### EFFECT OF SELENIUM AND CADMIUM IN CHLOROPHYLLS AND CAROTENOID CONTENT OF *PHASEOLUS AUREUS* ROXB

Rihab Edan Kadhim

Biology Department, Science College, Babylon University, Iraq. rihabedan@gmail.com

Article received 16.10.2017, Revised 21.11.2017, Accepted 29.11.201wer7

### ABSTRACT

In greenhouse, seeds of *Phaseolus aureus* Roxb. local class are germinated in pots. The seeds treated with different concentrations of Cd (0.0, 0.01 and 0.05M, as CdCl<sub>2</sub>), a unique concentration of Se (0.05  $\mu$ M, as Na<sub>2</sub>SeO<sub>3</sub>) and as a combination with Cd, in addition to a control (distilled H<sub>2</sub>O). In *P. aureus*, there was significant decreasing in all chlorophylls content. Generally, treatment with Se enhanced the content of chlorophyll which increased significantly in comparison with the control (dH<sub>2</sub>O). The combination of Se + Cd, 0.05M caused increasing in chlorophyll b and carotenoid significantly comparing with Cd alone at 0.05M. These results indicate the antagonistic effect between Se and Cd on chlorophyll and carotenoid contents.

Key words: Phaseolus aureus Roxb., Se, Cd, chlorophylls, carotenoid.

#### **INTRODUCTION**

The environmental pollution by heavy metals is the most interested subject for their effect on plants. Cadmium (Cd) is represented the most dangerous one, because the plant roots are able to absorb it and its effect on the plant metabolites and growth (Costa and Spitz, 1997). The toxicity of Cd belongs to its motility and toxicity at low concentration (Ye *et al.*, 2003) and its ability to transport through the nutrition chain (Hiroyuki and Mistuo, 2002). It's found in soil, water, and air, especially when these places are crowded with vehicles, factories or polluted with fertilizers, sewage, and pesticides (Wu *et al.*, 2004).

Selenium (Se) is one of the essential elements, it is important for animals and humans at low concentrations (Birringer *et al.*, 2002). Depending on some researchers' results (Pilon-Smits *et al.*, 2009; Malik *et al.*, 2012), Se is beneficial for plant at low concentration and harmful at high concentration. Ekelund and Danilov (2001) referred that Se serves as an antioxidant and is one of glutathione peroxidase components. Like Cd, toxicity of Se differs depending on plant species, growth medium, and presence of competitive ions as S and P (Põldma *et al.*, 2013). There is an antagonistic effect between Se and many of heavy metals including Cd (Fagasova *et al.*, 2006).

Chlorophyll content of leaf provides valuable information about physiological status of plants (Gitelson *et al.*, 2003). The chlorophylls a and b are essential pigments for the formation of stored chemical energy from light energy, and the solar radiation amount which absorbed by a leaf is determined by the photosynthetic pigment content; thus, chlorophyll content can determine photosynthetic potential and primary production directly (Curran *et al.*, 1990, Filella *et al.*, 1995, Abbas *et al.*, 2017). Furthermore, because much of leaf nitrogen is incorporated in chlorophyll, chlorophyll gives an estimation of the nutrient status indirectly (Moran *et al.*, 2000). Also, leaf content of chlorophyll is closely related to plant stress and senescence (Merzlyak *et al.*, 1999)

Carotenoids belong to the light-harvesting pigments that play a photo-protective role, preventing damage in the photosynthetic systems (Chappelle *et al.*, 1992; Merzlyak *et al.*, 2003). Some studies indicated that the chlorophyll a, b, total chlorophyll and carotenoid content decreased in some plants under stress of salinity (Abdul Jaleel and Azooz, 2009), or under stress of heavy metal (Hussain *et. al.*, 2013).

The target of this study is the detoxification of Cd by Se at low concentration by determination the chlorophylls and carotenoid contents.

#### MATERIALS AND METHODS

Under greenhouse conditions, seeds of mung bean (Phaseolus aureus Roxb.) local class were germinated in betmos soil in pots (high10 cm, diameter 7cm). Ten seeds grew in each pot, then treated with A: Cd (as CdCl<sub>2</sub>) at different concentrations (0.0, 0.01, 0.05 M), B: Se (as Na<sub>2</sub>SeO<sub>3</sub>) at 0.05µM (selected from a series of concentrations as an enhancement concentration), C: a combination between solutions A and B. After that, the seeds irrigated with distilled water along the time of experiment. After 17 days (age of experiment), about 0.05g of leaves were taken then put in vial containing 20ml acetone (85%), after that the vials were kept in dark container in refrigerator for 5-6 days (modified method from Kumar and Sharma, 2014). After 6 days, the chlorophylls a, b and total chlorophyll content from the supernatant, were estimated according to Arnon (1949) and carotenoid content according to (Lichtenthaler and Wellburn, 1983).

Statistically, the data were analyzed by oneway analysis of variance (ANOVA). The means of three replicates separated by using the least signifycant difference (LSD) at probability level = 0.05.

# **RESULTS AND DISCUSSION**

In figures 1, 2, 3 and 4 for *P. aureus*, there was a significant increasing in chlorophylls and carotenoids content. Mukherjee and Dalal (2014) indicated a presence of gradual decline in dry biomass with increasing doses of Cd. In a previous study (Shacklette, 1972) it was mentioned that the existence of Cd at low concentrations is as a normal constituent in plant tissues.

The reduction in pigments content by high concentration of Cd as may be belong to the destruction of the chlorophyll pigments (Megdiche *et al.*, 2008), or may be attributed to interfere of ions through *denovo* proteins syntheses of chloroplast structure component rather than the chlorophyll destruction (Levitt, 1980).

Se with a unique concentration  $(0.05\mu M)$  caused a significant inhibition in the chlorophyll a, b, total chlorophyll and carotenoids content (figures 1-4) comparing with control. This inhibition in chlorophylls content was lest comparing with Cd.

The combinations treatments (Se, 0.05  $\mu$ M + Cd, 0.05M) caused a significant increase in chlorophyll b & carotenoids (fig. 2 & 4) comparing with Cd at 0.05M, while figures 1 & 3 showed nonsignificant increasing. From these results, Se interact with the toxic effect of Cd in its both concentrations (low & high), and raised all pigments content. This result show that the Se concentration may be increasing in plant leaves by storage, and then competes with Cd ions lead to prevent the toxic effect of it. Malorgio et al., (2009) referred that no significant change in chlorophyll content of lettuce and chicory leaves when treated with Se (as antioxidant) at harvest, while after storage for 5day, chlorophyll content of lettuce and chicory increased in these plants. This indicates the increase of Se concentration in the plant leaves, which had a positive effect on plant yield. The current study data, confirmed the results of (Fagasova et al., 2006). They referred to reduce the unfavorable effect of Cd on chlorophylls content by Se.

In another study by Kadhim (2016), the Se  $(0.05\mu M)$  caused increasing in chlorophylls and carotenoids content of *Cucumis melo* comparing with control and Cd treatments. This result indicates that the plant (depending on its type) may have a special mechanism to detoxify the heavy metal and whether it is sensitive or not. Some researches revealed the possibility of alleviated some biotic stress effect as drought by heavy metal at determinate concentration (Adiko *et al.*, 2017)

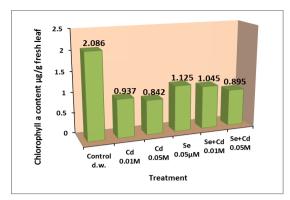


Figure 1: Chlorophyll a content  $(\mu g/g)$  with different treatments of Cd(M), Se  $(\mu M)$  & combination of Cd & Se, LSD (0.05)=0.329.

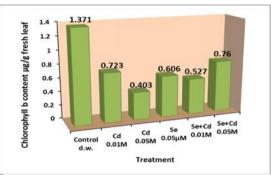


Figure 2: Chlorophyll b content  $(\mu g/g)$  with different treatments of Cd (M), Se  $(\mu M)$  & combination of Cd & Se, LSD (0.05) = 0.288.

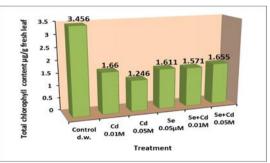


Figure 3: Total chlorophyll content ( $\mu g/g$ ) with different treatments of Cd (M), Se ( $\mu$ M) & combination of Cd & Se, LSD (0.05) =0.552.

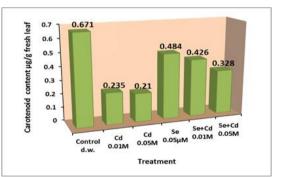


Figure 4: Carotenoid content ( $\mu$ g/g) with different treatments of Cd (M), Se ( $\mu$ M) & combination of Cd & Se, LSD (0.05) =0.106.

## CONCLUSION

Se antagonists with Cd element, and may be important in chlorophyll synthesis or protect it from destruction. Se in low concentration is beneficial in detoxification of Cd by increasing content of chlorophylls and carotenoid pigments in *P*. *aureus* which is sensitive to both Cd & Se alone.

## REFERENCES

- Adiko, N. N., Ratnadewi, D. and Miftahudin Physiological responses of rice (Oryza sativa L.) to zinc treatments under drought stress. Pak. J. Biote-chnol. 14 (2): 173-181 (2017).
- Abdul Jaleel, C. and M.M. Azooz, Exogenous calcium alters pigment composition, γ-glutamyl kinase and proline oxidase activities in saltstressed *Withania somnifera*. Plant Omics Journal 2(2): 85-90 (2009).
- Arnon, D. I., Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiology 24: 1 – 15 (1949).
- Birringer, M., Pilawa, S. and L. Flohe, Trends in selenium biochemistry. Natural Product Reports 19: 693–718 (2002).
- Chappelle, E.W., Kim, M.S. and J.E. McMurtrey, Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentrations of chlorophyll A, chlorophyll B, and carotenoids in soybean leaves. Remote Sens. Environ. 39: 239 – 247 (1992).
- Costa, G. and E. Spitz, Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured *Lupinus albus*. J. Plant Sci. 128: 131-140 (1997).
- Curran, P.J., Dungan, J.L. and H.L. Gholz, Exploring the relationship between reflectance red edge and chlorophyll content in slash pine. Tree Physiol. 7: 33- 48 (1990).
- Ekelund, N.G.A. and R.A. Danilov, The influence of selenium on photosynthesis and light-enhanced dark respiration (LEDR) in the flagellate Euglena gracilis after exposure to ultraviolet radiation. Aquatic Sciences 63:457-465 (2001).
- Fagasova, A., Pastierova, J. and K. Svetkova, Effect of Se-metal pair combinations (Cd, Zn, Cu, Pb) on photosynthetic pigments production and metal accumulation in *Sinapis alba* L. seedlings. Plant Soil Environ. 52(1): 8-15 (2006).
- Filella, I., Serrano, I., Serra, J. and J. Penuelas, Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. Crop Science 35: 1400 – 1405 (1995).
- Gitelson, A.A., Gritz, Y. and M.N. Merzlyak, Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non

-destructive chlorophyll assessment in higher plant leaves. J. Plant Physiol. 160: 271–282 (2003).

- Hiroyuki, H., Eriko, A. and C.H. Mistuo, Estimate of cadmium concentration in brown rice. 17<sup>th</sup> WCSS, 29: Pp. 1-5 (2002).
- Hussain, M.B., Ali, S., Azam, A., Hina, S., Farooq, A.M., Ali, B., Bharwana, S.A. and M.B. Gill, Morphological, physiological and biochemical responses of plants to nickel stress: A review. Afr. J. Agric. Res. 8(17): 1596-1602 (2013).
- Kadhim, R.E., Effect of Selenium and Cadmium in Chlorophylls and Carotenoid Content of *Cucumis melo* L. Iraqi Journal of Biotechnology 15(3): 25-30 (2016).
- Kumar, H. and Sharma, S., Determination of chlorophyll and carotenoid loss in *Dalbergia sissoo* caused by *Aonidiella orientalis* (newstead) [Homoptera: Coccoidea: Diapididae]. J. Entomology and Zoology Studies 2(1): 104-106 (2014).
- Levitt, J.J., Responses of Plants to Environmental Stresses, Radiation, Salt and Other Stresses. 2<sup>nd</sup> edition, Academic press Inc. London, United Kingdom (1980).
- Lichtenthaler, H.K. and A. Wellburn, Determination of total carotenoids and chlorophylls *a* and *b* of leaf extract in different solvents. Bioch. Soc. Trans. 603: 591-592 (1983).
- Malik, J.A., Goel, S., Kaur, N., Sharma, S., Singh, I. and H. Nayyar, Selenium antagonizes the toxic effects of arsenic on mung bean (*Phase-olus aureus* Roxb.) plants by restricting its uptake and enhancing the antioxidative and detoxification mechanisms. Environmental and Experimental Botany 77: 242–248 (2012).
- Malorgio, F., Diaz, K., Ferrante, A., Mensuali, A. and B. Pezzarossa, Effects of selenium addition on minimally processed leafy vegetables grown in floating system. J. Agri. Food Chem. 2243-2251 (2009).
- Megdiche, W., Hessini, K., Gharbi, F., Jaleel, C.A., Ksouri, R. and C. Abdelly, Photosynthesis and photosystem-2 efficiency of two salt-adapted halophytic seashore *Cakile maritima* ecotypes. Photosynthetica 46: 410–419 (2008).
- Merzlyak, M.N., Gitelson, A.A., Chivkunova, O.B. and V.Y. Rakitin, Nondestructive optical detection of leaf senescence and fruit ripening. Physiol. Plant 106: 135–141 (1999).
- Merzlyak, M. N., Solovchenko, A.E. and A.A. Gitelson, Reflectance spectral features and non -destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. Postharvest Biol. Technol. 27:197–211 (2003).

- Moran, J. A., Mitchell, A.K., Goodmanson, G. and K.A. Stockburger, Differentiation among effects of nitrogen fertilization treatments on conifer seedlings by foliar reflectance: a comparison of methods. Tree Physiology 20: 1113– 1120 (2000).
- Mukherjee, M. and T. Dalal, Early seedling growth and accumulation of proline and phenol in *Trigonella foenum-graecum* under heavy metal stress. Int. J. Sci. Res. 3(8): 1271-1273 (2014).
- Pilon-Smits, E.A.H., Quinn, C.F., Tapken, W., Malagoli, M. and M. Schiavon, Physiological functions of beneficial elements. Current Opinion Plant Biology 12: 267–274 (2009).
- Põldma, P., Moor, U., Tõnutare, T., Herodes, K. and R. Rebane, Selenium treatment under field conditions affects mineral nutrition, yield and antioxidant properties of bulb onion (*Allium cepa* L.). Acta Sci. Pol. Hortorum Cultus, 12 (6): 167-181 (2013).

- Shacklette, H.T., Cadmium in plants: U.S. Geological Survey Bulletin 1314-G, PP. 28 (1972).
- Wu, F.B., Chen, F., Wei, K. and G.P. Zhang, Effect of cadmium on free amino acid, glutathione and ascorbic acid concentrations in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. Chemosphere 57(6):447-54 (2004).
- Ye, H.B., Yang, X.E., He, B., Long, X. and W.Y. Shi, Growth response and metal accumulation of *Sedum alfredii* to Cd/Zn complex-polluted ion levels. Acta Bot. Sin. 45: 1030–1036 (2003).