Effect of heavy metals on in vitro growth and development of the *Momordica cymbalaria* Fenzl

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Abstract

Heavy metals have played a great role in the genesis of the present-day civilization. Human beings are affected when these metals are added to the food chain. Although these are the most important plant nutrients, they are phytotoxic at high concentrations. Heavy metals at super optimal concentrations affect different metabolic pathways in plants and result in their ceased growth and development. They may enter plants either by their root system or through foliar uptake; stunted growth, chlorosis, necrosis, and reddish-brown discoloration are visible symptoms of severe metallic phytotoxicity. The study of heavy metal stress tolerance on *Momordica cymbalaria* shows the effect on the plant growth and metabolism. All heavy metals treated with high concentrations affect the overall plant growth. The Murashige and Skoog (MS) basal medium with ZnSO₄ at 100 µM concentration resulted in healthy shoot development (9) with a maximum shoot length of 7.2 cm. MS basal medium with low concentration of CuSO₄ (50 µM) achieved a maximum shoot number (7) with healthy leaves and shoots. MS basal medium with higher concentration of CdCl₂ (150 µM) affects plant growth and reduced the regeneration capability completely.

Keywords *Momordica cymbalaria* · ZnSO₄ · CuSO₄ and CdCl₂ · Chlorophyll · UV-Spectrophotometer

Introduction

Heavy metal contamination in soils is caused by either natural processes or by human activities (Fidalgo et al. 2013). Heavy metals such as Ag, As, Be, Ni, Zn, Cu, Cr, Mn, Cd, Hg, Pb, Sb, Ti, etc., are responsible for the pollution of the environment (Sparks 2005). Plants are known to accumulate heavy metals from soil and deposit them in various parts of the plant body depending on their affinity to a particular metal (Bhat et al. 2010; Celik et al. 2010; Haribabu and Sudha 2011). Plants are the main bridge for the transfer of heavy metals from contaminated soil to humans (Shin et al. 2013; Souri et al. 2019). Heavy metal contamination of herbal remedies has previously been reported in many Asian, South American and African herbal products in various countries (Dghaim et al. 2015).

The ability of plants to absorb metals from the soil also depends on several other non-soil factors including cationic characteristics and metabolic processes (Steve et al. 2018). Among several heavy metals, Ni, Mn, Cr, Zn, Cu, Hg, As and Cd are common toxic metals regularly entering the food chain (Singh and Kalamdhad 2011; Wong and Selvam 2006). Bioremediation is a modern thought and safe practice in the elimination of heavy metals from the environment (Rahman and Singh 2020; Song et al. 2017). For example, it has been reported that water hyacinth is used in pollution treatment systems for the removal of heavy metals. It also serves as a structural component in ribosomes and appears to stabilize the ribosome particles for protein synthesis (Mary lissy and Madhu 2011).

The availability of heavy metals to plants is influenced by soil temperature, soil pH and ion exchange, soil organic matter and heavy metal concentration in soil. The ability of plants to absorb metals also depends on several other non-soil factors including cationic characteristics and metabolic processes (Dalvi et al. 2013). Among the several heavy metals, Ni and Cd are common toxic metals regularly entering...
the food chain, whereas Cu and Zn are essential for plant growth and development but can be toxic at high concentrations (Van Hoof et al. 2001; Hall 2002). Heavy metal analysis of medicinal plants should be prioritized so that the contamination cannot accumulate up to the finished products (Singh et al. 2014). In vitro plant tissue culture enables the selection of plants that are resistant to certain metal ions. However, the previous report on *M. charantia* revealed the accumulation and distribution of these heavy metals in different plant parts like Ni, Cu, Pb, Cr, Cd, Co, Fe and Al in roots; Zn in branches and stem; and Mn in leaves (Savsatliet al. 2016).

*Momordica cymbalaria* belongs to the Cucurbitaceae family and contains many bioactive compounds in different plant parts. These plants originated in the tropical regions of India and South East Asia. The plant has synonyms named *Momordica tuberosa* Roxb or *Luffa tuberosa* Roxb. It is commonly known as Athalakkai in Tamil and Kasarakaya in Telugu. It is used as an edible vegetable and also in various therapeutic treatments. *Momordica cymbalaria* arise from a small perennial tuber; it is an herbaceous climber and climbs on supports with the aid of tendrils or allowed to grow on the boundary of fields, fences and in the crop fields.

Previous studies on *M. cymbalaria* plant reported the anti-cancer (Jeevanantham et al. 2011), anti-diabetic (Firdous et al. 2009), neuroprotective and anti-ulcer (Bharathi Dasan et al. 2010), anti-ovulatory, abortifacient and cardioprotective (Raju et al. 2008), antioxidant (Prashanth et al. 2013), hypolipidemic (Ezra et al. 2014), anti-diarrheal and protective (Raju et al. 2008), antioxidant (Prashanth et al. 2016). In vitro plant tissue culture enables the nodal propagation of *M. cymbalaria*. Hence, the current study refers to be initial report on the assessment of the heavy metal effect on in vitro nodal propagation of *M. cymbalaria*.

### Materials and methods

*Momordica cymbalaria* healthy young nodes were collected, rinsed with fresh water for 15 min, subsequently washed with 10% tween 20 (liquid soap) for 5 min and then washed thrice with sterilized double-distilled water. Afterward, the explants were sterilized with 0.1% bavistin (a fungicide) for 5 min, followed by 0.1% mercuric chloride (HgCl₂) for 1–3 min in an aseptic environment. Then, the explants were washed with double sterile distilled water to vanish HgCl₂. These disinfected explants were inoculated on culture medium (Murashige and Skoog 1962) under aseptic conditions (Chaitanya et al. 2020).

### Heavy metal stocks

All the heavy metal chemicals were purchased from Merck India Ltd., Mumbai. The heavy metal stock solutions (1000 µM) were prepared as per the molecular weight of the chemicals (CdCl₂.2H₂O–183.32 g/mol, CuSO₄·5H₂O–249.69 g/mol, ZnSO₄·H₂O–179.45 g/mol) using sterile distilled water, and different concentrations (50 µM, 100 µM and 150 µM) of these heavy metals were prepared accordingly from these stock solutions.

### Culture media and conditions

MS medium augmented with vitamins, myo-inositol (100 mg/l), sucrose (30 g/l), agar (8 g/l) and different concentrations of heavy metals like CuSO₄/ZnSO₄/CdCl₂ (50 µM, 100 µM and 150 µM). The basal MS medium without any hormones and heavy metals was also prepared accordingly as per the standard protocol. The pH was set to 5.6–5.8 with 0.1 N NaOH or 0.1 N HCl and autoclaved at 121 °C for 15 min. The cultures were maintained in a sterilized culture room and incubated at 26±2 °C with a relative humidity of 60±10% and 16 h photoperiod and 8-h dark conditions (Perveen et al. 2012).

### Estimation of chlorophyll content

The chlorophyll content of the test samples was estimated by Arnon (1949) method. According to this method, 1 gr of the matured leaf sample was taken and blended with 20 ml of acetone (80%) in a pre-chilled motor and pestle. Later a pinch of MgCO₃ was added to the above leaf sample and blended again. The resultant mixture was maintained at 4 °C for about 3 h, followed by centrifugation at 3000 rpm for 5 min. The supernatant was collected in a 100-ml conical flask, and the volume was made up to 100 ml using 80% acetone. The absorbance of this test solutions was measured at 645 and 663 nm using a UV–Vis spectrophotometer, and the amount of chlorophyll was estimated by using the below formula.

\[
\text{Chlorophyll a (mg/g FW)} = \frac{12.7 \times A_{663} - 2.69 \times A_{645} \times V}{1000 \times W}
\]

\[
\text{Chlorophyll b (mg/g FW)} = \frac{12.9 \times A_{663} - 4.68 \times A_{645} \times V}{1000 \times W}
\]

\[
\text{Total Chlorophyll content (mg/g)} = \frac{20.2 \times A_{663} - 8.02 \times A_{645} \times V}{1000 \times W}
\]
Data collection and interpretation

The inoculated nodal explants were observed every week and noted down the changes/responses of explants toward each heavy metal under study. The data on the frequency of explants responded for callus formation, the average number of shoots and the length of shoots and roots were recorded regularly at weekly intervals. The data were analyzed statistically using analysis of variance (ANOVA). All the experiments were conducted with 30 explants and each was repeated thrice (Fig. 1).

Fig. 1 Node based in vitro plant regeneration of *Momordica cymbalaria* Fenzl with different concentrations of heavy metals. a Formation of shoots and leaves from node (Control). b Induction of shoots formation and leaves with ZnSO₄ (50 µm). c Formation of shoots and leaves with CuSO₄ (50 µm). d Green callus and shoot formation with CdCl₂ (50 µm). e Increasing number of leaves and shoots with ZnSO₄ (100 µm). f Increasing number of leaves and shoots with CuSO₄ (100 µm). g Shrinkening of shoots with CdCl₂ (100 µm). h Root initiation and shrinking leaves and shoots with ZnSO₄ (150 µm). i Root formation and shrinking leaves and shoots with CuSO₄ (150 µm). j Fully shrink dead shoot with no leaves with CdCl₂ (150 µm).
Results and discussion

Nodal explants of *Momordica cymbalaria* cultured on a basal MS medium with or without heavy metals were observed after 15, 30 and 45 days (Fig. 2). Plant growth on the basal medium (control) was found to be normal (Fig. 1a). MS basal medium was amended with heavy metals like ZnSO₄, CuSO₄ and CdCl₂ which showed great differentiation in in vitro regeneration. The percentage of response exhibited is more in nodal explants cultured on ZnSO₄ when compared to control and other heavy metals tested. The increase in the heavy metals concentration over the optimal concentration showed the detrimental growth of the explants cultured (Fig. 1).

**Effect of ZnSO₄**

The amount of ZnSO₄ supplemented in the basal MS medium promotes normal growth of the cultured explants. The enhancement of ZnSO₄ in the medium promotes enhanced growth. Among the tested concentrations of ZnSO₄, nodal explants cultured on MS medium amended with 100 µM ZnSO₄ exhibited high response with regard to the mean shoot number (9) and shoot length (7.2) after 45 days of culture (Fig. 1e; Table 1). As the concentration of ZnSO₄ increased from 50 µM to 100 µM, there is a drastic increase in the growth of the cultured explants (Fig. 1b). Further increase in the concentration of ZnSO₄ resulted in the root formation but shoot growth and proliferation declined (Fig. 1h).

The total chlorophyll content of the cultured explants ranged from 2.834 to 1.578 mg/g (Table 1). Among all the concentrations and combinations tested, 50 µM ZnSO₄ exhibited maximum chlorophyll content than the control and other heavy metals tested (Table 1). The amount of total chlorophyll content was found to gradually decrease as the concentration of ZnSO₄ increased.

**Effect of CuSO₄**

The nodal explants cultured on MS medium amended with 50 µM CuSO₄ showed a better response with an average shoot number (7) and shoot length (6.2 cm) per explant after 45 days of incubation (Fig. 1c). Further increase in the concentration of CuSO₄ beyond 50 µM resulted in the growth reduction of the cultured explants (Fig. 1f, i).

The chlorophyll content of regenerants was also found to gradually decrease with an increase in concentration of CuSO₄. The total chlorophyll content in all CuSO₄-treated samples showed a decrease from 2.743 to 1.405 mg/g over the control samples (Table 1).

![Fig. 2](image-url)  In vitro regeneration from nodal explants at different time intervals
Among the different concentrations of CdCl\textsubscript{2} tested, the nodal explants cultured on MS medium with 50 µM CdCl\textsubscript{2} developed only callus without any signs of growth and development after 45 days (Fig. 1d). Further increase in the concentration of the CdCl\textsubscript{2}, resulted in the slowdown of growth and eventually led to the death of the explants, clearly indicating the toxicity of Cd at high concentration on in vitro regeneration studies (Fig. 1g, j).

The total chlorophyll content of explants cultured on CdCl\textsubscript{2} (50 µM) was found to be 0.853 mg/g. Among all the samples tested, the lowest amount of chlorophyll content was exhibited by the cultures on CdCl\textsubscript{2} (50 µM) (Table 1).

### Discussion

In plants, the induction of secondary metabolites is triggered by both biotic and abiotic stresses. Metal stress can affect the plant growth performance and the yield of biomolecules. Bioactive molecule quercetin, found in the roots of *M. cymbalaria*, is used for the prevention or treatment of COVID-19 and other respiratory tract infections in humans (Aucoin et al. 2020). The quercetin concentration was reported to increase at 200 µM ZnSO\textsubscript{4} or 150 µM CuSO\textsubscript{4} in in vitro regenerated plantlets of *Pluchea lanceolata* (Kumar et al. 2004).

Metals are usually required in tiny quantities during plant growth and development, but at high concentrations, they become phytotoxic (Sarkar et al. 2010). Metals (Zn, Cu, Cd) present at adequate levels in the regeneration medium enhanced the growth and shoot regeneration of *M. cymbalaria* under in vitro study. Virginia Sarropoulou and Eleni Maloupa (2017) examined similar results at elevated levels of 3 different MS medium micronutrients CuSO\textsubscript{4}, MnSO\textsubscript{4} and ZnSO\textsubscript{4} on in vitro shoot proliferation of *Sideritis raeseri*.

Among the heavy metals tested, ZnSO\textsubscript{4} induced optimal response of growth. Zn plays a major role in protein synthesis, metabolism of carbohydrates, lipids and nucleic acids and maintains membrane integrity and detoxifies superoxide radicals (Tamta et al. 2021). Zinc promotes the formation of chlorophyll and carotenoids (Broadley et al. 2007). The optimal Zn concentrations in the regeneration medium have a good influence on the growth of the chloroplast membrane system and chlorophyll content (Ahmad et al. 2015). These findings were consistent with those obtained in *Pluchea lanceolata*, where ZnSO\textsubscript{4}
exhibits a positive response up to 150 μM. Thereafter, a further increase in the concentration resulted in the slowdown of the growth and proliferation (Kumar et al. 2004). Similar findings of the effect of ZnSO₄ were reported in Rauvolfia serpentina (Ahmad et al. 2015), Holarrhena antidysenterica (Agrawal and Sharma 2006); Withania somnifera (Fathima et al. 2011). Similar results were found in Jatropha curcas; when the explants were treated with a lower concentration of nickel (Ni), it may initiate growth and regeneration; however, as the concentration increased, it affected plant regeneration (Sarkar et al. 2010). The chlorophyll content of regenerants was also greatly influenced by the heavy metal stress. Similar effect of decrease in chlorophyll content of the heavy metal treated samples with the increasing heavy metal concentration was found in Albizia lebbeck (Perveen et al. 2012), Rauvolfia serpentina (Ahmad et al. 2015).

However, the concentration of copper across their optimal level brings about drastic changes clearly specifying the toxic effect of elevated copper. Kumar et al. (2004) also reported a similar result that an increase in the concentration of CuSO₄ beyond the optimum level resulted in the reduction of callus weight and growth. Such a stimulatory effect of CuSO₄ was also reported in Stevia rebaudiana (Jain et al. 2009); Capsicum annuum (Joshi and Kothari 2007). In contrast, an increase in the copper level resulted in the high regeneration of wheat and barley (Dahleen 1995). Addition of ZnSO₄ and CuSO₄ to the media promoted higher regeneration capacity when compared to control plants in many different species like Populus (Kalisova et al. 2003); Holarrhena antidysenterica (Agrawal and Sharma 2006); Withania somnifera (Fathima et al. 2011); Rauvolfia tetraphylla (Shahid et al. 2016).

Basically, Cu is involved in respiration, photosynthesis, synthesis of cell wall and protection against oxidative stress (Tamta et al. 2021). Elevated levels of Cu become toxic and inhibit the growth and regeneration of explants (Naik et al. 2015; Javed et al. 2017). This may be resulted due to oxidative stress generated by plants and reduced uptake of other essential metals (Javed et al. 2017). Copper may inhibit pigment production, photosynthesis, and harm to plasma membrane permeability in some species (Macnair 1992). In our investigation, lower copper concentrations (50 μM) increased chlorophyll content. When the CuSO₄ concentration was raised to 150 μM, the total chlorophyll content of the samples treated was found to decrease compared to the control samples. Cu is known to damage chloroplasts, disrupt their structure, functions and hinder chlorophyll production. It also prevents the uptake and transport of other elements such as Mn, Zn, and Fe, resulting in leaf necrosis (Liu et al. 2004). These might be the primary causes of the reduced chlorophyll content at high Cu concentrations in the regeneration medium. Similar findings have been observed in other species, notably Stevia rebaudiana (Jain et al. 2009) and Withania somnifera (Fathima et al. 2011). The concentration of CuSO₄ and ZnSO₄ above the optimum levels resulted in the decrease in regeneration capacity and the amount of chlorophyll content (Perveen et al. 2012; Ahmad et al. 2015; Das et al. 2018; Alam et al. 2020).

Cadmium is toxic for all living organisms including plants. However, plant species may show different tolerance to the presence of cadmium in their root system (Hatamian et al. 2020). The toxicity of CdCl₂ was clearly observed in plant regeneration at higher concentration. These findings are in agreement with the results obtained in Bacopa monniera, where the concentration of CdCl₂ beyond 50 μM resulted in the death of the regenerants (Ali et al. 1998). The inhibition of growth is sign of Cd-induced stress (Fernández et al. 2008). Prolonged treatment of high concentration of Cd resulted in the death of the tissue (Yemets et al. 2021). A similar inhibitory effect of CdCl₂ was also reported in different plant species like Holarrhena antidysenterica (Agrawal and Sharma 2006); woody species (Almeida et al. 2007); Vigna species (Ratheesh Chandra et al. 2010); Albizia lebbeck (Perveen et al. 2012); Rauvolfia tetraphylla (Shahid et al. 2016); Celtis australis (Hatamian et al. 2020). Similar effect of Cd on the reduction of the chlorophyll content was reported earlier by Iqbal et al. (2015).

Correspondingly, chromium also exhibited similar toxic effects of growth and yield reduction and decrease in chlorophyll content as the concentration increased and was reported earlier in several plant species like Lycopersicon esculentum (Shekar et al. 2013), blackgram (Lakshmi and Sundaramoorthy 2003), tomato and brinjal (Purohit et al. 2003), blackgram, soybean and paddy (Ganesh et al. 2006).

**Conclusion**

The present investigation was undertaken with a view to gain a deep insight into the effect of heavy metals like Zn, Cu and Cd on in vitro regeneration of Momordica cymbalaria. The study of heavy metal stress tolerance showed the effect on plant growth and metabolism of M. cymbalaria. All treated heavy metals at higher concentrations above 100 μM affected the plant growth and development. ZnSO₄ and CuSO₄ at optimal concentrations exhibited the profound growth, whereas CdCl₂ at a higher concentration affected plant growth and development and reduced the regeneration capability completely. This is a model experiment and helps to evaluate the effect of different heavy metals on different crop systems.

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Author Contributions G. Chaitanya and T. Shashthree contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript. Ch. PAVani designed the table and analyzed the data.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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