difference (LSD) test.

3.5 Melatonin-Mediated Regulation of Pb Bioaccumulation, Translocation with enhanced Polyphenol content, and Nutrient Homeostasis in Brinjal

Melatonin significantly modulated Pb uptake and partitioning in brinjal plants. Under Pb stress, roots accumulated a high Pb concentration about 251.3 µg g^{-1} DW, indicating strong metal uptake and limited exclusion capacity. However, melatonin application reduced root Pb accumulation to 209.2 µg g^{-1} DW, suggesting that melatonin restricts Pb influx and/or enhances its sequestration within root tissues, thereby limiting its systemic availability (**Figure 5a**). Additionally, Pb translocation to aerial tissues was markedly influenced by melatonin. Pb-treated plants showed substantial Pb accumulation in shoots (35.7 µg g^{-1} DW) and fruits (28.5 µg g^{-1} DW) in total, highlighting efficient root-to-shoot transfer and potential risk to edible organs. In contrast, melatonin supplementation significantly reduced Pb levels to 21.7 µg g^{-1} DW in shoots and 18.4 µg g^{-1} DW in fruits, indicating that melatonin suppresses Pb mobility, likely via enhanced chelation, compartmentalization, or regulation of metal transport systems (**Figure 5b**). To understand the mechanism, we also assessed the total polyphenol content in shoot and root of brinjal plant that's strongly affected by both Pb stress and melatonin application **(Figure 5c-d)**. In shoot, total polyphenol content (TPC) was 29.2 µg GAE mg^{-1} DW in control plants and increased to 34.5 µg GAE mg^{-1} DW under melatonin treatment, reflecting stimulation of secondary metabolism. Pb exposure significantly reduced TPC (16.5 µg GAE mg^{-1} DW), indicating inhibition of phenolic biosynthesis under oxidative stress. However, exogenous melatonin treatment partially restored TPC 24.3 µg GAE mg^{-1} DW), suggesting that melatonin reactivates phenolic pathways and enhances antioxidant capacity (**Figure 5c**). A similar trend was observed in roots, where melatonin enhanced TPC (32.5 µg GAE mg^{-1} DW), Pb reduced it (15.3 µg GAE mg^{-1} DW), and combined treatment resulted in partial recovery (22.5 µg GAE mg^{-1} DW) (**Figure 5c**).

Nutrients	Control	Melatonin	Lead	Lead + Melatonin
Protein (mg g^{-1} DW)	56.2 ± 0.36^{c}	75 ± 0.72^{a}	37.5 ± 1.08^{d}	62.5 ± 0.36^{b}
K (mg g^{-1} DW)	38.5 ± 0.07^{b}	40 ± 0.12^{a}	36.4 ± 0.09^{c}	39.1 ± 0.28^{b}
Ca (mg g^{-1} DW)	3.12 ± 0.005^{b}	4.43 ± 0.01^{a}	2.34 ± 0.0^{d}	2.88 ± 0.01^{c}
Mg (mg g^{-1} DW)	1.74 ± 0.001^{b}	1.94 ± 0.001^{a}	1.68 ± 0.001^{c}	1.73 ± 0.001^{b}
P (mg g^{-1} DW)	6.66 ± 0.18^{b}	7.67 ± 0.31^{a}	4.57 ± 0.10^{c}	6.41 ± 0.09^{b}
S (mg g^{-1} DW)	27.5 ± 0.14^{b}	35.4 ± 0.23^{a}	12.3 ± 1.05^{d}	23.5 ± 0.19^{c}
Cu (mg g^{-1} DW)	20.5 ± 0.31^{ab}	19.3 ± 0.17^{b}	21.7 ± 0.45^{a}	13.5 ± 0.14^{c}
Fe (µg g^{-1} DW)	183 ± 0.20^{c}	190 ± 0.44^{b}	201 ± 0.41^{a}	150 ± 0.24^{d}
Mn (µg g^{-1} DW)	20.2 ± 0.15^{c}	16.1 ± 0.19^{d}	25 ± 0.57^{a}	23.4 ± 0.23^{b}
Zn (µg g^{-1} DW)	28.9 ± 0.23^{b}	32.8 ± 0.24^{a}	19.6 ± 0.19^{d}	27.1 ± 0.13^{c}
Pb (µg g^{-1} DW)	N/A	N/A	28.5 ± 1.25^{a}	18.2 ± 0.45^{b}

Table 1: Effect of melatonin on the nutritional properties of brinjal fruits under Pb stress. Values are presented as mean ± standard error of the mean (SEM). Means followed by different letters are significantly different at $p < 0.05$.

Fruit nutrient composition (**Table 1**) further demonstrated melatonin-mediated mitigation of Pb toxicity. Melatonin treatment enhanced key nutritional parameters, including protein (1.33-fold), Ca (1.42-fold), P (1.15-fold), and S (1.29-fold), while maintaining or slightly improving K, Mg, Fe, and Zn levels. In contrast, Pb exposure disrupted nutrient homeostasis, leading to reductions in essential macro- and micronutrients and increased accumulation of Cu and Pb. Importantly, melatonin co-application reduced Pb accumulation in fruits by 1.6-fold relative to Pb alone and partially restored nutrient balance, indicating improved ionic regulation and metabolic stability. Post-harvest soil nutrient dynamics (**Supplementary Table S1**) also reflected treatment-dependent changes. Melatonin application slightly enhanced soil nutrient availability (K, Mg, P, S, Fe, Mn, Zn), whereas Pb stress reduced overall nutrient levels, likely due to impaired root function and altered rhizosphere processes. Melatonin supplementation under Pd treatment showed partial recovery of several nutrients, particularly Mg and Cu, while some elements (e.g., P and S) remained below control levels. Pb residues were detected only in Pb-treated soils, with comparatively lower accumulation under melatonin supplementation. Collectively, these findings indicate that melatonin plays a critical role in mitigating Pb toxicity by regulating metal uptake and translocation, enhancing polyphenol-mediated antioxidant defense, and maintaining nutrient homeostasis, thereby improving plant performance and reducing Pb contamination in edible tissues.

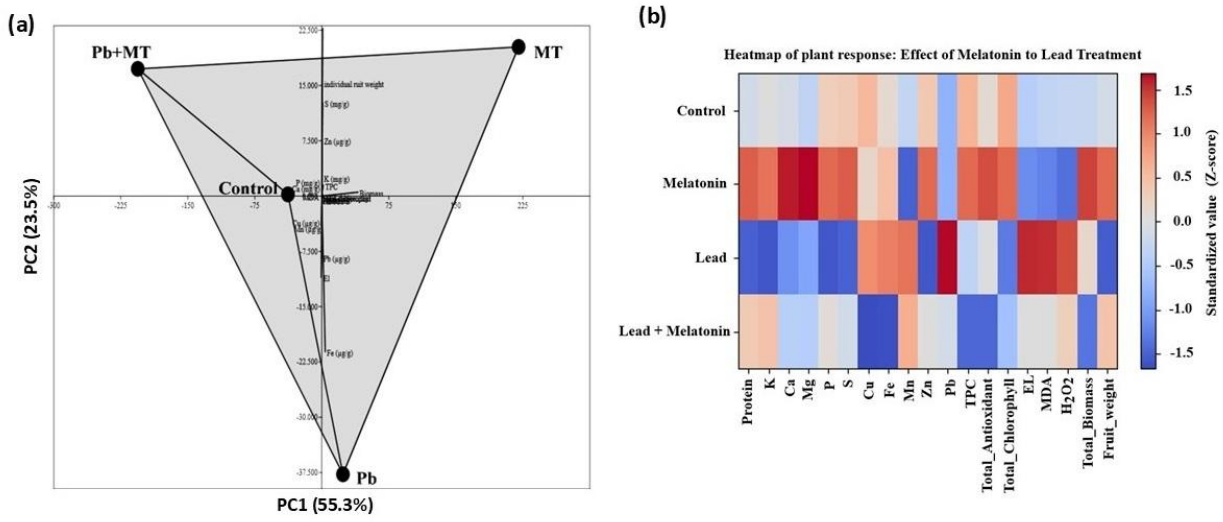


Figure 6: Principal component analysis (PCA) and correlation matrix heatmap showing the effects of melatonin on nutritional properties, physicochemical traits, and yield attributes of brinjal under Pb stress: (a) PCA plot and (b) correlation heatmap of plant responses to melatonin under Pb stress.

3.6 Principal Component Analysis (PCA) and Multivariate Response of Brinjal to Melatonin under Pb stress

Principal Component Analysis (PCA) revealed a clear discrimination among treatments, with the first two components explaining 78.8% of the total variance (PC1: 55.3%, PC2: 23.5%). The Pb-treated plants were distinctly separated along the negative axis of PC2 and strongly associated with stress-related variables, including Pb accumulation, electrolyte leakage (EL), malondialdehyde (MDA), and H_2O_2 content (**figure 6**). These parameters collectively indicate severe oxidative stress, membrane damage, and disruption of cellular homeostasis. Additionally, Pb treatment showed negative associations with essential macro- and micronutrients, reflecting impaired nutrient uptake and metabolic dysfunction under heavy metal toxicity.

In contrast, melatonin-treated plants were positioned in the positive region of both PC1 and PC2, closely correlated with growth-promoting and physiological parameters such as total biomass, fruit weight, chlorophyll content, total antioxidant capacity, and essential nutrients (K, Zn, and S). This distribution highlights the role of melatonin in enhancing metabolic efficiency, maintaining redox balance, and supporting nutrient assimilation. The Control plants were clustered near the origin, representing a balanced physiological state without pronounced stress or stimulation effects. Importantly, the Pb + melatonin treatment occupied an intermediate position, clearly separated from Pb alone and oriented toward the Control and melatonin clusters. This shift indicates that melatonin substantially mitigates Pb-induced toxicity by modulating plant physiological and biochemical responses, however full restoration to control conditions is not achieved.

Heatmap analysis further corroborated these findings by illustrating coordinated multivariate responses. Positive shifts (neutral to red) were observed for growth attributes, chlorophyll content, total antioxidant capacity, and key nutrients (K, Ca, Mg, P), indicating improved metabolic activity and nutrient homeostasis. Conversely, stress biomarkers (Pb accumulation, MDA, H_2O_2, EL) remained relatively elevated (blue), suggesting residual oxidative burden. Differential responses among micronutrients (e.g., Cu, Fe) further indicate selective regulatory effects. Collectively, the findings indicate that exogenous melatonin alleviates Pb-induced toxicity by improving redox homeostasis, nutrient balance, photosynthetic performance, antioxidant defense, and growth-related processes, ultimately enhancing plant tolerance to Pb stress.

4. Discussion

The present study demonstrates that exogenous melatonin plays a pivotal role in mitigating Pb-induced phytotoxicity in brinjal through coordinated physico-chemical adjustments (**Figure 1-6)**. Lead stress severely impaired plant growth, photosynthetic performance, antioxidant capacity, and yield, which is consistent with previous findings highlighting Pb as a potent inhibitor of plant metabolism (Hamim et al., 2019; Kohli et al., 2019). The observed reduction in chlorophyll content and net photosynthesis under Pb stress indicates disruption of chloroplast integrity and photosynthetic machinery (Cunha et al., 2020). This decline may be attributed to Pb-induced

inhibition of chlorophyll biosynthesis, enhanced pigment degradation, and interference with electron transport processes (Rahman et al., 2024). However, melatonin supplementation significantly restored chlorophyll content and photosynthetic rate, suggesting its protective role in maintaining chloroplast structure and function (**Figure 1**). This improvement may be linked to melatonin-mediated stabilization of photosynthetic pigments and enhancement of carbon assimilation efficiency. Furthermore, melatonin is known to modulate photosynthesis-related gene expression, thereby sustaining carbon fixation under stress conditions (Ebrahimi et al., 2023; Hasan et al., 2025a; Hasan et al., 2023; Xie et al., 2021).

A major consequence of Pb toxicity is the excessive accumulation of reactive oxygen species (ROS), leading to oxidative stress and cellular damage (Huihui et al., 2020). Accordingly, in the present study, Pb exposure resulted in pronounced accumulation of H_2O_2 and superoxide radicals, accompanied by elevated malondialdehyde (MDA) levels and electrolyte leakage, indicative of lipid peroxidation and membrane destabilization (**Figure 3**). Histochemical staining further confirmed enhanced ROS generation under Pb stress. These findings underscore the role of oxidative damage as a central mechanism of Pb toxicity (Huihui et al., 2020). In contrast, melatonin application significantly attenuated ROS accumulation and reduced oxidative biomarkers, highlighting its dual role as a direct free radical scavenger and an indirect regulator of the antioxidant defense network (Khan et al., 2020). This effect is likely mediated through both direct scavenging of ROS and upregulation of antioxidant defense systems, including increased total antioxidant capacity and DPPH activity (Altaf et al., 2022; Barand et al., 2020). Additionally, the observed increase in total polyphenol content under melatonin treatment suggests activation of secondary metabolism, contributing to enhanced redox buffering capacity and metal chelation (Bhattacharya and Jha, 2020; Xing et al., 2023).

Biomass accumulation and growth parameters were significantly suppressed under Pb stress, reflecting impaired cell division, nutrient uptake, and metabolic activity (Rahman et al., 2024). The marked reduction in fresh and dry weights confirms the inhibitory effects of Pb on plant development (**Figure 1**). However, melatonin application substantially improved biomass accumulation, indicating its role in promoting growth under stress conditions. This improvement may be associated with enhanced nutrient uptake, improved water relations, and maintenance of cellular homeostasis (Xing et al., 2023; Zhang et al., 2022).

An important aspect of this study is the modulation of Pb uptake, bioaccumulation, and translocation by melatonin, highlighting its critical role in mitigating Pb-induced stress. The present findings reveal that melatonin significantly reduced Pb accumulation in roots, shoots, and fruits, accompanied by lower bioaccumulation (BAF) and translocation factors (TF) values (**Figure 5**). These results indicate that melatonin effectively limits Pb uptake and translocation within the plant. Although the present study did not directly investigate the underlying molecular mechanisms, the observed reductions in Pb accumulation and translocation suggest that melatonin may restrict Pb mobility through mechanisms such as enhanced metal chelation, sequestration in root tissues, and modulation of metal transporter activity (Arnao and Hernández-Ruiz, 2019; Karpagam and Godwin, 2025; Xing et al., 2023). As recent studies have demonstrated that melatonin-mediated suppression of the metal transporter genes *OsMTP1*,

OsHMA9, and *OsNRAMP5*, together with the upregulation of the phytochelatin biosynthetic gene *OsPCS1*, significantly enhances Pb tolerance in rice by limiting Pb uptake and promoting metal detoxification (Khan et al., 2025a; Khan et al., 2025b). The reduced Pb content in brinjal fruits is particularly important from a food safety perspective, as it minimizes the risk of dietary exposure to toxic metals. Pb stress also disrupted nutrient homeostasis, leading to reduced concentrations of essential macro- and micronutrients (table-1). Such imbalances can further aggravate metabolic dysfunction and growth inhibition. However, melatonin treatment partially restored nutrient levels (Table-1. For example, melatonin supplementation enhanced sulfur (S) accumulation in brinjal by 1.9-fold compare to Pb only treatment suggesting improved ion uptake, transport, and utilization. This recovery may be linked to enhanced root system functionality and membrane stability, facilitating selective ion transport under stress conditions (Karpagam and Godwin, 2025; Xing et al., 2023).

Reproductive performance and yield were significantly affected by Pb stress, as evidenced by reduced fruit number, weight, and overall yield (**Figure 4)**. These reductions are likely linked to impaired photosynthesis, hormonal imbalance, and oxidative damage affecting reproductive organs (Hasan et al., 2025b; Javed et al., 2018; Rahman et al., 2024). Notably, melatonin application significantly improved fruit set, fruit weight, and yield under Pb stress, demonstrating its role in sustaining reproductive development. The enhancement of yield attributes may result from improved photosynthetic efficiency, better assimilate partitioning, and protection of reproductive tissues from oxidative damage (Harimadhav et al., 2026; Zhu et al., 2026).

Multivariate analyses (PCA and heatmap) provided integrative insights into treatment responses. The clear separation of Pb-treated plants with stress-related parameters confirms the detrimental effects of Pb toxicity. In contrast, the clustering of melatonin treated plants with growth and antioxidant traits highlights its positive regulatory role (**Figure 6)**. The intermediate positioning of melatonin supplementation under Pb treatment indicates partial but substantial recovery, suggesting that melatonin modulate plant responses toward stress tolerance (Shah et al., 2025). However, the present study was conducted under controlled net-house pot conditions using a single brinjal cultivar, one Pb concentration (30 mg kg^{-1} soil), and one melatonin concentration (100 μM). Furthermore, the study did not include molecular analyses or field validation. Therefore, future studies should evaluate multiple cultivars and dose combinations under field conditions and incorporate molecular approaches to elucidate the mechanisms underlying melatonin-mediated Pb tolerance. Nevertheless, the present the findings demonstrate that melatonin mitigates Pb toxicity through a multifaceted mechanism involving regulation of ROS homeostasis, enhancement of antioxidant defense and polyphenol metabolism, protection of photosynthetic machinery, maintenance of nutrient balance, and restriction of Pb uptake and translocation as shown in **figure 7** (Harimadhav et al., 2026; Xing et al., 2023). These integrated responses collectively contribute to improved growth, physiological stability, and productivity of brinjal under Pb stress conditions.

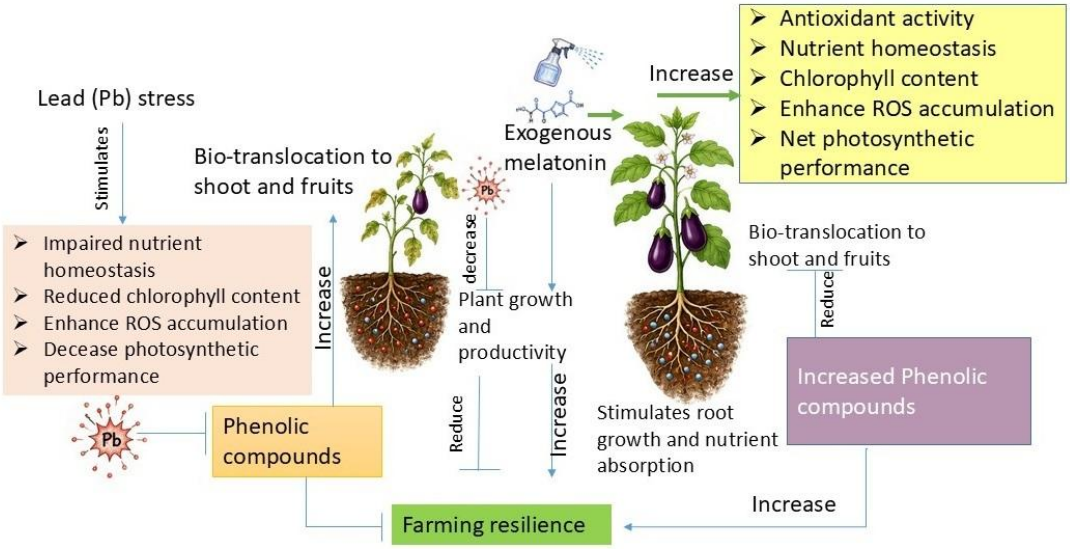


Figure 7: Schematic representation of melatonin-mediated alleviation of Pb-induced phytotoxicity in brinjal (*Solanum melongena* L.).

Conclusion

This study provides mechanistic insights into the role of melatonin as a multifunctional regulator of Pb stress tolerance in brinjal. Lead toxicity induced severe physiological and biochemical disruptions, including impaired photosynthesis, oxidative stress, nutrient imbalance, and reduced growth and yield. Exogenous application of melatonin effectively mitigated these adverse effects by enhancing antioxidant defense systems, reducing ROS accumulation, stabilizing membrane integrity, and preserving photosynthetic efficiency. Importantly, melatonin significantly reduced Pb accumulation and translocation, particularly to edible fruit tissues, while improving nutrient homeostasis and polyphenol metabolism, thereby contributing to enhanced plant performance under Pb stress. Although complete recovery to control levels was not achieved, melatonin markedly improved plant resilience and productivity under Pb stress. In a nutshell, while improving nutrient homeostasis and polyphenol metabolism, thereby contributing to enhanced plant performance under Pb stress (**figure 7)**. Although complete recovery to control levels was not achieved, melatonin substantially improved plant resilience and productivity. Overall, these findings suggest that melatonin is a promising and environmentally friendly approach for mitigating Pb toxicity in brinjal under controlled conditions. However, its agronomic effectiveness and practical applicability require further validation across multiple cultivars and Pb levels under field conditions. Future studies should also investigate the molecular signaling networks and gene regulatory mechanisms underlying melatonin-mediated Pb tolerance to facilitate its effective application in sustainable crop production.

CRedit author contribution statement:

Shafiul Karim: Investigation and writing-original draft, **Md. Kamrul Hasan:** Conceptualization, writing-original draft, writing-original revised, funding acquisition and supervised the study. **Tushar Kanti Das:** Investigation and formal analysis. **Bidya Bhussan Dey**: Investigation and formal analysis, **Joyita Roy**: Investigation and formal analysis. **Sabrina Mehenaz Tan:** Investigation and formal analysis. **Munmun Saha**: Conceptualization, formal analysis and resources. **Biswojit Debnath:** Writing-review and editing. **Biplop Kumar Shaha:** Writing-review and editing. **Md. Sirajul Islam Khan:** Writing-review and editing. All authors have read and agreed to the published version of the manuscript. **Jannat Shopan:** Formal analysis and writing-review and editing.

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