Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5933

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (1):** 903-909 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Cadmium Induced Stress Mitigation in Tomato by Exogenous Melatonin

M. Umapathi[1*], M. K. Kalarani[2], M. Udhaya Bharathi[1] and P. Kalaiselvi[3]

[1*]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, India

[2]Tapioca and Castor Research Station, Yethapur, Tamil Nadu Agricultural University, India

[3]Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore, India

*Corresponding Author E-mail: umapathi182@gmail.com

Received: 25.10.2017 | Revised: 29.11.2017 | Accepted: 6.12.2017

ABSTRACT

Melatonin is a ubiquitous signal molecule, playing crucial roles in plant growth and stress tolerance. Its beneficial effects include better water conservation in leaves, more chlorophyll contents, enhanced endogenous melatonin, synthesis of phytochelatins (PCs), over production of antioxidants, greater photosynthetic performance under stress conditions. In the present study, melatonin application significantly increases cadmium tolerance in tomato plants. This study was undertaken with PKM 1 variety of tomato was selected to evaluate the melatonin function under cadmium stress. Pre-treatment with melatonin significantly induced endogenous melatonin content in plant but foliar spray 25 ppm of melatonin had more marked effect on endogenous melatonin biosynthesis and also it reduces cadmium accumulation in the economical part of the plant through its phytochelating process.

Key words: *Melatonin, Cadmium, Phytochelatins, Stress, Antioxidants*

INTRODUCTION

Vegetables (Spinach, Cabbage, Beetroot, Raddish, Okra, Tomato and Cucumber) are an important part of human's diet. In addition to a potential source of nutrients, vegetables constitute important functional food components by contributing proteins, vitamins, iron and calcium which have marked health effects[2]. Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crop belongs to family Solanaceae and grown throughout the world because of its wider adaptability, high yielding potential and suitability for variety of uses in fresh as well as processed food industries. It is grown in farm and kitchen garden for slice, soup, sauce, ketch-up and vegetable. When tomato growing area is contaminated with heavy metals by waste and sewage water irrigation in urban areas of vegetable cultivation, heavy metals are the important types of contaminants that can be found on the surface and in the tissue of fresh vegetables. Prolonged human consumption of unsafe concentrations of heavy metals in food stuffs may lead to the disruption of numerous biological and biochemical processes in the human body.

Cite this article: Umapathi, M., Kalarani, M.K., Bharathi, M.U. and Kalaiselvi, P., Cadmium Induced Stress Mitigation in Tomato by Exogenous Melatonin, *Int. J. Pure App. Biosci.* **6(1):** 903-909 (2018). doi: http://dx.doi.org/10.18782/2320-7051.5933

The rising concern on cadmium (Cd) as a toxic heavy metal is not only due to its detrimental effects on crop production but also for potential health hazards associated with food chain contamination[1,7,14]. Cd accumulated and disrupts normal metabolism leading to diverse morphological, physiological, biochemical and cellular changes in crop plants[12,15] . Cd has been shown to interfere with the uptake, transport, and utilization of essential nutrients and water to decrease photosynthesis, changes in enzyme activities and also to cause symptoms such as chlorosis, necrosis and root browning in tomato[13]. Inhibition of Calvin cycle enzymes, photosynthesis and carbohydrate metabolism by Cd results in low biomass accumulation in plants[14]. On the other hand, Cd accumulation in edible plant parts ultimately poses threat to human health as Cd toxicity is closely associated with various health hazards including kidney damage, bladder and lung cancer[1]. Melatonin (*N-acetyl-5-methoxytryptamine*) was discovered in 1958 in the bovine pineal gland. It is one of the best studied biological molecules and its role has been explored in mammals, birds, amphibians, reptiles and fish. Melatonin has many physiological roles in animals, influencing circadian rhythms, mood, sleep, body temperature, loco motor activity, food intake, retina physiology, sexual behaviour, seasonal reproduction and the immune system. In plants, research efforts over the past decade have focused on determining its many roles in plant physiology. Solid evidence implicates melatonin as a growth promoter, rooting agent and stress tolerance[10]. Heavy metal stress especially Cd stress positively alleviated by exogenous application of melatonin. The contents of Cd and melatonin were gradually increased over time under Cd stress. However, such increase in endogenous melatonin was incapable to reverse detrimental effects of Cd. Exogenous melatonin spray to the plant that would be increase the endogenous melatonin content and mitigate the cadmium toxicity[4]. Although PCs (phytochelatins) are well known to bind many toxic metals including Cd to promote metal detoxification and subsequent stress tolerance in plants, information relating to melatonin-induced PC biosynthesis and stress tolerance in higher plants is missing. The objective was to examine the influence of exogenous melatonin can increase the endogenous melatonin and then reduce the accumulation of Cd content in plant parts.

MATERIAL AND METHODS

Experiment was performed at the Department of Crop Physiology, Tamil Nadu Agricultural University**,** Coimbatore with tomato PKM 1 during 2015-2016. The nursery was maintained in protrays and vermicompost used as medium for raising seedlings. Seeds were sown in protrays and irrigation was given through rose cane periodically up to 25 days and transplanted to pots. Cadmium spiking was done with red sandy soil and used for pot culture experiment. Single pot contains 16 kg of soil. Before preparing pot mixture, water holding capacity (WHC) of experimental soil was measured, based on the WHC, known quantity of cadmium salt (0.48 g/pot) dissolved in known quantity of water (40 ml) and cadmium containing solution was prepared. Prepared cadmium solution was poured into the experimental soil and thoroughly mixed (spiking). It was done for each pot individually. Spiked soil was filled in the experimental pots. Twenty five days aged seedlings were transplanted and one plant was maintained in each pot. Crop was applied with recommended dose of fertilizers and other cultivation operations including plant protection measures were carried out as per recommended package of practices of Tamil Nadu Agricultural University, Coimbatore. The experiment was laid out in completely randomized block design (CRD) with three replications and nine melatonin treatment viz. seed treatment with 250 ppm (T_3), foliar spray 25 ppm (T_4), 50 ppm (T_5), 100 ppm (T_6) other three treatments were comprised with these foliar sprays used individually along with seed treatment of 250 ppm (T_7, T_8, T_9), control pot with only Cd and no melatonin (T_2) and absolute control pot (T_1) maintained with no Cd and melatonin for comparison purpose.

Foliar spray was given in two times at vegetative and flowering stage. The following observations were recorded at vegetative (30-45 DAT), flowering (45-60 DAT) and fruit formation (65-90 DAT) stages.

Endogenous Melatonin Content

Leaf and fruit endogenous melatonin content was determined by using HPLC available at Department of Microbiology, TNAU, Coimbatore. Direct sample extraction method with ethyl acetate using the method described by Arnao and Hernandez[3]. Fresh leaf samples was collected (0.1-1g), cut into small sections (3-5mm) and placed in vials containing ethyl acetate (3 ml) over night (15 hours) at 4°C in darkness with shaking, the sections were discarded after being washed with the respective solvent (0.5 ml).The extract and washing from each sample were evaporated to dryness under vacuum using a Speed Vac (Thermo Savant SPD111V) coupled to a refrigerated RVT400 (USA) vapour trap. The residue was dissolved in acetonitrile (0.5 ml), filtered (0.2 μm) and analysed with HPLC fluorescence detection and expressed in ppm.

Estimation of Cadmium Heavy Metal Content

Root, leaf and fruit cadmium content was estimated by using Atomic Absorption Spectrometer (AAS) available at Department of Environmental Science, TNAU, Coimbatore. The amount of total cadmium in the effluent was determined by digesting the sample with aqua regia (Nitric and Sulphuric acid in the ratio of 1:3) in a hot plate for about 110^0C for 2 h. The concentration of Cd in the digests was measured by Atomic Absorption Spectrophotometer (AAS, Perkin Elmer) using air- acetylene flame . The adsorption percentage is also calculated by using the formula,

$$\% \text{ Adsorption} = (C_i - C_f)/C_i \times 100$$

C_i (mg/L) and C_f (mg/L) are the initial and final metal concentrations and express in ppm.

The data on various parameters were analysed statistically as per the procedure suggested by Gomez and Gomez[6]. Wherever the treatment differences are found significant, critical differences were worked out at five per cent probability level and the values were furnished.

RESULTS AND DISCUSSION

Endogenous melatonin content was recorded in leaf and fruit. Leaf melatonin was recorded at three different stages and average was taken and tabulated in Table 1. Plants under absolute control had less capacity to accumulate melatonin in leaf (1.0 ppm) and also in fruit (2.0 ppm). Plants under cadmium stress also increased the melatonin content in leaf (1.6 ppm) and in fruit (4.0 ppm) compared to unstressed plants. Melatonin application had greater effect on improving endogenous melatonin was found in this study. Foliar spray of 25 ppm alone raised the melatonin level to 9.0 ppm in leaf and 4.8 ppm in fruit which was highly significant from other treatments followed by melatonin seed treatment with 250 ppm plus 25 ppm foliar spray (7.1 ppm and 4.6 ppm). Foliar spray 50 ppm and 100 ppm of melatonin were on par with each other.The exposure of tomato seedling to Cd induced substantial increases in endogenous melatonin concentrations gradually over time. However, such increase in endogenous melatonin was unable to ameliorate oxidative stress possibly due to insufficient ROS scavenging by melatonin in parallel with Cd-induced ROS generation. Nevertheless, induction of endogenous melatonin by Cd may indicate an adaptive response in plants as melatonin is well-recognized as a ubiquitous signal molecule[4]. In contrast, exogenous application of melatonin efficiently ameliorates Cd-induced oxidative stress and also noticed that exogenous melatonin greatly induced endogenous melatonin content (Fig. 1). The present investigation confirmed that exogenous melatonin could stimulate endogenous melatonin level to confer Cd tolerance. It is worth mentioning that, melatonin showed significantly different roles in regulating plant growth and development even in the same species, which support our current observation[17]. The study was also supported by Hasan *et al*.[8].Cd content was observed in root, leaf, fruit and post harvested soil and data presented in Table 2. The result on Cd content revealed that, significant differences were observed between the treatments. Among the melatonin treatments, foliar spray 25 ppm melatonin registered the highest accumulation of Cd content in root (10.3 ppm) and concomitant reduction in leaf (0.3 ppm)and below deducting level (BDL) in fruits was observed compared to control which recorded maximum amount of Cd in root, leaf and fruit of 15, 1.5 and 2.8 ppm respectively (Fig. 1). Remaining Cd metals were accumulated in the residual soil (post-harvest soil).When the tomato plants are exposed to Cd stress, the uptake of Cd content was more because Cd metals are easily assimilated in plant parts (root, leaf and fruit) due to specificity of ion channels and divalent metal transporters[11]. Cd leads to disturb the plant morphological, physiological, biochemical and cellular changes[15] and also it inhibits Calvin cycle enzymes, photosynthesis, and carbohydrate metabolism by Cd results in low biomass accumulation in plants[14]. Plants have intrinsically evolved sophisticated cellular mechanisms for Cd detoxification and tolerance such as immobilization, exclusion, chelation and compartmentalization of metal ions and the repair of cell structural alteration[7]. The earlier findings corroborated well with the present study. The root cell wall, which is in direct contact with heavy metals in soil, can first release chelating compounds, such as phytosiderophores, nicotianamine and organic acids, to bind Cd and other heavy metals in the epidermis[16]. Reason behind the melatonin treated plants accumulate less amount of Cd is melatonin had a direct function as antioxidant, melatonin-induced biosynthesis of metabolites like organic acids, vitamin A, squalene and subsequent compartmentalization of Cd in cell wall and vacuole might play a critical role to confer Cd tolerance in tomato[9] . These earlier findings provide a valid support for the result obtained in the present study.

Table 1: Effect of melatonin on endogenous melatonin (ppm) content of tomato under cadmium toxicity

Treatments	**Leaf (ppm)**	**Fruit (ppm)**
T_1: Absolute control	1.0	2.0
T_2 : Control (Soil : 30 ppm Cd)	1.6	4.0
T_3: Soil (30 ppm Cd) + Seed treatment (250 ppm melatonin)	6.4	3.7
T_4: Soil (30 ppm Cd) + Foliar spray (25 ppm melatonin)	9.0	4.8
T_5: Soil (30 ppm Cd) +Foliar spray (50 ppm melatonin)	4.6	2.8
T_6: Soil (30 ppm Cd) + Foliar spray (100 ppm melatonin)	4.8	2.6
T_7: Soil (30 ppm Cd) + Seed treatment (250 ppm melatonin) + Foliar spray (25 ppm melatonin)	7.1	4.6
T_8: Soil (30 ppm Cd) + Seed treatment (250 ppm melatonin) + Foliar spray (50 ppm melatonin)	4.9	1.0
T_9: Soil (30 ppm Cd) + Seed treatment (250 ppm melatonin) + Foliar spray (100 ppm melatonin)	4.7	1.3
Mean	**4.90**	**2.97**
SEd	**0.28**	**0.15**
CD (0.05)	**0.60**	**0.32**

*Foliar spray at Vegetative (30 DAT) and Flowering stage (45 DAT)

Table 2: Effect of melatonin on cadmium content (ppm) of tomato under cadmium toxicity

Treatments	Root (ppm)	Leaf (ppm)	Fruit (ppm)	Post-harvest soil (ppm)
T_1: Absolute control	0.3	0.1	BDL	0.6
T_2: Control (Soil : 30 ppm Cd)	15.0	1.5	2.8	9.0
T_3: Soil (30 ppm Cd) + Seed treatment (250 ppm melatonin)	9.8	0.8	BDL	18.4
T_4: Soil (30 ppm Cd) + Foliar spray (25 ppm melatonin)	10.3	0.3	BDL	18.9
T_5: Soil (30 ppm Cd) +Foliar spray (50 ppm melatonin)	9.2	1.2	BDL	17.7
T_6: Soil (30 ppm Cd) + Foliar spray (100 ppm melatonin)	9.0	1.1	BDL	18.5
T_7: Soil (30 ppm Cd) + Seed treatment (250 ppm melatonin) + Foliar spray (25 ppm melatonin)	9.1	0.5	BDL	19.8
T_8: Soil (30 ppm Cd) + Seed treatment (250 ppm melatonin) + Foliar spray (50 ppm melatonin)	9.5	1.0	BDL	18.7
T_9: Soil (30 ppm Cd) + Seed treatment (250 ppm melatonin) + Foliar spray (100 ppm melatonin)	9.3	1.3	BDL	17.6
Mean	**9.05**	**0.86**	**0.31**	**15.53**
SEd	**0.44**	**0.04**	**0.02**	**0.85**
CD (0.05)	**0.93**	**0.08**	**0.04**	**1.80**

*Foliar spray at Vegetative (30 DAT) and Flowering stage (45 DAT), BDL- Below Detecting Level

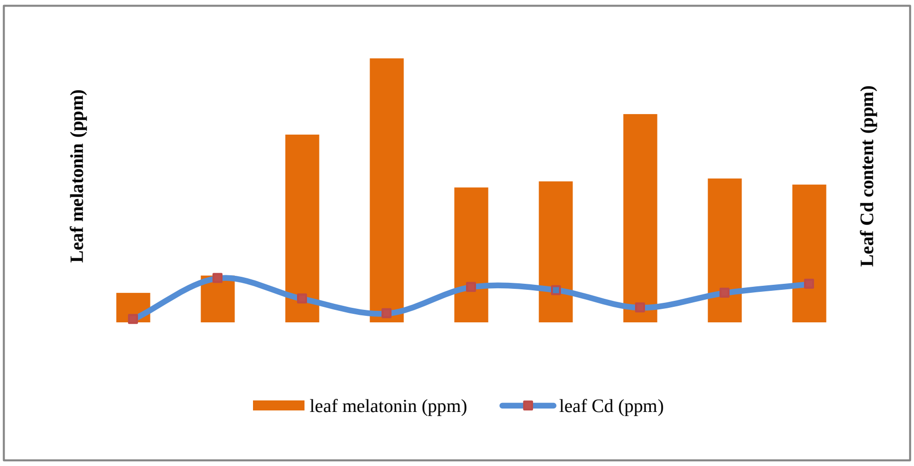

Fig. 1: Effect of melatonin on cadmium and melatonin accumulation in (ppm) tomato leaf under cadmium toxicity

Treatment details

T1	:	Absolute control	T6	:	Foliar spray 100 ppm of melatonin
T2	:	Control	T7	:	T3+ Foliar spray 25 ppm of melatonin
T3	:	Seed treatment 250 ppm of melatonin	T8	:	T3+ Foliar spray 50 ppm of melatonin
T4	:	Foliar spray 25 ppm of melatonin	T9	:	T3+ Foliar spray 100 ppm of melatonin
T5	:	Foliar spray 50 ppm of melatonin			

CONCLUSION

This study showed that both exogenous melatonin and Cd stress could induce endogenous melatonin accumulation. However, exogenous melatonin had complex and influential effects on Cd immobilization in cell walls and vacuoles under Cd stress. Even though, exogenous melatonin had no effect on root Cd content but drastically reduced leaf Cd content, which indicates its potential role in regulating Cd translocation and tolerance. Therefore, these findings can be implicated for developing new strategies to produce safe food as an eco-friendly manner particularly in marginal areas where Cd contamination is a limiting factor for crop production.

REFERENCES

1. Ajjimaporn, A., Botsford, T., Garrett, S.H., Zhou, X.D. and J.R. Dunlevy, J.R., ZIP8 expression in human proximal tubule cells, human urothelial cells transformed by Cd^{+2} and As^{+3} and in specimens of normal human urothelium and urothelial cancer. *Cancer Cell Int.* **12:** 16 (2012).
2. Arai, S. Global View on Functional Foods: Asian Per- spectives. *B. J. Nutr*. **88(2):** 139-143 (2002).
3. Arnao, M.B. and J. Hernandez-Ruiz, J., Assessment of Different Sample Processing Procedures Applied to the Determination of Melatonin in Plants. *Phyto chem. Anal.,* **(20):** 14–18 (2008).
4. Byeon, Y., Lee, H.Y., Hwang, O.J., Lee, H.J., Lee, K. and Back, K., Coordinated regulation of melatonin synthesis and degradation genes in rice leaves in response to cadmium treatment. *J. Pineal Res.* **58:** 470–478 (2015).
5. DalCorso, G., Farinati, S. and Furini, A., Regulatory networks of cadmium stress in plants. *Plant Signal Behav***. 5:** 663–667 (2010.).
6. Gomez, K.A. and Gomez, A.A., Statistical procedures for agricultural research. (2nd Ed.) John Wiley and sons, NewYork, USA (1984).
7. Hall, J.L., Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot,* **53:** 1–11 (2002).
8. Hasan, M.K., Ahammed, G., Yin, L., Shi, K., Xia, X. Zhou, Y., Yu, J. and Zhou, J., Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration and antioxidant potential in tomato (*Solanum lycopersicum* L.). *Frontiers in Plant Sci.* **6(601): (**2015**).**
9. Hasan, S.A., Hayat, S. and Ahmad, A., Brassinosteroids protect photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. *Chemosphere*. **84:** 1446–1451 (2011).
10. Hernandez, R.J., Cano A, Arnao M.B., Melatonin acts as a growth-stimulating compound in some monocot species. *J. Pineal Res.* **39:** 137–142 (2005).
11. Liu, J.G., Liang, J.S., Li, K.Q., Zhang, Z.J., Yu, B.Y. and Lu, X.L., Correlations between cadmium and mineral nutrients in absorption and accumulation in various genotypes of rice under cadmium stress. *Chemosphere*. **52:** 1467–1473 (2003).
12. Llamas, A., Ullrich, C.I. and Sanz, A., Cd^{2+} effects on transmembrane Electrical potential difference, respiration and

membrane permeability of rice (*Oryza sativa* L) roots. *Plant Soil*. **219:** 21–28 (2000).

13. Lopez, M.A.F., Sagardoy, R., Solanas, M., Abadia, A. and Abadia, J., Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics. *Environ. Exp. Bot*. **65:** 376–385 (2009).
14. Mobin, M. and Khan, N.A., Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. *J. Plant Physiol.* **164:** 601 610 (2007).
15. Pietrini, F., Iannelli, M.A., Pasqualini, S. and Massacci, A., Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplast of *Phragmites australis* (Cav.) *Trin. exsteudel*. *Plant Physiol.* **133:** 829–837 (2003).
16. Xiong, J., An, L.Y., Lu, H. and Zhu, C., Exogenous nitric oxide enhances cadmium tolerance of rice by increasing pectin and hemi cellulose contents in root cell wall. *Planta*. **230:** 755–765 (2009).
17. Zhang, N., Sun, Q., Zhang, H., Cao, Y., Weeda, S. and Ren. S., Roles of melatonin in abiotic stress resistance in plants. *J. Exp. Bot.* **66:** 647-656 (2015).