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Role of Cd and Hg on biochemical contents of fennel and its reduction by exogenous treatment of nitrogen

Mohsin Anayat, Rajneesh Kumar Agnihotri, Rashmi Vamil, Sanjay Kumar and Rajendra Sharma

Department of Botany, School of Life Sciences, Khandari, Agra, Dr. B.R. Ambedkar University Agra-282002

Abstract- Heavy metal contamination is a serious environmental problem that limits crop production and endangerous for human health. The present study was conducted at Department of Botany, School of Life Sciences, Dr. B.R. Ambedkar University, Agra for assessing the toxic effect of cadmium and Mercury at different concentration viz. 10, 20 and 50 µm on the chlorophylls, proline and protein content of *Foeniculum vulgare* L. The standard solution was prepared using standard metal of inorganic ventures. These heavy metals affected the chlorophylls, carotenoids, protein and proline content of fennel seedlings as compared to control. Carotenoids were less effected as compared to chlorophyll 'a' and 'b' at low concentration (10µm), while at higher concentrations i.e. 20 and 50µm, the chlorophylls, carotenoids and protein content of the seedlings were reduced drastically. However the addition of nitrogen (5 mM) minimize the effect of these heavy metals to some extent. The proline content of plants was increased under Cd and Hg treatments at all concentrations. However in this case additional supply of nitrogen in the form of Ammonium nitrate decreased proline content of plants at all concentrations of these heavy metals (Cd and Hg).

Index Terms- Chlorophyll, Fennal, Heavy metals, Proline and Protein

I. Introduction

Foeniculum vulagre (L.) is a biennial or short lived perennial herb attaining a height of up to 2 meter. Fennel prefers loamy soil, rich in organic matter with a pH between 6.5 and 8.0 and soil temperature between 50 – 75 ºF. It is a native of mediterranean region and Europe but is commonly cultivated through out India especially in Assam, Maharashtra, Punjab and Gujarat (Kaur and Arora, 2006). It is used as a spice and also as an important ingredient in various folklore medicines throughout the world. India is well known historically as a land of spices and aromatic plants and continues to be one of the leading producers of spices and medicinal plants in the world (Prajapati *et al*., 2005). Spices are dried parts of the plants, which have been used as diet components often to improve colour, arome and acceptability of food. With the current emphasis of eating healthy diets that are low in fat and salts, people are turning to various herbs and spices to flavour their food. But the overall growth and productivity of these plants have been reduced to considerable extent by the heavy metal pollution in the air, soil and water (Husain *et al.*, 1995). **T**oxic heavy metal interfere with several metabolic processes, causing toxicity to the plants revealed by reduced root growth and phytomass, chlorosis, photosynthetic impairing, stunting and finally plant death (Sinha *et al*., 2007). Among list of various heavy metals, the Cd and Hg top the relative toxicity of metals to flora and fauna. Mercury has been found to reduce phytomass, total chlorophyll, photosynthesis, nitrogen and phosphorous contents in aquatic plants. Mercury (Hg) has become a problem of current interest as a result of environmental pollution on global scale (Aliu *et al*., 2013). Cd is a non essential heavy metal that does not have any metabolic function in plants (Bavi *et al*., 2011). It can be incorporated and accumulated by all organisms in large amounts and disturb physiological metabolism in plants like transpiration, photosynthesis, respiration and nitrogen assimilation (Wang *et al*., 2008). Proteins are the main components of nucleic acid, cell membrane and other cell organelles. Heavy metals are known to reduce protein content of various plants (Bavi *et al*., 2011; Balestrasse *et al*., 2003). Most common form of nitrogen i.e. NO_3^- are highly reactive and mobile and are generally susceptible to losses to heavy metal stress condition. NH_4^+, which are water soluble and are easily available for absorption by plants.

II. Material and Methods

The seeds of *Foeniculum vulgare* L. were obtained from National Seed Corporation, Sikandara, Agra. Seeds with uniform size, colour and weight were chosen for the experimental purpose. Two types of experiments – petridish experiment and pot and sand culture experiment were conducted in triplicate form. The seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) to prevent any contamination. After washing with distilled water they were soaked in 5% bavistin (a systemic fungicide) for 10 – 12 minutes. The selected seeds were placed in 10 cm diameter petridishes lined with filter paper Whatman No. 1 to this 5 ml solution of $NH_4(NO_3)$ at different levels (control, 5 mM) with various concentration of Cd and Hg (control, 10, 20 and 50 µM) was applied. Distilled water was used as control. The seeds were allowed to germinate for studying various parameters for 10 – 15 days. Three replicates of each treatment were maintained. The chlorophyll and carotenoids were estimated by Arnon's (1949) technique using double beam UV-visible spectrophotometer (Systronics). Proline estimation was carried out by Bates *et al.*, (1973) method, transmittance was read at 520 nm by using double beam UV-visible spectrophotometer (Systronics) and the standard curve was prepared by using pure proline (BDH). Protein estimation was carried out by Folin and Lowry (1975) method, transmittance was read at 750 nm by using double beam UV-visible spectrophotometer (Systronics) and the standard curve was prepared by using BSA.

III. Result and Discussion

The effect of Hg was more pronounced than Cd and all concentration and among different types of chlorophyll, chlorophyll 'b' was more effected than chlorophyll 'a'. Data presented in Table 1 indicate that chlorophyll 'a' was reduced upto 0.320 and 0.343 mgg^{-1} Fw at 10µM concentration of Hg and Cd respectively as compared to control (0.354 mg g^{-1} Fw). Chlorophyll 'b' was reduced upto 0.330 and 0.376 mgg^{-1} Fw at 10µM concentration of Hg and Cd treatment as compared to control (0.421mg g^{-1} Fw). Similarly at 20µM concentration chlorophyll 'a' reduced upto 0.288 and 0.330 mgg^{-1} Fw for Hg and Cd as compared to control (0.354 mgg^{-1} Fw), while chlorophyll 'b' was reduced upto 0.311 and 0.347% mgg^{-1} Fw as compared to 0.421mgg^{-1} Fw (control) under Hg and Cd treatment respectively and at 50µM concentration chlorophyll a was reduced upto 0.252 and 0.290 mg g^{-1} Fw as compared to control (0.354 mg g^{-1} Fw), while chlorophyll 'b' was reduced upto 0.290 and 0.309 mg g^{-1} Fw as compared to control (0.421mg m^{-1} Fw). The application of nitrogen (Ammonium nitrate) in the nutrient medium proved beneficial for all pigments. Increase in chlorophyll 'b' was more than the chlorophyll 'a'. Carotenoids appears to be more tolerant to heavy metals as compared to other pigments thus showed an increase in the presence of the nitrogen. Thus chlorophyll a, b, and total chlorophyll were drastically reduced under both metal treatments especially at higher level. Similar results have been obtained by Xin Chen *et al*., 2011 working under Cd stress on pakchoi and mustard plants. Tantrey and Agnihotri (2010) found total chlorophyll reduced considerably due to heavy metal Cd and Hg on Gram (*Cicer arietinum*). Shekar *et al*. (2011) have shown a reduction in chlorophyll content in tomato plants when treated with mercury. With the application of nitrogen carotenoids showed about 4% and 3% increase as compared to control at 50 µM concentration of Cd and Hg . From the data presented in Table 1 it is clear that after the supply of nitrogen all the chlorophyll and carotenoid contents increased as compared to control. Thus in agreement with earlier reporter (Sun *et al*., 2008, Vajpayee *et al*., 2000; Mobin and Khan, 2007). Similar results have been obtained in other laboratory studies (Jiang *et al*., 2007). In this study we find that chlorophyll 'a' content exceeded that of chlorophyll 'b' in all treated plants, which has been proven by other researchers (Mobin and Khan, 2007; Singh *et al.*, 2012). Moreover, the application of heavy metals shows that there occurs a decline in the carotenoid contents in all treated plants and has been proven by various workers (Thapar *et al*., 2008; Da-Lin *et al*., 2011; Xin-Chen *et al*., 2011; Singh *et al*., 2012). Proline, an amino-acid is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress (Saradhi *et al*., 1993). A kind of amino acid which could play a therapeutic role in plants. Fennal Plants shows an increase of upto 0.207, 0.268 and 0.468 mg g^{-1} proline as compared to control (0.182 mg g^{-1} proline) when treated with the 10µM, 20µM and 50µM concentration of Cd. When treated with Hg, the proline concentration was further enhanced and it was 0.221, 0.282 and 0.498 mg g^{-1} proline when treated with 10, 20 and 50µM of Hg as compared to control (0.182 mg g^{-1}) proline. However with the application of nitrogen the proline content was reduced to some extent. At higher concentration of Cd and Hg (50µM) proline was reduced upto 64% and 65% over control under the application of nitrogen. Similar results were reported by (John *et al.,* 2009; Singh *et al.,* 2012). Heavy metals are known to reduce the protein content of the plants. Data presented in the Table2 demonstrated the deleterious effects of Cd and Hg on protein content. This detrimental effect was more due to Hg as compared to the Cd. As evident from the Table 2 control plants exhibit maximum 0.60 mg/g, protein content and the minimum 0.42 mg/g and 0.39 mg/g, reported in 50µM Cd and Hg concentration. Protein content was reduced upto 0.55 mg/g under 10µM concentration of Cd, 0.50 mg/g under 20µM concentration of Cd. Hg proved more harmful for protein content, and reduced it by 0.52 mg/g under 10µM Hg, 0.48 mg/g under 20µM of Hg. In contrary to Cd and Hg, inclusion of nitrogen (NH_4NO_3) 0.5mM in nutrient medium increases protein content and proved to be beneficial for plants. Plants showed 24% increases in protein content at 50µM concentration of Cd and 38% increase at 50µM of Hg when treated with nitrogen. Our findings are similar to the findings of various workers that there occurs reduction in the protein content by the application of heavy metals (Balestrasse *et al*., 2003; Bavi *et al*., 2011). The reduction in the amount of protein could be due to decrease in protein synthesis or an increase in protein degradation (Balestrasse *et al*., 2003).

IV. Conclusion

In the present study, exposure of heavy metals (Cd and Hg) to fennel plants affected different parameters like chlorphyll, carotenoids, proline and protein content of this plant. Exposure of metals to the seedlings decreased the total chlorophyll content, carotenoids and proteins contents as the metal concentration goes on increasing. The present results shows that Cd and Hg toxicity increases the proline content of the fennel seedlings with the imposition of low concentration of Cd and Hg (10µm) less amount of proline was increased and as the plants are treated with higher doses (20 and 50 µm) of Cd and Hg there seems to be more increase in the accumulation of proline. In contract to other parameters, in this case the additional supply of nitrogen reduces proline content of the plant.

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AUTHORS

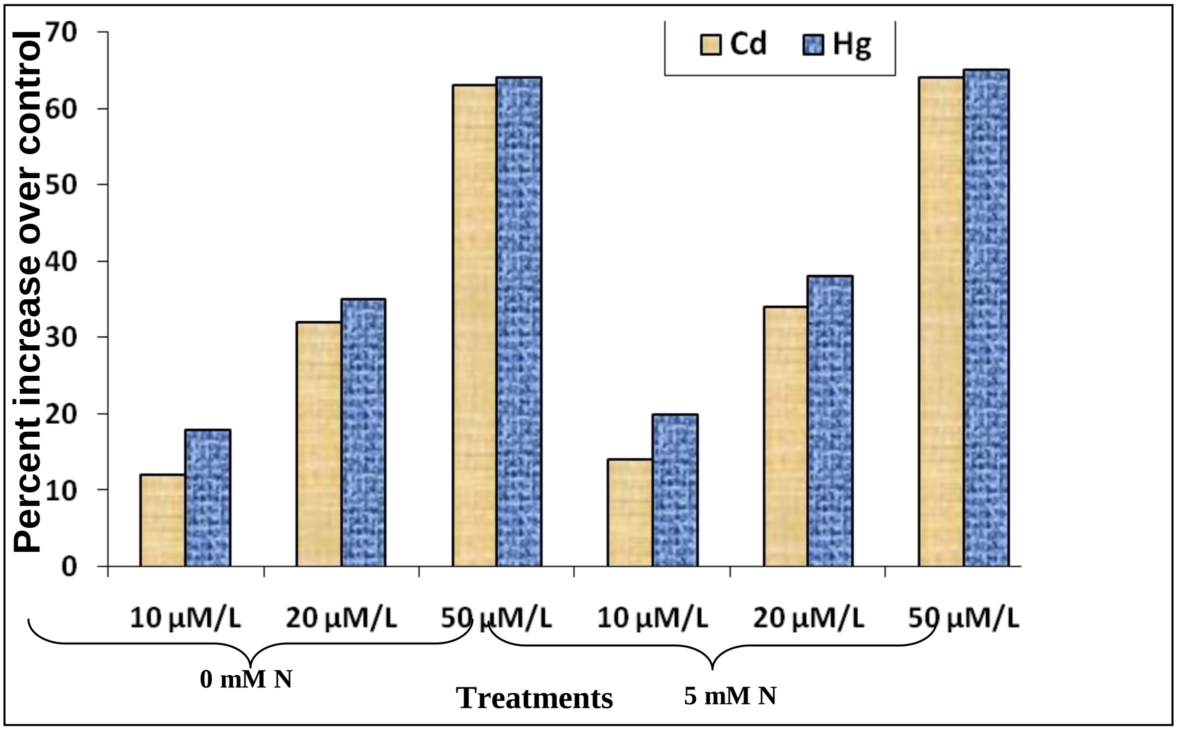
First Author – Mohsin Anayat, Department of Botany, School of Life Sciences, Khandari, Agra, Dr. B.R. Ambedkar University Agra-282002

Second Author – Rajneesh Kumar Agnihotri, Department of Botany, School of Life Sciences, Khandari, Agra, Dr. B.R. Ambedkar University Agra-282002, Email id : rk_agnihotri@rediffmail.com

Third Author – Rashmi Vamil, Department of Botany, School of Life Sciences, Khandari, Agra, Dr. B.R. Ambedkar University Agra-282002

Fourth Author –Sanjay Kumar, Department of Botany, School of Life Sciences, Khandari, Agra, Dr. B.R. Ambedkar University Agra-282002

Fifth Author – Rajendra Sharma, Department of Botany, School of Life Sciences, Khandari, Agra, Dr. B.R. Ambedkar University Agra-282002



PROLINE CONTENT

Fig 1. : Effect of heavy metals and nitrogen interaction on proline content (mg g^{-1} FW) in *Foeniculum vulgare* L.

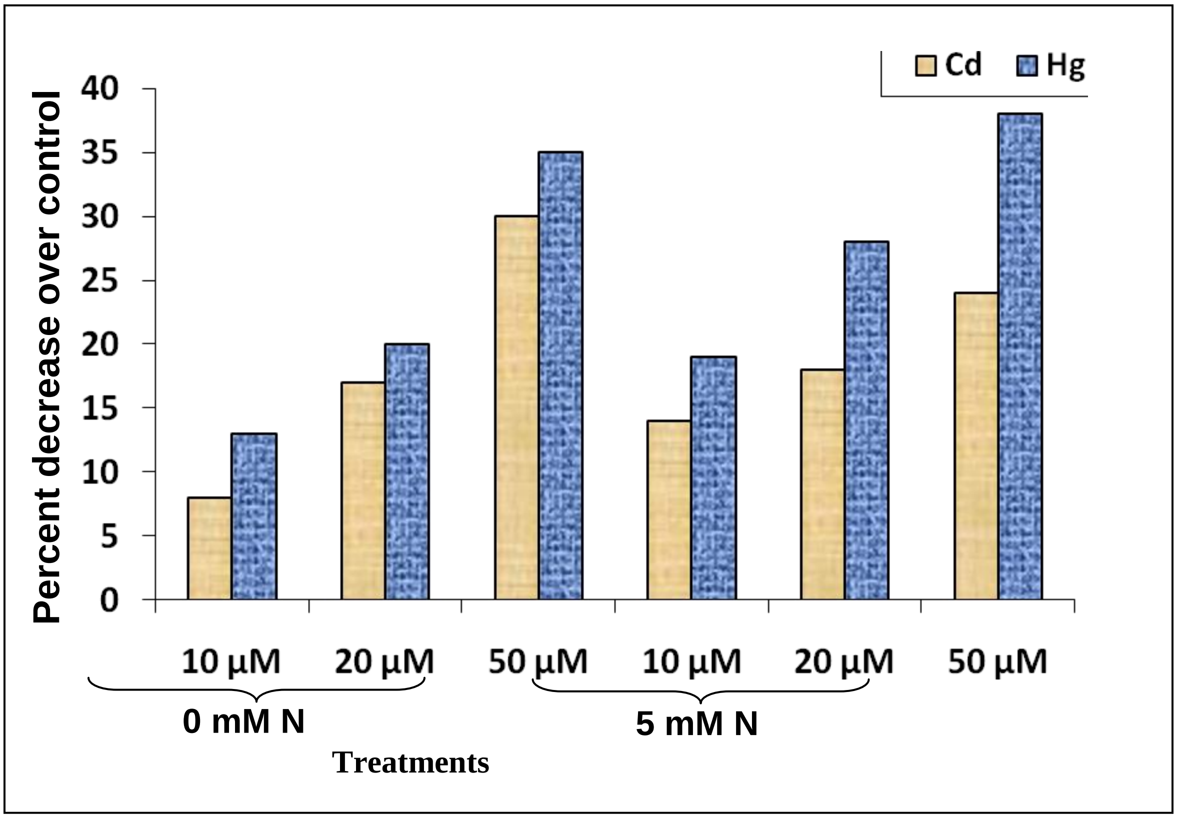


Fig. 2 : Effect of heavy metals interaction on protein content in *Foeniculum vulgare* L. with and without nitrogen

Table 1 : Effect of Cadmium and mercury on pigment composition (mg g^{-1} FW) in Fennel (*Foeniculum vulgare* L.) grown with or without nitrogen.

Metals	**Concentration (µM)**	**Chlorophyll a**		**Chlorophyll b**		**Total Chlorophyll**		**Carotenoids**	
		0 mM N	**5 mM N**	**0 mM N**	**5 mM N**	**0 mM N**	**5 mM N**	**0 mM N**	**5 mM N**
Control	0	0.354	0.402	0.421	0.483	0.775	0.885	1.547	1.646
Cd	10 µM	0.343	0.389	0.376	0.465	0.719	0.854	1.520	1.603
	20 µM	0.330	0.370	0.347	0.412	0.677	0.782	1.492	1.562
	50 µM	0.290	0.350	0.309	0.391	0.599	0.741	1.418	1.497
Hg	10 µM	0.320	0.380	0.330	0.415	0.650	0.795	1.485	1.579
	20 µM	0.288	0.337	0.311	0.400	0.599	0.737	1.439	1.490
	50 µM	0.252	0.310	0.290	0.363	0.542	0.673	1.376	1.421

N – Nitrogen
Data are average of 3 replicates.

Table 2 : Effect of Cadmium and mercury on proline content (mg g^{-1} FW) and protein content in Fennel (*Foeniculum vulgare* L.) in shoots grown with or without nitrogen.

Metals	Concentration (µM)	Proline content (mg g^{-1} FW)		Protein content (mg g^{-1} FW)	
		0 mM N	**0 mMN**	**5 mMN**	**5 mM N**
Control	0	0.182	0.60	0.72	0.165
Cd	10 µM	0.207	0.55	0.62	0.192
	20 µM	0.268	0.50	0.59	0.250
	50 µM	0.486	0.42	0.55	0.462
Hg	10 µM	0.221	0.52	0.58	0.206
	20 µM	0.282	0.48	0.52	0.265
	50 µM	0.498	0.39	0.45	0.477

N – Nitrogen
Data are average of 3 replicates.