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Original Article

Effect of Increasing Concentrations of Chromium on *In-vitro* Culture of *Datura inoxia*

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ABSTRACT

The World's industrialization has spectacularly increased the overall environmental 'load' of heavy metal toxins in such a way that people dependent upon them for proper functioning. Industrial processes have actively manufactured, mined, burned, and refined heavy metal compounds for many reasons. Today heavy metals are profuse in drinking water, air and soil. They are exists in almost every area of modern consumer products such as in materials of construction materials, medicines, agents of destruction, processed foods, and in fuel sources. Their presence in the ecosystems causes accumulation by living organisms in their bodies. Industrial activities produce large amount of wastewater polluted by metals. Thus, environmental cleanup in the form of removal of metals from industrial effluents was a thrust area in today's research. The present piece of research is aimed to make an in-depth investigation about effect of various chromium concentrations on *In vitro* culture of *Datura inoxia*. Here percentage of survival and shoot length was analyzed on heavy metal supplemented M. S. Media. Nodal and shoot tip explants were used. *Datura inoxia* shown survival up to 45 mg/l, but at higher concentration of chromium causes adverse effect on shoot length and percentage of survival was decreased.

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INTRODUCTION

Soil pollution by metals differs from pollution of air and water, because these heavy metals exist in soil for long term than in other compartments of the biosphere[4]. In recent decades, the annual worldwide release of heavy metals reached 22,000 Metric Ton for cadmium, 783,000 Metric Ton for lead and 1,350,000 Metric Ton for zinc[5]. Major sources of heavy metal contaminants in soils include smelting, metalliferous mining, sewage sludge treatment, waste disposal sites, Agricultural fertilizers, warfare and military training, and electronic industries etc. These industrial activities are responsible for very high heavy metal concentrations in the environment, which may be 100 to 1000 fold higher than natural concentration in the Earth's outer crust[7]. Existence of heavy metals in high concentration into the environment results in severe ecological and health problems[8]. They are become very prevalent in our environment. By polluting food chain, these elements became a risk to environmental and human health.

Datura inoxia is an erect, bushy herb some what branched, generally grows up to 1.5 m height. Leaves are supported by a long stalk and they are simple and alternate. Crushed leaves have unpleasant smell. Its stem is smooth, hairy and purplish. Flowers are white, tubular and erect. Fruit is a capsule hanging from the branched with long and slender spines, contains numerous small black seeds[11]. It occurs on riverine, disturbed areas. It requires low rain. It mainly prefers rich land and warm region. Basically it is native to Central Europe and South America. It can be cultivated as a garden ornamental and for medicinal purposes. It is a poisonous plant and deemed dangerous to human and animals also and can cause nausea, skin diseases by simple contact. Varied species of Datura have been used in traditional medicine worldwide, among them *Datura inoxia*, *Datura metel*, and *Datura stramonium* are commonly used. Datura is potent member of the *Solanaceae* family.

Plants also have some capacity to decrease the threats of pollutants[9]. According to researchers plant tissue culture is a suitable laboratory tool for studies on phytoremediation. Established *In vitro* cultures can be cultured indefinitely and are made available when required. In contrast, whole plants studies in soil or in hydroponic systems have a limited lifespan. Thus, the time required for experimental investigations may be considerably reduced using plant tissue culture technique than whole plants. There are various advantages of plant tissue cultures also as compared with whole plants. Plant tissue cultures are grown and maintained in sterilized and controlled environment, thus they can be used to study how the responses and metabolic capabilities of plant cells differ from those of microorganisms of rhizosphere[1,2]. Thus here effect of chromium concentrations on *Datura inoxia* tested *In vitro* culture.

Chromium toxicity

Chromium is a hard metal, brittle and lustrous. Its colour is silver-gray and it can be highly polished, and the green chromic oxide is formed when heated due to its burning. Chromium is unstable in oxygen therefore it immediately produces a thin oxide layer that is impermeable to oxygen and protects the metal present below in soil.

The main human activities that increase the concentrations of chromium (III) are steal, textile and leather industry. The main human activities that increase chromium (VI) concentrations are leather, chemical, electro painting, and textile manufacturing. These applications will mainly increase concentrations of chromium in water. Coal combustion causes chromium release in air and through waste disposal chromium will end up in soils. Chromium generally occurs in the three most common forms: metallic (Cr^0) – does not exist naturally, trivalent ($Cr+^3$) – nutrient, limited solubility and hexavalent ($Cr+^6$) – water soluble, highly toxic. It is widely used in industry due to its anticorrosive property like metal surface plating, leather tanning, glassware cleaning, and textile production, Chromium mainly used in alloys such as stainless steel, in metal ceramics and in chrome plating.

Disposition of fly ash onto land and urban sewage sludge can introduce considerable quantities of chromium into soils. The most stable and common forms of chromium are chromium (III) and the phytotoxic chromium (VI). Phytotoxicity of chromium to plants is decreased by reducing the strongly oxidising chromium (VI) species to chromium (III). Uptake of chromium by roots and its transportation to aerial tissue is low at or near-neutral pH. Foliar concentrations show little relationship with overall soil chromium concentrations[3].

Acute poisoning of chromium causes acute renal failure, nausea, vomiting, contact dermatitis, irritation, eczema, allergies, eczema and reproductive toxicity etc. Through drinking water such as metal contaminated well, dangerous chromium (IV); hexavalent chromium enters in the body. Most of the chromium enters through food because chromium (III) occurs naturally in many vegetables, fruits, meats, yeasts and grains. Food preparation and packaging may alter the chromium contents of food. Even storage of food in steel tanks or cans also raises the chromium concentration. High concentration of chromium (III) can cause health effects like skin rashes. Chromium (VI) in leather products can cause allergic reactions like ulcerations, dermatitis, and allergic skin reactions and upon inhalation it can cause nose irritations and nosebleeds, bronchospasms, asthmatic bronchitis, and edema. It is also carcinogenic. Other health problems that are caused by chromium (VI) are respiratory problems, kidney and liver damage, alteration of genetic material, weakened immune systems, lung cancer etc.

MATERIAL AND METHODS

Experimental methodology included the establishment of *Datura inoxia* on M. S. Media by standardization and formulation of specific composition[10]. Various concentrations of plant growth regulators and their combinations were tried for initiation of plantlets in culture conditions.

Explant collection and sterilization

Shoot tip meristem and nodal explants were collected from contaminated industrial sites and used for initiation of culture. These explants were washed with tap water thrice and then dipped in antifungal Bavistin for 30 min then again washed with water and treated with soap solution for 10 min, after washing it was surface sterilized by Mercuric Chloride and inoculated on heavy metal supplemented M. S. Media.

Trials were also done for survival rate and contamination of the inoculated explants. Table 1 shows the percentage of survival of explant and average shoot length in response to the various combinations of plant growth regulators such as BAP, NAA and KN.

In preliminary studies conducted it was found that the response of the explants of *Datura inoxia* in culture was dependent upon the age of explants and season of collection. Shoots excised from the plant shows better results during August to February were more vigorous and responded more. The time period of the treatments of surface sterilizing agent 0.1% $HgCl_2$ was standardize from 2-3 minutes at which 85-90% explants were sterilized. The explants dehydrated when treated with 70% ethanol solution may be due to the softness of shoots[6].

The inoculated cultures were maintained at 250 C and 15-16 hrs photoperiods. After 6 to 7 days bud breaking from the axillary and apical meristem was observed. The best response was observed on medium supplemented with combination of BAP, NAA and KN.

Effect of chromium on *in-vitro* cultures of *Datura inoxia*

Selection of Chromium accumulator plantlets were performed on MS medium supplemented with 0.5 BAP, NAA and Kinetin and also the addition of Chromium at concentrations 0.1-50 mg/l. The cultures were exposed to increasing concentrations of toxic metal for several months to increase the accumulation capacity during *In vitro* cultivation. The cultures were examined on a medium with increasing concentrations of Chromium, which was already a lethal level for other plants in the tissue cultures.

RESULTS AND OBSERVATIONS

It has been found that as the Chromium concentration increases the percentage survival rate was decreased. Shoot length was also affected by higher concentrations of Chromium in the medium. The proportion of growth in shoot length was decreases at the higher concentration of Chromium in the medium. Some percentage of yellowing of the shoots was observed in the higher concentrations due to death of some tissues in cultures. Due to increased amount of the external Cr concentration considerably there was remarkable decrease the shoot length and vigorous growth whereas more than 90% of response was shown by culture of Datura on control medium.

As the Chromium concentration increases in medium the rate of growth of shoots were decreased or stunted due to its toxic effect as tabulated in Table 2. According to Graph 1, as the heavy metal concentration increases growth of *Datura inoxia* reduces but due to accumulation capability Datura cultures are shown the survival up to 45 mg/l. Figure 1 shows effect of chromium treatements on shoots of *Datura inoxia.*

CONCLUSION

In recent era the importance of biodiversity is increasingly considered for the cleanup of the heavy metal contaminated soil. Present research paper is an effort to fulfill the emerging thrust area of phytoremediation research gaining commercial significance in the contemporary field of environmental biotechnology. This technique of plant tissue culture also can be used in study of metal tolerance and accumulation of a plant by exposing it in culture media containing known quantities of the specific heavy metal like Chromium. The plants identified and screened by this method can then be tested for phytoremediation at polluted land. The research also has the potential to study the effect of metal on whole plants. Here *Datura inoxia* cultures were shown the excellent accumulation capacity for chromium.

REFERENCES

1. Lebeau, T., Braud A., and Jezequel, K. (2008). Performance of bioaugmentation assisted phyto-extraction applied to metal contaminated soils. *Environ Pollut,* 153:497–522.
2. Chaudhry, Q., Blom-Zandstra, M., Gupta, S., and Joner, E. J. (2005). Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. *Environ. Sci. Pollut Res*, 12:34–48.
3. Mcgrath, S.P., (1995). Chromium and Nickel. *In* "Heavy Metals in Soils" (Ed. Alloway B.J.), 152-178.
4. Lasat, M. M., (2002). Phytoextraction of toxic metals- A review of biological mechanisms. *Journal of Environmental Quality*, 31, 109-120.
5. Singh, O. V., Labana, S., Pandey, G., Budhiraja, R., & Jain. R. K., (2003). Phytoremediation: an overview of metallicion decontamination from soil. *Applied Microbiology and Biotechnologv*, 61. 405-412.
6. Waoo, A. A., Khare, S. S., and Ganguli, S., (2013). In-vitro Propagation of *Datura inoxia* from Nodal and Shoot Tip Explants.*World Journal of Environmental Engineering*. Vol. 1, No. 1, 1-4, 02 May 2013, DOI: 10.12691/wjee-1-1-1, © Science and Education Publishing , ISSN 2372-3084
7. Carral, E., Puente, X., Villares, R., *Sci. Total. Environ*., 172 (1995) 175
8. Cerbasi, I.H., and Yetis, U., (2001). Biosorption of Ni (ii) and Pb (ii) by Phanerochaete chrysosporium from binary metal system – Kinetics. *Water Res.,* 27, 15–20.
9. Cunningham, S. D., Shann, J. R., Crowley, D.E., and Anderson, T.A., Phytoremediation of contaminated water and soil, *Kruger*, E.L.
10. Engvild, K. C., (1973). Shoot Differentiation in Callus Cultures of *Daturainoxia*, *Physiologia Plantarum*. 28(1), 155-9.
11. Preissel, Hans-Georg. Brugmansia and Datura: Angel's Trumpets and Thorn Apples Buffalo, New York: Firefly Books. pp. 117–119. ISBN1-55209-598-3.

Table 1. Effect of various concentrations of BAP, NAA and KN on percentage of survival and average shoot length of *Datura inoxia*

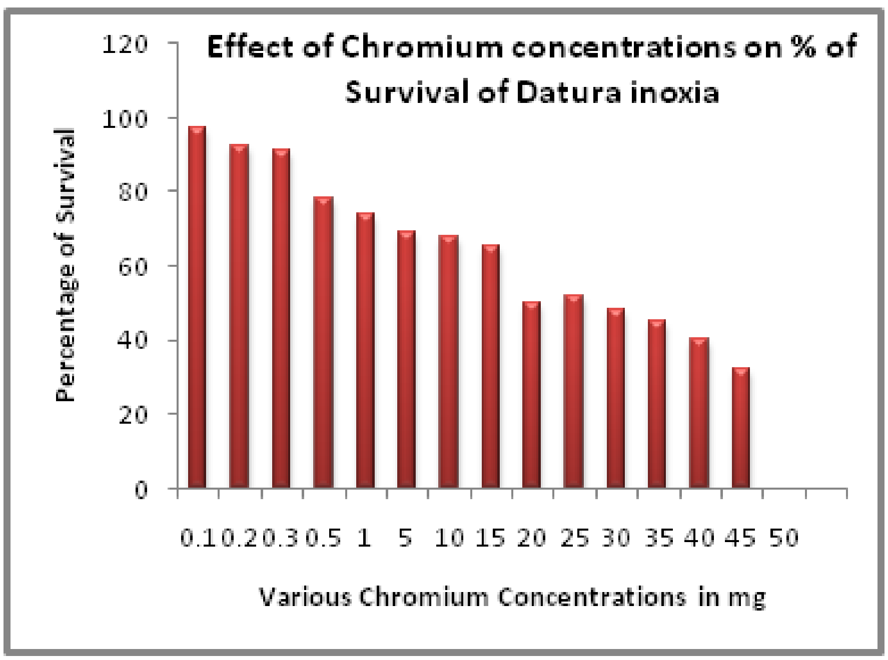
MS + Growth Regulators (mg/l)			% of Initiation	Average Shoot Length in cm
BAP	NAA	KN		
0.1	-	-	-	-
0.5	-	-	10	0.5
1	-	-	20	0.8
2	-	-	24	1
0.5	0.1	-	25	1.5
0.5	0.5	-	40	2.5
1	0.5	-	50	0.75
2	0.5	-	62	2
0.1	0.5	0.5	55	2.5
0.5	0.5	0.5	94	4.1
1	0.5	0.5	70	1.22
2	0.5	0.5	75	1
0.5	-	0.5	10	0.5
1	-	0.5	15	0.5
2	-	0.5	15	0.5

Table 2. Effect of different concentrations of chromium on % survival of *Datura inoxia*

0.5 MS + $CrNO_3$ (mg/l)	% of survival*	Average shoot length* (in cm)	Average no. of shoots*
0.1	97	3.8	1-2
0.2	92	3.81	1-2
0.3	91	3.83	2
0.5	78	3.85	2
1	74	3.87	1
5	69	3.90	1
10	68	3.91	1
15	65	3.92	1
20	50	3.96	1
25	52	3.96	1
30	48	3.97	1
35	45	3.97	1
40	40	3.97	1
45	32	3.97	1
50	-	-	-
SD (±)	25.86	1.01	
SE (±)	6.68	0.26	

* Values are mean of 3 replicates

Figure 1. Effect of chromium treatments on shoots



Graph 1. Effect of Increasing Concentrations of Chromium on *Datura inoxia*