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Optimization of Melatonin to Mitigate Cadmium Stress at Seedling Level in Tomato

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Heavy metals are important environmental pollutants in nature. Crops and vegetables growing in soils contaminated with heavy metals have greater accumulation of heavy metals than those grown in uncontaminated soil. Melatonin is auxins like hormone which serves many physiological functions in plants and protects the plants from adverse environment through the up regulation of metabolic compounds and plays an important role in seed germination and heavy metal tolerance index at the seedling level. A study was undertaken to assess the optimum concentration for imposing cadmium stress and melatonin for mitigating Cd stress and improving seedling health index in tomato (PKM 1). Cadmium chloride ($CdCl_2$) was used to impose cadmium (Cd) heavy metal stress in five different concentrations 10, 20, 30, 40 and 50 ppm of Cd. Seeds sown in 10 and 20 ppm Cd solution recorded 75 and 68 per cent germination respectively while 50 per cent germination was observed in 30 ppm Cd. Based on the results, 30 ppm Cd concentration was selected to induce Cd toxicity in laboratory and pot culture experiments for the mitigation of Cd. To optimize melatonin concentration for mitigation of Cd, different concentration of melatonin ranging from 25, 50, 100 and 250 ppm was applied as seed treatments with 30 ppm Cd. Different parameters of seedling health characters were observed. The result revealed that, seed treatment with 250 ppm of melatonin showed the maximum root length (3.7 cm), promptness index (51.2) and total dry matter production (0.284g/seedling) compared to other treatments. This laboratory experiment concluded that 30 ppm cadmium is optimum concentration to impose cadmium stress and 250 ppm melatonin is the optimum for mitigating Cd toxicity.

Key words: Auxin, Cadmium, Seedling health index

Heavy metals are naturally present in the environment. Their concentration has gradually increased due to the natural and anthropogenic activities such as industrialization. Copper and Zn are important micronutrients if present in low concentration, while in high concentration, these two metals become toxic to plants. The role of Cd and Pb as nutrients is not yet well known (Jeliazkova and Craker, 2001). Plants readily accumulate them in their system. Cadmium is also produced as a by-product of the process of smelting (heating and melting ores to extract metals). Crops and vegetables growing in soils contaminated with heavy metals. Tomato is one of the important horticulture crops, cultivated in all over the world. 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 tissue of fresh vegetables (Umapathi *et al*., 2017). Cd toxicity causes delay in germination, induce membrane damage, and impair food reserve mobilization by increased cotyledon or embryo ratios of total soluble sugars, glucose, fructose and amino acids (Rahoui *et al*., 2010). Cd toxicity led to stimulated expression of Glutathione peroxidase (GPX) (a thioredoxin dependent enzyme in plants) and a drastic reduction in glutathione reductase (GR) activity thereby modulating the level of thiol during the germination. Many investigations reported inhibiting effect of Cd on fresh and dry mass accumulation, height, root length, leaf area and other biometric parameters of plants. Hediji *et al*. (2010) reported fresh weight reduction of *Solanum lycopersicum* under high Cd levels. Cd stress effect can be elicited by the several phytohormones such as abscisic acid (ABA), brassinosteroids (BRs), jasmonic acid (JA), ethylene (EHT) and salicylic acid (SA) was found to be associated with Cd stress response. Exogenous 24-epibrassinolide (EBR, a biologically active BR) could not only alleviate Cd-induced photosynthetic inhibition and oxidative stress, but also could reduce Cd content in tomato plants (Ahammed *et al.,* 2013).

Most recently, Cd has been reported to regulate melatonin content in rice, a ubiquitous signal molecule, well accepted as a new plant growth regulator or bio stimulator rather than plant hormone (Byeon *et al*., 2015). Posmyk *et al*. (2008) found that, melatonin pre-treatment increased the germination of seeds from *Brassicaoleracea rubrum* L. by about 17 per cent in water and by about 12–14 per cent in the presence of copper. Melatonin plays a role as plant growth regulator. In consider the molecular structure

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of melatonin and IAA, the relationship between the molecular structure and biological activity is close. Melatonin has also proven its protective role against drought, ultraviolet radiation, heavy metals and chemicals stress. Melatonin is also useful to save plants from heavy metals stress, as pre sowing seed treatment of red cabbage seed (*Brassicaoleracea rubrum* L.) eliminated the toxic effects of copper ions (0.5 and 1mM) during germination and early seedling growth (Posmyk *et al*., 2008). The present work was conducted to assess the optimum concentration of cadmium and melatonin for improving seedling health index and cadmium tolerance in tomato.

Material and Methods

This laboratory study was carried out at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore to mitigate the cadmium toxicity in tomato (*Solanum lycopersicum* L.) by exogenous application of melatonin. Petri plates were sterilized using 0.01 per cent $HgCl_2$ and 70 per cent ethanol and finally washed with distilled water. Tomato variety PKM 1 was obtained from Horticulture College and Research Institute, Periyakulam for this investigation. The duration of variety is 140 days.

Standardization of cadmium

Cadmium chloride ($CdCl_2$) salt aqueous solution was used to impose the Cd stress in five different concentration *viz* 10,20,30,40,50 ppm for standardization of cadmium. Germination percentage and vigour index of the seedling was calculated based on the germination count, which was taken every fourth day after germination.

Melatonin standardization

Based on the experiment I, 30 ppm Cd was selected for inducing Cd toxicity in experiment II. Tomato seeds were soaked in 25, 50, 100, 250 ppm melatonin concentration for 12 hrs and the seeds were dried under shade for four hours. Later the treated seeds were placed on germination paper in each petridish separately. The germination paper was moistened with regular interval by 30 ppm CdCl2 solution for toxicity and water for absolute control. The petridishes were kept in laboratory under room temperature. The seeds were allowed to germinate by pouring the 10 ml of 30 ppm of CdCl2 solution once in three days. Distilled water was used for maintaining the absolute control. Three replicates were used for each treatment and the observations were taken periodically. Germination percentage was recorded at every 24 hrs interval up to 15 days. Seeds were considered as germinated when the radicle was at least 2 mm long. Finally germination was recorded on the 15th day and number of seeds germinated was expressed as per cent. The vigour index of the seedlings was calculated using the formula proposed by Abdul and Anderson (1973). Promptness index and germination stress index were observed on ten days after. The number of germinated seeds was recorded and promptness index (PI) and germination stress index (GSI) were calculated using the formula proposed by Sapra et al. (1991) and Bouslama and Schapaugh (1984) respectively.

Results and Discussion

Effect of cadmium stress on germination percentage and vigour index

Cadmium concentration was standardized under the laboratory condition. Maximum percentage of germination was observed in 10 ppm followed by 20 ppm Cd concentration (75%) and (68%). Seed germination is an important growth cycle of the plant, it determines the standard establishment and final yield of the plant. In the present study, germination percentage and vigour index were drastically reduced in higher concentration of Cd. The results showed that 30 ppm Cd concentration recorded the optimum percentage of seed germination (52%) with the highest vigour index (274) compared to

Table 1. Cadmium effect on germination percentage and vigour index

Cadmium concentration	Germination percentage (%)	Vigour index
10 ppm Cd	75	351
20 ppm Cd	68	312
30 ppm Cd	52	274
40 ppm Cd	43	220.4
50 ppm Cd	40	210.3
Mean	55.59	273.54
SEd	2.10	10.40
CD(0.05)	4.68	23.19

40 ppm (220.4) and 50 ppm (210.3) of Cd concentration (Table1). Similar results have also been reported by Aimen *et al*. (2009). The decrease in water intake by seed in heavy metal toxicity and by the ion toxicity with accumulation of Cd around the seed and prevent seed germination (Murillo *et al*., 2002). Based on the results, 30 ppm Cd concentration was selected to induce Cd toxicity in laboratory. This result was also supported by the Jaja and Odoemena (2004), who stated that increased metal concentration in seed increases the suppression of germination.

Effect of melatonin on seedling health characters of tomato seedling under cadmium stress

The results exhibit the seedling health characters of tomato seedling in different melatonin

concentration under Cd stress. Based on the results, absolute control recorded the maximum germination percentage (86%) because the seedlings were not exposed to the Cd stress while Cd exposed (control) seedling showed minimum germination (52%) (Fig 1). The germination and rooting of seedlings are the important stages of whole plant growth and it is also the most sensitive stage of plants to changes of their surrounding environment. This result was supported by Sfaxi *et al.*(2010). Among the melatonin treated seedlings, 250 and 100 ppm showed the highest germination percentage (83% and 75%), which was highly significant with absolute control. Seeds treated with 250 and 100 ppm melatonin resulted in 59.6 and 44.2 per cent increase in germination over control. Posmyk *et al.* (2008) stated that, melatonin seed treatment enhances the germination percentage in cabbage under $CuSO_4$ stress. In green algae, exogenous melatonin can relieve cadmium-induced stress (Tal *et al.*, 2011).The present study confirms the earlier findings.

Table 2. Effect of melatonin on seedling health characters of tomato seedlings under cadmium stress

Parameters	Absolute Control (without Cd) (T_1)	Control (with Cd) (T_2)	Different conc. of Melatonin seed treatment (ppm) + 30 ppm Cd				Mean	SEd	CD (p=0.05)
			25 (T_3)	50 (T_4)	100 (T_5)	250 (T_6)			
Germination percentage (%)	86	52	68	69	75	83	72.16	2.91	6.35
Shoot length (cm seedling^{-1})	4.7	3.0	3.2	3.4	3.7	4.1	3.71	0.14	0.31
Root length (cm seedling^{-1})	2.9	1.1	3.5	3.4	3.3	3.7	2.98	0.13	0.28
Vigour index	680.2	234.0	442.0	469.2	525.0	647.4	499.63	20.99	45.73
Promptness index	82.5	37.2	46.2	46.5	50.0	51.2	52.26	2.12	4.62
Germination stress index	-	45.0	56.0	56.3	60.6	62.0	54.15	2.15	4.69
Total dry matter production (g seedling^{-1})	0.293	0.123	0.185	0.197	0.205	0.284	0.21	0.00	0.01

Root length and shoot length are important traits against any abiotic stress and in general, variety with longer root growth has resistant ability for abiotic stress (Leishman and Westoby, 1994). The results revealed that, root length and shoot length of all the melatonin treatment plants was highly significant from control plants. Among the treatments, seed treatment of 250 ppm melatonin recorded maximum shoot length (4.1 cm) and root length (3.7 cm), which was on par with seed treatment of 25 ppm melatonin (3.5 cm). In a recent study of *Arabidopsis* treated with melatonin and grown at cadmium treated solution, melatonin treated plants had significantly greater fresh weight, primary root and shoot length compared with untreated plants, the effect being both time and concentration dependent (Bajwa *et al.*, 2014). Control seedling shows the minimum root growth (1.1 cm) followed by absolute control (2.9 cm). Based on the mean values, root length (2.98 cm) was more inhibited than the shoot length (3.71 cm). Sujing *et al*. (2012) stated that, in cadmium stress, it is interesting to note that, the root length was inhibited more than shoot length. The loss of these may be due to inhibition of cell division, impairment of PSII activity, directly or indirectly inhibits physiological processes resulted in poor biomass and yield.

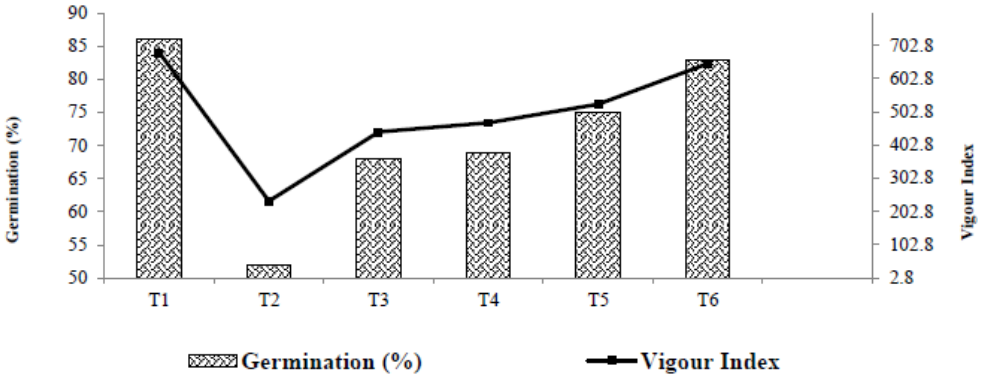

Fig. 1. Effect of melatonin on seedling health index of tomato under cadmium stress

T_1: Absoluute control; T_1 Control; $T_3 – T_6$ Seed treatment with melatonin @ 25,50,100 and 250 ppm, respectively.

All the treatments were significantly different from others in recording total dry matter production (TDMP). Excluding absolute control (0.29 g), all the treatments reduced the TDMP under Cd stress, the reduction was high in control plant (58 %) but the seed treatment with 250 ppm melatonin treated plants recorded more or less same TDMP of 0.28 g. In this experiment, root and shoot length were reduced in tomato seedling by 62 per cent and 36.1 per cent respectively and the total dry matter production also get reduced (58.6%) due to cadmium stress respectively. The present study confirmed by earlier findings. Irrespective of

the treatments, seed treatment of 250 ppm melatonin showed its supremacy in recording higher per cent of root and shoot length and total dry matter production than other treatments. Reason behind the increased root, shoot and TDMP was melatonin reduced the intercellular pH and inducing cell wall loosening, which is responsible for the cell wall expansion and elongation in lupin (Arnao and Hernandez, 2007). The present investigation supported by these findings.

Vigour index is a product of germination percentage and seedling length. The vigour index was significantly inhibited by cadmium stress. The result of vigour index indicates (Table. 2), the maximum vigour index recorded in unstressed plants (680.2) followed by 250 ppm melatonin seed treatment (647.4), which was significantly similar to the absolute control. Cd stress caused a greater adverse effect on vigour index of the seedlings. Due to the cadmium stress, 65.5 per cent reduction was observed in vigour index over absolute control. Increased melatonin concentration leads to maximize vigour index of seedlings. Hernandez *et al*., (2004) reported that, pre-treatment of seeds with melatonin, produced the higher per cent of seedling vigour index in lupin tissue. Pre-treatment of seeds with melatonin may induce the germination and high seedling length, this might be a reason for high vigour index under Cd stress. This finding provides a valid support for the result obtained in the present study.

Assessment of promptness index, absolute control noticed the highest promptness index (82.5). The control (37.2) recorded the 54.90 per cent reduction in promptness index compared to absolute control. Seed treated with 250 ppm melatonin obtained the maximum promptness index (51.2), when compared to other melatonin treatment. Seeds treated with 250 ppm melatonin recorded the highest GSI (62.0) which was on par with the seed treatment of 100 ppm melatonin (60.6). 100, 50 and 25 ppm melatonin seeds were on par with each other in recording GSI. PI was highly affected by Cd, which leads to drastic reduction in speediness of seedling germination. The GSI of plants was calculated based on promptness index and it showed inhibition of growth and biomass synthesis. High Cd concentration leads to prevent water uptake and water movement in the embryo axis and that might be the main reason for the poor seedling development. This result was confirmed with early study of Haouari *et al*. (2012) in tomato and Vijayaragavan *et al*. (2011) in cowpea. Chen *et al*. (2009) reported that, a low level of melatonin stimulated the speediness of seedling germination and root growth. The earlier findings corroborated well with the present study.

The study concluded that, cadmium stress drastically reduced the germination percentage and vigour index of the seedling under high Cd concentration. 30 ppm Cd had the optimum per cent (50 %) of germination and vigour index and it was selected to induce Cd toxicity in laboratory and pot culture experiment. Seed treatment 250 ppm of melatonin under 30 ppm Cd imposed condition, performed well in recording germination percentage, root and shoot length, vigour index, promptness index, GSI and TDMP.

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