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Does Exogenous Application of Melatonin Ameliorate Lead Toxicity in *Eruca vesicaria* Plants?

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MELATONIN (N-acetyl-5-methoxytryptamine) is receiving a considerable interest due to its prospected role in alleviating the heavy metals stress in plants. Thus a step toward the profitable use of melatonin (ME) in agriculture, the present study investigated the exogenous application of ME in *Eurica vesicaria* grown in contaminated soil amended with lead nitrate $Pb(NO_3)_2$ to produce Pb levels of 350mg/kg. Melatonin was applied by two methods: priming of seeds for 12hrs [0.0µM (P_0); 25µM (P_1) and 50µM (P_2)] and by foliar spray at seedling foliage [0.0µM (F_0); 25µM (F_1) and 50µM (F_2)].

Rocket plants affected by Pb toxicity exhibited significant reduction in the growth characteristics (fresh and dry weights of shoot and root, and leaf area/plant), photosynthetic pigments (chlorophyll *a,* chlorophyll *b* and carotenoids), photosynthetic activity ($^{14}CO_2$-assimilation), and photosynthetic enzymes (phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate-carboxylase/oxygenase). Meanwhile, exogenous application of ME appeared to ameliorate toxic effects of lead by improving the above criteria as compared to the control or Pb treated plants. These results showed that ME can promote the growth of *Eurica vesicaria* growing in Pb-contaminated soil by stimulating the photosynthetic activity, and the foliar spray method is the optimum at a dose of ME (50µM).

Keywords: *Eurica sativa*, Lead, Melatonin, Photosynthetic activity ($^{14}CO_2$-fixation).

Introduction

Rocket (*Eruca vesicaria*), belongs to Brassicaceae and its leaves are regarded as an excellent nutritive source of minerals and vitamins. Crops with such promising potential require much research for improving production, quantity and quality especially that grown in contaminated soil (Moussa, 2006). Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring compound present in roots, leaves, fruit, and seed of a large number of plant species (Dubbels et al., 1995). Melatonin has a role in regulation of plant growth and development (Arnao, 2014). It has been demonstrated that melatonin has the ability to alleviate effects of abiotic stresses such as low temperature, heavy metals stress, and suboptimal light conditions during seed germination (Tiryaki & Keles, 2012; Moussa & El-Gamal, 2017).

Melatonin may possess some auxin-like effects (Kolár & Machackova, 2005) and act as a regulatory molecule in plants (Van Tassel et al., 2001). It effectively reduces reactive oxygen species (ROS) accumulation and alleviates oxidative stress (Galano et al., 2013). It is amphiphilic, this property enabling it to easily cross cell membranes (Reiter et al., 2013). Melatonin may have a role in regulating flowering, photosynthesis, chlorophyll synthesis (Tan et al., 2012; Moussa & El-Gamal, 2017), callus formation and root regeneration (Zhang et al., 2013; Zhang et al., 2014), and senescence in leaves, where it has been reported that melatonin delays senescence (Wang et al., 2011; Wang et al., 2013).

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Lead is non-essential nutrient without any biological benefits, however it is extremely common contaminant in the environment (Moussa & El-Gamal, 2017). Pb is a very malleable, heavy and non-corrosive metal (Csuros, 1994). Pb toxicity has been reported to inhibit the growth of various plant species (Liu et al., 2008; Zhou et al., 2010). It exerts adverse effects on morphology, growth and photosynthetic processes. It also causes inhibition of enzyme activities, water imbalance, perturbation of membrane permeability and disturbance of mineral nutrition (Maestri et al., 2010). Many authors reported that Pb toxicity is known to cause oxidative cell damage by generating reactive oxygen species (Grčman et al., 2003; Sharma & Dubey, 2005; Islam et al., 2011).

The Goal of this study is to assess the capability of melatonin to alleviate the phytotoxicity of lead, on the growth, the activities of carboxylating enzymes and photosynthetic activity ($^{14}CO_2$-fixation) of *Eurica vesicaria* plants.

Materials and Methods

Seeds of Rocket (*Eruca vesicaria* subsp. *sativa*), cv. Baladi, were obtained from the Crop Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation, Giza, Egypt. The soil was collected, dried, crushed and sieved through 2mm sieve. Some soil samples were filled in standard plastic pots (40cm high × 35cm diameter), each filled with 10 kg heavy clay soil and all pots received 60, 50 and 40mg/kg N, P_2O_5 and K_2O as urea, super phosphate and potassium sulphate, respectively. To simulate soil pollution, the soil was amended with lead nitrate $Pb(NO_3)_2$ to produce Pb levels of 350mg/kg**.** The soil was watered to field capacity and incubated in green house for 10 days to allow the soil chemical reactions to equilibrate.

Seeds were surface sterilized in 0.1% (w/v) sodium dodecyl sulphate solution and then thoroughly rinsed with sterile deionized water. The seeds were then primed by soaking overnight (12hrs) in either distilled water or the different concentrations of freshly prepared melatonin solution [0.0µM (P_0), 25µM (P_1) and 50µM (P_2)].

Seeds were sown in the soil. Plants were grown in a controlled environment growth chamber with 15hrs photoperiod; 65%–75% relative humidity; and at 22 and 20°C day and night temperatures, respectively. Photosynthetic photon flux density at maximum plant height was about 440µmol$m^{-2}s^{-1}$.

When the plants were at the stage of 2 to 3 true leaves after 13 days, freshly prepared melatonin solutions with different concentrations [0.0µM (F_0), 25µM (F_1) and 50µM (F_2)] was added to Tween 20 to fix the solutions to the leaf surface. Water spray treatment was taken as a control. The solutions were sprayed once in the morning every day using a manual pump for one week.

Cultural practices, such as weed control and irrigation, were performed as needed. The pots were rinsed with water once a week to avoid salt accumulation. The experimental design was randomized complete block design with three replicates.

The plants were sampled at 30 days after sowing to assess their growth characteristics (shoot fresh and dry weights, root fresh and dry weights, and leaf area). The dry weight (DW) was measured after the shoots and roots were dried at 60˚C to constant weight. The leaf area was measured with the help of portable area meter, LI-COR, Model LI-3000, USA.

All measurements and physiological determinations were carried out at the harvest stage (30 days after sowing), photosynthetic pigments (Chl. *a*, and Chl. *b*) and carotenoids were estimated using the method of Moran (1982); photosynthetic activity ($^{14}CO_2$-fixation) was measured in the Radioisotope Department, Atomic Energy Authority, Cairo, Egypt, according to Moussa (2008); the activities of carboxylating enzymes ribulose-1,5-bisphosphate-carboxylase/oxygenase content (RuBPCase, EC 4.1.1.39) was determined following Warren et al. (2000). Phosphoenol pyruvate carboxylase (PEPC, EC 4.1.1.31) was measured using the method adopted by Cánovas & Kornberg (1969).

Statistical analysis

All data were statistically described in terms of mean ± standard deviation, frequencies or median, range and percentages. Comparison between the study groups was done by the usage of student t-test for independent samples in comparing 2 groups. Correlation between different variables was done by using Pearson moment correlation

equation for linear relation. P values less than 0.05 was taken in the consideration as a statistically significant. All the calculations were determined using computer programs Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA (SPSS) version 15.0 (Norusis, 2006).

Results

Rocket (*Eruca vesicaria*) plants cultivated in soil contaminated with 350mg Pb/kg, showed a significant decrease in the growth characteristics (shoot fresh and dry weights, root fresh and dry weights and leaf area plant^{-1}), as compared to the control as shown in Table 1. However, the plants treated with melatonin applied by two methods, seed priming and foliar spray, significantly increased these parameters as compared to the control and plants grown in soil contaminated with 350mg Pb/kg). Also, the maximum values were obtained by the foliar treatments of rocket plants at a dose of melatonin 50µM.

Plants grown in soil polluted with 350mg pb/kg significantly declined the level of chlorophyll (Chl. *a*, and Chl. *b*) carotenoids and photosynthetic activity ($^{14}CO_2$-assimilation); as compared to the control (Table 2).

However, the plants treated with melatonin applied either by priming or soaking significantly enhanced these parameters as compared to the control and Pb stresed plants. Also, the maximum values were obtained by the foliar treatments at a dose of melatonin 50µM.

The activities of carboxylating enzymes (phosphoenol pyruvate carboxylase (PEPC, EC 4.1.1.31) and ribulose1,5-bisphosphate carboxylase (RuBPC, EC 4.1.1.39); decreased to significant level in rocket plants cultivated in soil contaminated with 350mg pb/kg as compared to the control as represented in Table 3.

Meanwhile, the plants primed or sprayed with melatonin significantly increased these parameters as compared to the control plants and plants grown in soil contaminated with 350mg Pb/kg. Whereas, the most favorable results were recorded by the foliar treatments of rocket plants at a dose of melatonin 50µM as shown in Table 3.

TABLE 1. Changes in some growth characteristics (shoot and root fresh and dry weights and leaf area/plant) of *Eruca vesicaria* grown for 30 days in soil contaminated with lead with and without melatonin applied as priming or foliar spraying.

Parameters	Lead (350 mg/kg^{-1})	Priming ME			Foliar ME		
		P_0	P_1	P_2	F_0	F_1	F_2
Shoot fresh weight plant^{-1} (mg)	L_0	389 ±3.8^c	552±11^b	924±27.7^a	385±3.8^c	613±12.2^b	1150±34.5^a
	L_1	210±2.1^c	313±6.2^b	617±18.5^a	212±2.1^c	408±8.1^b	726±21.7^a
Shoot dry weight plant^{-1} (mg)	L_0	5.0±0.05^c	6.3±0.12^b	11.4±0.34^a	5.2±0.05^c	8.2±0.16^b	15.4±0.46^a
	L_1	2.8±0.02^c	5.0±0.10^b	6.1±0.18^a	2.9±0.02^c	6.3±0.12^b	8.5±0.25^a
Root fresh weight plant^{-1} (mg)	L_0	112±1.12^c	208±4.16^b	367±11^a	115±1.15^c	285±5.7^b	400±12^a
	L_1	86±0.86^c	100±2^b	227±6.81^a	88±0.88^c	112±2.24^b	317±9.51^a
Root dry weight plant^{-1} (mg)	L_0	2.0±0.02^c	2.3±0.04^b	2.9±0.08^a	2.2±0.02^c	2.6±0.05^b	3.5±0.10^a
	L_1	0.88±0.08^c	1.84±0.03^b	2.33±0.06^a	0.90±0.09^c	2.34±0.04^b	2.96±0.08^a
Leaf area plant^{-1} (cm^2)	L_0	0.70±0.07^c	1.31±0.02^b	2.21±0.06^a	0.77±0.07^c	1.82±0.03^b	2.91±0.08^a
	L_1	0.49±0.04^c	0.99±0.01^b	1.90±0.05^a	0.51±0.05^c	1.31±0.02^b	2.42±0.07^a

-ME: Melatonin, where lead concentration (L_0) 0.0 and (L_1) 350mg/kg; (P_0) 0.0, (P_1) 25 and (P_2) 50µM of priming melatonin solutions; (F_0) 0.0, (F_1) 25 and (F_2) 50µM of foliar melatonin solutions.

- Data are means of three replicates.

- Values in a row followed by the same letter are not significantly different, P< 0.05, Tukey's test.

TABLE 2. Changes in photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) and photosynthetic activity ($^{14}CO_2$-fixation).) of rocket plants grown for 30 days in soil contaminated with lead with and without melatonin applied as priming or foliar spraying.

Parameters	Lead (350 mg/ kg^{-1})	Priming ME			Foliar ME		
		P_0	P_1	P_2	F_0	F_1	F_2
Chlorophyll (*a*) (mg·g^{-1}FW)	L_0	1.28±0.02^c	1.53±0.06^b	2.11±0.12^a	1.25±0.02^c	2.08±0.08^b	2.92±0.17^a
	L_1	0.77±0.01^c	1.08±0.04^b	1.96±0.11^a	0.75±0.01^c	1.58±0.06^b	2.55±0.15^a
Chlorophyll (*b*) (mg·g^{-1}FW)	L_0	0.55±0.01^c	0.89±0.03^b	1.32±0.07^a	0.61±0.01^c	1.32±0.05^b	1.87±0.11a
	L_1	0.23±0.04^c	0.50±0.02^b	0.87±0.05^a	0.26±0.05^c	0.78±0.03^b	1.15±0.06^a
Total Chlorophyll (*a*+*b*) (mg·g^{-1}FW)	L_0	1.83±0.03^c	2.42±0.09^b	3.43±0.2^a	1.86±0.03^c	3.40±0.13^b	4.79±0.28^a
	L_1	1.00±0.02^c	1.58±0.06^b	2.83±0.16^a	1.01±0.02^c	2.36±0.09^b	3.70±0.2^a
Carotenoids (mg·g^{-1}FW)	L_0	0.79±0.01^c	1.40±0.05^b	2.41±0.14^a	0.82±0.01^c	1.85±0.07^b	2.87±0.17^a
	L_1	0.51±0.01^c	1.09±0.04^b	2.08±0.12^a	0.48±0.09^c	1.32±0.05^b	2.46±0.14^a
Photosynthetic activity (KiloBecquerel·mg^{-1}FW),	L_0	16.6±0.3^c	22.8±0.9^b	28.4±1.7^a	16.2±0.3^c	25.7±1^b	36.8±2.2^a
	L_1	9.7±0.19^c	18.8±0.75^b	23.2±1.3^a	9.5±.19^c	18.3±0.73^b	29.3±1.7^a

-ME: Melatonin, where lead concentration (L_0) 0.0 and (L_1) 350mg/kg; (P_0) 0.0, (P_1) 25 and (P_2) 50µM of priming melatonin solutions; (F_0) 0.0, (F_1) 25 and (F_2) 50µM of foliar melatonin solutions.

- Data are means of three replicates.

- Values in a row followed by the same letter are not significantly different, P< 0.05, Tukey's test.

TABLE 3. Changes in photosynthetic enzymes (ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBPCase) and phosphoenolpyruvate carboxylase (PEPCase) of rocket plants grown for 30 days in soil contaminated with lead with and without melatonin applied as priming or foliar spraying.

Parameters	Lead (350 mg/ kg^{-1})	Priming ME			Foliar ME		
		P_0	P_1	P_2	F_0	F_1	F_2
RuBPCase (mg·g^{-1} FW)	L_0	53.80.53±^{c}	61.41.2±^{b}	65.81.9±^{a}	54.0±0.54^c	68.91.3±^{b}	77.82.3±^{a}
	L_1	41.70.41±^{c}	43.70.87±^{b}	50.1±1.5^a	41.90.41±^{c}	45.3±0.9^b	58.21.7±^{a}
PEPCase (µmol CO_2 min^{-1} mg^{-1} protein)	L_0	16.80.16±^{c}	17.9±0.35^b	20.00.6±^{a}	16.9±0.16^c	18.80.37±^{b}	23.1±0.69^a
	L_1	11.30.11±^{c}	13.70.27±^{b}	14.7±0.44^a	11.5±0.11^c	15.20.3±^{b}	17.9±0.53^a

-ME: Melatonin, where lead concentration (L_0) 0.0 and (L_1) 350mg/kg; (P_0) 0.0, (P_1) 25 and (P_2) 50µM of priming melatonin solutions; (F_0) 0.0, (F_1) 25 and (F_2) 50µM of foliar melatonin solutions.

- Data are means of three replicates.

- Values in a row followed by the same letter are not significantly different, P< 0.05, Tukey's test.

Discussion

Pb toxicity has been reported to inhibit the growth of various plant species (Liu et al., 2008; Zhou et al., 2010). Such findings agreed with our results which showed that Pb treatment decreased the growth parameters, the content of chlorophylls (*a* and *b)*, carotenoids and photosynthetic activity ($^{14}CO_2$-fixation) as compared with the control. Photosynthesis is one of the most Pb sensitive plant processes and it is harmfully affected by Pb toxicity (Grčman et al., 2003; Sharma & Dubey, 2005; Farahat & Linderholm, 2015; Malar et al., 2014).

This reduction in growth could be attributed to interference of absorbed Pb with metabolic and biochemical processes associated with normal growth and development of the plants (Hegazi et al., 2010; Piyanast et al., 2014). A considerable decrease in dry weights of plant parts is observed under Pb treatment (Kosobrukhov, 2004).

Additionally, one of the most frequently mentioned functions of melatonin is considered to be an antioxidant for protection against heavy metal stress (Tan et al., 2007; Posmyk et al., 2008). The present results agreed with those of Fleta-Soriano et al. (2017) who stated that melatonin

also enhanced chlorophyll content in tomato. Melatonin has an important role in preservation of chlorophyll, efficiency of photosynthesis, and improvement of crop production (Tan et al., 2012). Moussa & El-Gamal (2017) reported that treatment with melatonin in spinach plants under stress helped preserved chlorophyll, growth characteristics and promoted photosynthesis due to increasing antioxidant enzyme activities and antioxidant contents and inhibiting production of reactive oxygen species. Arnao & Hernández-Ruiz (2009) stated that melatonin lowered chlorophyll degradation and slowed the senescence process. Beneficial effects of melatonin may result from its signaling function, through induction of metabolic pathways and stimulation of production of substances preferably operating under stress (Moussa & El-Gamal, 2017; Manzer et al., 2019). Our results showed the potential of melatonin for improvement of growth and seed production in different crops (Wei et al., 2015). Tan et al. (2012) concluded that a primary function of melatonin in plants is to serve as the first line of defense against internal and environmental oxidative stressors.

Pb toxicity has been reported to inhibit the photosynthetic enzymes involved in the first step of carbon assimilation (ribulose-1,5-bisphosphate-carboxylase/oxygenase and phosphoenolpyruvate carboxylase (Arena et al., 2017). Melatonin increased rubisco in slat stressed tomato (Manzer et al., 2019). Also, Agnieszka et al., 2018 reported that melatonin increased phosphoenolpyruvate carboxylase probably by activates alternative pathway of sugar synthesis.

Conclusion

Therefore, it can be concluded that Pb-induced toxicity particularly in the photosynthetic machinery has been overcome with the exogenous application of melatonin that manifested here by enhanced growth parameters, $^{14}CO_2$-assimilation, photosynthetic pigments and enzymes.

Conflict of interest: The authors reported no potential conflict of interest.

Authors contribution: H.R. Moussa and Hanan Abdallah Mohamed conceived the original ideas and designed the experiments with the collaboration of Eman Selem; the manuscript was, for the most part, written by H.R. Moussa and Eman Selem; Mona Hosam El-Deen Sayed Ragab did the physiological data acquisition; H.R. Moussa and Mona Hosam El-Deen Sayed Ragab analyzed the data; H.R. Moussa revised the manuscript.

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References

Agnieszka, K., Sławomir, B., Małgorzata, M.P. (2018) Melatonin redirects carbohydrates metabolism during sugar starvation in plant cells. *Journal of Pineal Research,* **64**(4), 1-14.

Arena, C., Figlioli, F., Sorrentino, M.C., Izzo, L.G., Capozzi, F., Giordano, S., Spagnuolo, V. (2017) Ultrastructural, protein and photosynthetic alterations induced by Pb and Cd in *Cynara cardunculus* L., and its potential for phytoremediation. *Ecotoxicology and Environmental Safety,* **145**, 83-89.

Arnao, M.B., Hernández-Ruiz, J. (2009) Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. *Journal of Pineal Research,* **46**(1), 58-63.

Arnao, M.B. (2014) Phytomelatonin: discovery, content, and role in plants. *Advances in Botanical,* **31**, 1-11.

Cánovas, L.J., Kornberg, L.H. (1969) Phosphoenol pyruvate carboxylase from *Escherichia coli. Methods in Enzymology*, **13**, 288.

Csuros, M. (1994) Introduction to metals. In: "*Environmental Sampling and Analysis for Technicians*", pp. 209-210. Boca Raton: Lewis Publishers.

Dubbels, R., Reiter, R.J., Klenke, E. (1995) Melatonin in edible plants identified by radioimmunoassay and by high-performance liquid chromatography–mass spectrometry. *Journal of Pineal Research*, **18**, 28-31.

Farahat, E., Linderholm, H.W. (2015) The effect of long-term wastewater irrigation on accumulation and transfer of heavy metals in *Cupressus sempervirens* leaves and adjacent soils. *Science* of the *Total Environment*, **512**, 1-7.

Fleta-Soriano, E., Diaz, L., Bonet, E., Munne-Bosch, S. (2017) Melatonin may exerta protective role against

drought stress in maize. *Journal* of *Agronomy* and *Crop Science*, **203**(4), 286-294.

Galano, A., Tan, D.X., Reiter, R.J. (2013) On the free radical scavenging activities of melatonin's metabolites, AFMK and AMK. *Journal of Pineal Research*, **54**, 245-257.

Grčman, H., Vodnik, D., Velikonja-Bolta, Sˇ, Lesˇtan, D. (2003) Ethylenediamine dissuccinate as a new chelate forenvironmentally safe enhanced lead phytoextraction. *Journal of Environmental* Quality, **32**, 500-506.

Hegazi, Amal Z., Mostafa, Soha S.M., Hamdino, M.I. (2010) Influence of different cyanobacterial application methods on growth and seed production of common bean under various levels of mineral nitrogen fertilization. *Nature and Science*, **8**(11).

Islam, E., Liu, D., Li, T., Yang, X., Jin, X., Khan, M.A., Mahmood, Q., Hayat, Y., Imtiaz, M. (2011) Effect of Pb toxicity on the growth and physiology of two ecotypes of *Elsholtzia argyi* and its alleviation by Zn. *Environmental Toxicology*, **26**(4), 403-416.

Kolár, J., Macha´cˇkova, I. (2005) Melatonin in higher plants: occurrence and possible functions. *Journal of Pineal Research*, **39**, 333-341.

Kosobrukhov, A., Knyazeva, I., Mudrik, V. (2004) *Plantago major* plants responses to increase content of lead in soil: growth and photosynthesis. *Plant Growth Regulation,* **42**, 145-151.

Liu, D., Li, T.Q., Jin, X.F., Yang, X.E., Islam, E., Mahmood, Q. (2008) Lead induced changes in the growth and antioxidant metabolism of the lead accumulating and non-accumulating ecotypes of *Sedum alfredii*. *Journal of Integrative Plant Biology,* **50**(2), 129-140.

Maestri, E., Marmiroli, M., Visioli, G., Marmiroli, N. (2010) Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. *Environmental and Experimental Botany*, **68**(1), 1-13.

Malar, S., Vikram, S.S., Favas, P.J.C., Perumal, V. (2014) Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes*(Mart.)]. *Botanical Studies,* **55**, 54-65.

Manzer, H.. Siddiqui, Saud Alamri, Mutahhar, Y., Al-Khaishany, M., Nasir Khan, Abdullah Al-Amri, Hayssam, M., Ali Ibrahim, A., Alaraidh, Abdulaziz, A. (2019) Exogenous melatonin counteracts NaCl-induced damage by regulating the antioxidant system, proline and carbohydrates metabolism in tomato seedlings. *The International Journal of Molecular Sciences*, **20**, 353.

Moran, R. (1982) Formula for determination of chlorophyllous pigments extracted with N.N. dimethylformamide. *Plant Physiology*, **69**(6), 1371-1381.

Moussa, H.R. (2006) Role of gamma irradiation in regulation of NO_3 Level in Rocket *(Eruca vesicaria* sub sp. *sativa*) plants. *Russian Journal of Plant Physiology*, **53**, 193.

Moussa, H.R. (2008) Gamma irradiation effects on antioxidant enzymes and G_6PDH activities in *Vicia faba* plants. *Journal of New Seeds*, **1**, 89-99.

Moussa, H.R., El-Gamal, S.M. (2017) Does exogenous application of melatonin ameliorate boron toxicity in Spinach (*Spinacia oleracea* L.) plants? *International Journal of Vegetable Science,* **23**(3), 233-245.

Norusis, M.J. (2006) Multiple Regression Diagnostics. SPSS 14.0 Guide to Data Analysis. Upper Saddle River, NJ: Prentice Hall Inc., pp. 557-575.

Piyanast, S., Narinthip, S., Yaneeporn, S., Arm, U., Siriluck, I. (2014) Effect of *Sprirulina maxima* aqueous extract on seed germination and seedling growth of *Mung been*, *Vigna radiate* and rice, *Oryza sativa* var. *Journal of the International Society for Southeast Asian Agricultural Sciences,* **20**(2), 77-84.

Posmyk, M.M., Kuran, H., Marciniak, K., Janas, K.M. (2008) Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. *Journal of Pineal Research*, **45**, 24-31.

Reiter, R.J., Tan, D.X., Rosales-Corral, S. (2013) The universal nature, unequal distribution and antioxidant functions of melatonin and its derivatives. *Mini*-Reviews in *Medicinal Chemistry*, **13**, 373-384.

Sharma, P., Dubey, R.S. (2005) Lead toxicity in plants.

Brazilian Journal of Plant Physiology, **17**(1), 35-52.

Tan, D.X., Hardeland, R., Manchester, L.C., Korkmaz, A., Ma, S., Rosales-Corral, S., Reiter, R.J. (2012) Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *Journal of Experimental Botany,* **63**, 577-597.

Tan, D.X., Manchester, L.C., Terron, M.P., Flores, L.J., Reiter, R.J. (2007) One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? *Journal of Pineal Research*, **42**, 28-42.

Tan, D.X., Hardeland, R., Manchester, L.C., Korkmaz, A., Ma, S., Rosales-Corral, S., Reiter, R.J. (2012) Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *Journal of Experimental Botany,* **63**(2), 577-597.

Tiryaki, I., Keles, H. (2012) Reversal of the inhibitory effect of light and high temperature on germination of *Phacelia tanacetifolia* seeds by melatonin. *Journal of Pineal Research*, **52**, 332-339.

Van Tassel, D.L., Roberts, N., Lewy, A., O'Neill, S.D. (2001) Melatonin in plant organs. *Journal of Pineal Research*, **31**(1), 8-15.

Wang, P., Yin, L., Liang, D. (2011) Delayed senescence of apple leaves by exogenous melatonin treatment: Toward regulating the ascorbate–glutathione cycle. *Journal of Pineal Research*, **53**, 11-20.

Wang, P., Sun, X., Li, C., Wei, Z., Liang, D., Ma, F. (2013) Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. *Journal of Pineal Research*, **54**, 292-302.

Warren, C.R., Adams, M.A., Chen, Z. (2000) Is photosynthesis related to concentrations of nitrogen and Rubisco in leaves of Australian native plants? *Australian Journal Plant Physiology,* **27**, 407-416.

Wei, W., Li, Q.T., Chu, Y.N., Reiter, R.J., Yu, X.M., Zhu, D.H., Zhang, W.K. (2015) Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *Journal of Experimental Botany,* **66**, 695-707.

Zhang, H.J., Zhang, N., Yang, R.C., Wang, L., Sun, Q.Q., Li. D.B., Cao, Y.Y., Weeda, S., Zhao, B., Ren, S., Guo, Y.D. (2014) Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA4 interaction in cucumber (*Cucumis sativus* L.). *Journal of Pineal Research*, **57**, 269-279.

Zhang, N., Zhao, B., Zhang, H.J., Weeda. S., Chen, Y., Yang, Z.C., Ren, S., Guo, Y.D. (2013) Melatonin promotes water-stress tolerance, lateral root formation, seed germination in cucumber (*Cucumis sativus* L.). *Journal of Pineal Research*, **54**, 15-23.

Zhou, Y., Huang, S., Yu, S., Gu, J., Zhao, J., Han, Y., Fu, J. (2010) The physiological response and subcellular localization of lead and cadmium in *Iris pseudacorus* L. *Ecotoxicology*, **19**(1), 69-76.

هل الاستخدام الخارجى للملاتونين يقلل من سميه الرصاص فى نباتات الجرجير؟

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يلاقي الميلاتونين (N-acetyl-5-methoxytryptamine) اهتمامًا كبيرًا نظرًا لدوره المتوقع في تخفيف الإجهاد التأكسدي في النباتات. ولذلك جاءت هذه الدراسه كخطوة لإستخدامه النافع في الزراعة. تمت زراعة نباتات الجرجير فى تربة ملوثة بالرصاص بتركيز 350 ملليجرام لكل كيوجرام من وزن التربة المستخدمة. أما بالنسبة للملاتونين فقد تم استخدامة في فترتين من عمر النبات بطريقتين: الطريقة الأولى بنقع البذور لمدة 12 ساعة فى تركيزات صفر (العينات الضابطة) و 25 و50 ميكرومول لكل لتر والطريقة الثانيه بالرش على أوراق البادرات بتركيزات صفر (العينات الضابطة) و 25 و50 ميكرومول لكل لتر. ولقد أظهرت النباتات التى تم معاملتها بعد 13 يوم بالرصاص نقصا ملحوظا فى الصفات الخضريه لنبات الجرجير وهى الوزن الطرى والجاف للنبات والمساحة الكليه للأوراق فى النبات وأيضا الأصباغ النباتيه وهى كلوروفيل أ & ب والكاروتينيدات الكليه ومعدل نشاط عمليه البناء الضوئى (معدل تثبيت ثانى أكسيد الكربون المشع) ونشاط الانزيمات الأساسية فى عمليه البناء الضوئى وهى (phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate-carboxylase/oxygenase). وعلى العكس أظهرت النباتات التى تم معاملتها بالملاتونين و المزروعه فى تربه ملوثة بالرصاص تحسنا ملحوظا فى المعاملات السابقة واظهرت أثرا واضحا ايجابيا فى التقليل من الأثر الضار لسمية الرصاص وذلك مقارنه بعينات الكنترول وأيضا مقارنه بالنباتات التى تم معاملتها بالرصاص فقط. وقد أظهرت النتائج النهائية أن معامله نباتات الجرجير بتركيز 50 ميكرومول لكل لتر بطريقة الرش على البادرات هى أنسب وأفضل الطرق المستخدمة فى معالجة الأثر الضار لسميه الرصاص حيث أنه من الواضح أن هناك تحفيز لعملية البناء الضوئى عن طريق زياده نشاط الانزيمات الأساسية الهامه لاتمام هذه العمليه فى النبات.