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Research Article

Effect of copper, nickel and lead on callus growth dynamics of *Solanum lycopersicum*

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Abstract
Solanum lycopersicum cv. (Punjab Kesar Cherry) (Tomato) belonging to family Solanaceae has been priced for the presence of lycopene, low sugar content and curative properties. However, the productivity of tomatoes has been observed to be low due to both biotic as well as abiotic stress. Considering the toxicity of copper (Cu), lead (Pb) and nickel (Ni) as environmental contamination and the economic importance of *S. lycopersicum*, the present study was undertaken to investigate the effects of these heavy metals on various growth parameters of callus cultures and plantlet regeneration. Callus induced through nodal segments was inoculated on Murashige and Skoog medium containing different concentrations (0 µM, 50 µM, 100 µM, 200 µM, 300 µM, 400 µM and 500 µM) of copper, lead and nickel. Decrease in different growth parameters was observed as 91.66-16.66 %, 76.38-11.11 %, 81.94-13.88 % for percent callus survival, 90.90-25 %, 72.72-37.50 %, 79.66-20 % for percent callus multiplication, 79.54-0 %, 82.75-33 %, 63.8-50 % for percent root regeneration and 73.33-33 %, 72.50-33 %, 79.6- 20 % for percent shoot generation with increasing concentrations of Cu, Pb and Ni, respectively. Decrease in the average fresh and dry weight of callus was observed for all the metals. The order of toxicity was observed as Pb > Ni > Cu at the highest concentrations used for treating *S. lycopersicum*. The present study revealed that all three metals induced stress in the studied plant and need attention to developing methods to mitigate the consequences of metal toxicity in crop plants.

Keywords: Environmental pollution, Heavy metals, Soil pollution, Tomato, Toxicity

INTRODUCTION

Solanum lycopersicum (Tomato) is a member of the family Solanaceae and is considered a vegetable crop and nutrient food for most culinary uses. Due to the presence of potassium, various antioxidants, vitamin C and choline content and low sugar content in tomato fruits, their consumption is considered good for heart health (Kim *et al*., 2011). The antioxidants in *S.lycopersicum* help the plant to combat stress caused by free radicals when given harsh or stressed conditions.(Kristina*et al.* 2019) Nowadays, increasing pollution level in agricultural soil is one of the major concerns because contaminants significantly cause abiotic stress, thereby decreasing the productivity of food crops(Das and Das, 2019).

Among different environmental contaminants, heavy metals are a group of elements non-biodegradable in nature and can bioaccumulate in both plant and animal systems. Some industrial and biologically important heavy metals include aluminium, cadmium, chromium, copper, lead, manganese, nickel, and zinc (Horsfall and Spiff, 2004; Rehman *et al.,* 2021). Heavy metals are commonly encountered in agricultural soil through industrial wastes and pose serious environmental concerns that can not be overlooked (Guo*et al*., 2006; Okereafor*et al.,* 2020). Due to release of heavy metals via industrial wastes has become a matter of serious

concern (Vijayaraghavan *et al*., 2004). Some heavy metals are essential micronutrients (Zn, Cu, and Ni) at low concentrations for plants, but at higher concentrations, they cause inhibition of growth and development as well as various metabolic disorders (Chojnacka *et al*., 2005).

Copper is reported to be an essential micronutrient required for normal growth and development of plants at lower doses, whereas it is potentially toxic at higher concentrations causing leaf chlorosis, necrosis, inhibition of root growth, retardation of plant growth, stress and injury to plants (Pichhode and Nikhil 2015; Kumar *et al.,* 2021). Some important functions of copper in plants are ATP synthesis, CO_2 assimilation, component of plastocyanin and cytochrome oxidase. The occurrence of Cu is increasing daily in the ecosystem due to different human activities, industrial activities, mining and smelting of Cu-containing ores. Cu generates oxidative stress and ROS (Husain *et al.,* 2019). Higher doses of copper in plants generate ROS and oxidative stress, consequently causing damage to macromolecules and disturbance of metabolic pathways (Pichhodeand Nikhil, 2015). Consequently, Cu exposure results in a profound reduction in growth rates and biomass production. Reduction of root growth in (Rhodes grass) *Chlorisgayana* was reported in some studies (Pichhode and Nikhil 2015). Copper toxicity in black bindweed caused plant death and a decrease in seed production (Pichhode and Nikhil, 2015). Root formation and malformation reduction were reported in beans or *Phaseolus vulgaris* (Katare *et al.,* 2015).

Among various heavy metal pollutants, lead (Pb) is of particular concern because its concentration is increasing continuously in cultivated areas causing human health risks due to the consumption of contaminated food via the food chain (Kumar *et al.,* 2020). In excessive amounts, Pb can cause various toxicity symptoms in plants such as blackening of the root system, chlorosis, stunted growth, inhibition of photosynthesis, altered hormonal functioning, hindered mineral nutrition and adversely affect cell membrane structure and permeability (Sharma and Dubey, 2005).

Nickel is a heavy metal present in soil and water in trace amounts and is considered an essential micronutrient for the improvement of yield and quality of plants (Anjum *et al*., 2015). It is a component of the urease enzyme which is required for nitrogen metabolism in higher plants. Nickel deficiency depressed urease enzyme activity (Barcelose *et al.,* 2018) and other enzymes responsible for nitrate reduction. Phytotoxicity of nickel occurs at different levels, *viz*. morphological, physiological and biochemical. Different symptoms of Ni toxicity include inhibition of growth and development, seed germination, sugar transport, and photosynthesis. Chlorosis, necrosis and wilting (Dubey and Pandey, 2011; Pandey and Sharma, 2002). The EU Commission raised awareness of Ni and has given recommendations to monitor this metal in food crops, including tomatoes. Due to Ni deficiency, disruption in protein synthesis and reduction in the level of total nitrogen has been observed (Ameen *et al.,* 2019). This was accompanied by the accumulation of urea, nitrate and several amino acids (Shimada and Watanab, 2004), which caused leaf chlorosis and meristem necrosis in several plants species such as soybeans (Eskew *et al*., 1984), parsley and cucumber plants (Shimada and Watanab, 2004).

Tomato shoot and root growth were observed to be improved under nickel treatments when compared to those of control samples (Rahmatullah *et al*., 2001). Nalini-Panday and Sharma (2003) observed that exposure to an excess concentration of nickel decreased uptake and translocation of Fe from roots to leaves, chlorophyll content and activities of the peroxidase, and catalase and Fe enzymes in tomato plants.

Plant tissue culture is a *in vitro* process through which the desired traits can be produced through mass propagation from various explants such s embryos, cotyledons, calluses or other tissues of the juvenile and mature plants. Economically important plant species which cannot be produced and regenerated by conventional methods can be conserved and multiplied by this technique. Millions of plantlets can be produced under *in vitro* conditions in a short space and time (Ashrafzadeh and Leung, 2015). Attempts to standardize the micropropagation protocol for *in vitro* regenerated plants were made by Chandra *et al*. (2013). In many studies, *in vitro* culture of the tomato has been successfully used for colonel propagation to raise high-value commercial cultivars, virus-free plants, and genetic transformation (Namitha and Negi, 2013; Hanus-Fajerska, 2006; Yarra *et al.,* 2012). Moreover, *in vitro* studies provides sterilized and controlled conditions such as nutrients through a liquid and solid medium, pH, humidity and temperature for plant growth and multiplication. Plant tissue culture is an advantageous tool over the traditional methods of propagation through cuttings seeds and grafting (Hussain *et al*., 2012).

Environmental contamination on account of heavy metals is a serious global concern. In plants, heavy metals lead to deleterious effects, while the uptake of heavy metals in food crops leads to adverse health effects in human beings. Hence, it is the need of the hour to generate the methods to combat the heavy metal stress in plant systems and plant tissue culture is a technique used for quick propagation and production of healthy plants under stressed conditions. Considering the medicinal and nutritional importance of the tomato plant as well as the toxicity of copper, lead and nickel metal ions, the present study was planned to study the effects of these heavy metals on callus growth parameters and regeneration dynamics of *Solanum lycopersicum.*

MATERIALS AND METHODS

Plant Material

Seeds of *S. lycopersicum* cv. (Punjab Kesar Cherry) were collected from Punjab Agriculture University, Ludhiana (Punjab). The seeds were grown in the greenhouse of "Mata Kaulan" Botanical Garden, Guru Nanak Dev University, Amritsar. Explants (leaves, nodal segments and intermodal segments) taken from healthy plants were used for *in vitro* studies. Different explants, such as the leaf, intermodal and nodal segments, were used for callus induction. Best responding explants (nodal segment) were used for callus multiplication. *In vitro* nodal segments were inoculated on MS Medium (Murashige and Skoog, 1962) fortified with different concentrations of 2, 4-dichlorophenoxyacetic acid (2, 4-D). Observing the best response at 0.5 mg/l concentration, it was used in further studies. After callus induction, it was transferred aseptically to the test tubes containing MS Medium for multiplication.

Heavy metal treatments

Stock solutions of Cu, Pb and Ni of 1M were prepared by using copper sulphate penta hydrate ($CuSO_4.5H_2O$), lead nitrate ($Pb(NO_3)_2$) and nickel chloride hexahydrate ($NiCl_2.6H_2O$) salts, respectively under aseptic conditions in a laminar flow hood. Mother stock solutions were stored at 4˚C in amber colour reagent bottle for further use. Different concentrations of Ni, Pb and Cu in the range from 0 µM, 50 µM, 100 µM, 200 µM, 300 µM, 400 µM and 500 µM were prepared from the mother stock solution by using the serial dilution method. MS medium was prepared and different concentrations of Ni, Pb and Cu were added before setting its pH. MS medium was poured into test tubes and culture flasks sealed and marked with the respective concentrations of heavy metals. Marked test tubes and flasks were then placed in the culture room under controlled conditions such as temperature of 25±2ºC and 70% relative humidity under cool white fluorescent lights by keeping 16 h photoperiod and 8h dark period daily for solidification. Approximately 500 mg of 30 days old callus induced and multiplied from nodal segments were inoculated in test tubes having different concentrations of heavy metals. IC_{50} value was decided on the basis of a 50 % decrease in the percent callus survival and other growth parameters concerning the control on the basis of the regression equation.

Determination of callus growth parameters and regeneration

Observation of the callus was done every day and the contaminated callus was removed. Experiment was terminated after 30 days and different growth parameters such as percent callus induction, percent callus survival, percent callus multiplication, average fresh and dry weight of callus and percent dry matter content of callus were measured. Each experiment was set up in triplicates.

Calculations

Percent callus survival was calculated as:

Callus survival (%) = Callus survived in each treatment/ Callus survived in control x 100 Eq. 1

Percent callus multiplication was calculated as:

Callus multiplication (%) = Number of explants with callus multiplication / Total number of explants x 100 Eq. 2

The percent callus dry matter content was determined using protocol given by Khater *et al*. (2013):-

Callus dry matter content (%) = Average dry weight of callus/ Average fresh weight of callus x 100 Eq. 3

The fresh weight of callus was calculated in grams by taking an average of three callus cultures grown in three different test tubes. Then, fresh callus was oven dried at 80˚C for 48 hours. Dry weight was calculated by subtracting the initial weight of the empty petriplate from the final weight of petriplate containing dried callus.

Shoot regeneration percentage

Ni, Pb and Cu-treated callus were cultured on MS Medium fortified with PGR optimum for shoot regeneration in callus. The percent shoot regeneration was calculated as:

Shoot regeneration (%) = Number of cultures with shoot regeneration/Total number of cultures x 100 Eq. 4

Root regeneration percentage

The shoots regenerated were sub cultured on MS medium supplemented with plant growth regulators optimum for root regeneration. The percent shoot regeneration was calculated as:

Root regeneration (%) = Number of cultures with root regeneration / Total number of cultures x 100 Eq. 5

Data analysis

Data are presented as mean and standard error and were analyzed by using Analysis of Variance (One Way ANOVA) and Tukey's test (SPSS version 16.0, SPSS Inc., USA).

RESULTS AND DISCUSSION

Abiotic stress on account of heavy metals is a prime global concern these days due to serious problems leading to limiting crop productivity. Elevated concentrations of toxic heavy metals such as arsenic, chromi-

um, copper, mercury, nickel, lead and zinc in soil and water bodies represent a growing threat to the ecosystem. These metals at super optimal levels severely affect the metabolic pathways in plants and result in retarded growth. Heavy metals enter the plant system through foliar uptake or root system, which leads to phytotoxicity with chlorosis symptoms, stunted growth, necrosis and discoloration (Chaitanya *et al*., 2022). Considering the same, the results on the effects of different concentrations of copper, lead and nickel on various growth parameters *viz*., percent callus survival, percent callus multiplication, average fresh and dry weight of callus, and callus dry matter content, root and shoot regeneration of callus cultures, are shown in Tables 1-3. Declines in all the growth parameters were observed with increased concentrations *viz*., 0 µM, 50 µM, 100 µM, 200 µM, 300 µM, 400 µM and 500 µM of metals studied during the present study.

It was seen that callus survival percentage for Cu, Pb and Ni treatment in *S. lycopersicum* was maximum as 91.66 %, 81.94% and 76.38 %, respectively at 50 µM which was decreased up to 16.66%, 13.88% and 11.11%, respectively at 500 µM concentration. Among three metals, lead was found to be highly toxic and callus survival percentage under different treatments at the highest concentration, i.e. 500 µM was observed to be in the order of Cu > Ni >Pb (Table 1). Percent callus multiplication was observed in the range of 90.90 % at 50 µM to 18.75% at 400 µM for Cu, 72.72% at 50 µM to 33.33% 300 µM and 400 µM for lead and 79.66% at 50 µM to 20% at 500 µM for Ni treatment of *S. lycopersicum* and order of toxicity was observed as Cu > Ni >Pb. The toxicity of copper was also reported in earlier studies. A study on the effects of copper sulphate in tissue culture of *Triticumtim opheevii* was conducted by Miroshnichenko et al. (2021) and it was reported that media containing higher levels of copper ions posed negative effects on the regeneration of green plants and caused albinism in plantlets. Similarly, Dalilah *et al*. (2022) highlighted the dynamic responses in terms of alterations of biochemical and mitochondrial mechanisms of *O. stamineus* cells upon exposure to different

Table 1. Effects of copper, lead and nickel on callus survival (%) and callus multiplication (%) during *in vitro* studies of *Solanumlycopersicum* after 30 days of inoculation period

Treatment (µM)	Percent callus survival (Mean ± S.E.)			Percent callus multiplication (Mean ± S.E.)		
	Copper	Lead	Nickel	Copper	Lead	Nickel
0	95.83±0.57^{d}	98.61±0.33^{f}	97.22±0.33^{e}	91.30±0.57^{d}	94.36±0.66^{e}	94.28±0.99^{e}
50	91.66±0.57^{cd}	76.38±1.33^{e}	81.94±0.66^{d}	90.90±0.57^{d}	72.72±0.81^{d}	79.66±0.33^{d}
100	81.94±0.66^{c}	62.50±0.57^{d}	62.50±0.99^{c}	79.66±0.66^{c}	60.00±0.99^{c}	60.00±0.52^{c}
200	47.22±0.33^{b}	48.61±0.33^{c}	50.00±0.57^{c}	47.05±0.33^{b}	45.71±0.33^{b}	47.22±0.66^{bc}
300	37.50±0.57^{b}	33.33±0.57^{b}	34.72±0.66^{b}	29.62±0.33^{a}	33.33±0.66^{bc}	32.00±0.33^{ab}
400	22.22±0.33^{a}	16.66±0.57^{a}	20.83±0.57^{a}	18.75±0.00^{a}	33.33±0.66^{a}	26.66±0.33^{a}
500	16.66±0.57^{a}	11.11±0.33^{a}	13.88±0.33^{a}	25.00±0.00^{a}	37.50±0.00^{a}	20.00±0.33^{a}

S.E Standard Error;Values followed by the different letters are statistically different at p ≤0.05

Table 2. Effects of copper, lead and nickel on root and shoot regeneration (%) of *in vitro* cultures of *S. lycopersicum* after 30 days of inoculation period.

Treatment (µM)	Root regeneration (%) (Mean ± S.E.)			Shoot regeneration (%) (Mean ± S.E.)		
	Copper	Lead	Nickel	Copper	Lead	Nickel
0	84.74±0.88^{d}	81.66±1.66^{d}	71.2±1.20^{d}	93.65±0.33^{d}	89.55±0.57^{d}	94.2±0.99^{d}
50	79.54±0.66^{c}	82.75±0.57^{c}	63.8±0.99^{c}	73.33±1.20^{c}	72.50±1.20^{c}	79.6±0.85^{c}
100	61.29±1.20^{b}	76.47±0.88^{bc}	55.5±0.57^{b}	65.95±1.33^{b}	62.96±0.88^{b}	60.0±0.00^{b}
200	54.54±0.57^{a}	71.42±0.33^{ab}	47.0±0.33^{ab}	68.75±0.33^{a}	43.75±0.33^{a}	47.2±1.76^{ab}
300	60.00±0.00^{a}	66.66±0.33^{ab}	37.5±0.40^{a}	62.50±0.88^{a}	37.50±0.00^{a}	32.0±0.33^{a}
400	50.00±0.33^{a}	33.33±0.33^{a}	25.0±0.33^{a}	66.66±0.33^{a}	33.33±0.33^{a}	26.6±0.33^{a}
500	0.000±0.00^{a}	33.33±0.33^{a}	50.0±0.33^{a}	33.33±0.33^{a}	33.33±0.33^{a}	20.0±0.33^{a}

S.E Standard Error; Values followed by the different letters are statistically different at p ≤0.05

Table 3. Effects of copper, lead and nickel on fresh weight (g), dry weight (g) and dry matter content (%) of *in vitro* cultures of S. *lycopersicum* after 30 days of inoculation period.

Treatment (µM)	Fresh weight (%) (Mean ± S.E.)			Dry weight (%) (Mean ± SE)			Callus dry matter content (%) (Mean ± SE)		
	Copper	Lead	Nickel	Copper	Lead	Nickel	Copper	Lead	Nickel
0	1.717±0.00^{e}	1.66±0.00^{e}	1.63±0.01^{d}	0.091±0.00^{g}	0.080±0.00^{e}	0.082±0.001^{f}	5.298±0.07^{e}	4.840±0.06^{d}	5.04±0.10^{d}
50	1.684±0.01^{e}	1.578±0.00^{d}	1.60±0.02^{d}	0.080±0.00^{f}	0.071±0.00^{e}	0.069±0.001^{e}	4.748±0.03^{d}	4.518±0.02^{cd}	4.35±0.09^{c}
100	1.601±0.00^{d}	1.343±0.01^{c}	1.54±0.00^{cd}	0.069±0.00^{e}	0.060±0.00^{d}	0.056±0.002^{d}	4.329±0.07^{d}	4.483±0.01^{cd}	3.68±0.13^{bc}
200	1.537±0.00^{d}	1.273±0.03^{c}	1.49±0.03^{c}	0.058±0.00^{d}	0.050±0.00^{cd}	0.052±0.001^{cd}	3.808±0.03^{c}	3.980±0.18^{bc}	3.51±0.12^{b}
300	1.410±0.03^{c}	1.114±0.00^{b}	1.34±0.01^{b}	0.049±0.00^{c}	0.041±0.00^{bc}	0.045±0.003^{bc}	3.502±0.10^{bc}	3.748±0.20^{bc}	3.36±0.23^{b}
400	1.304±0.01^{b}	0.990±0.00^{a}	1.29±0.03^{ab}	0.040±0.00^{b}	0.032±0.00^{ab}	0.040±0.001^{ab}	3.113±0.20^{b}	3.276±0.17^{ab}	3.13±0.01^{ab}
500	1.217±0.01^{a}	0.970±0.00^{a}	1.23±0.05^{a}	0.030±0.00^{a}	0.025±0.00^{a}	0.032±0.001^{a}	2.5171±0.08^{a}	2.607±0.27^{a}	2.59±0.00^{a}

S.E Standard Error; Values followed by the different letters are statistically different at p ≤0.05

concentrations of copper.

In the present study, shoot regeneration percentage was observed to maximum as 73.33 %, 72.50 % and 79.6 % at 50 µM and a minimum of 33.33 %, 33.33 % and 20 % at 500 µM of Cu, Pb and Ni, respectively. Root regeneration percentage was observed to be maximum as 79.54 %, 82.75 % and 63.8 % at 50 µM, which decreased to 0 %, 33.33 % and 25 %at 500 µM of Cu, respectively, Pb and Ni, respectively (Table 2). Mean fresh weight of the callus was observed to the maximum as 1.684 g, 1.578 g and 1.60 g at 50 µM and minimum as 1.217 g, 0.97 g and 1.23 g at 500 µM of Cu, Pb and Ni, respectively. Dry weight of the callus was observed to be maximum as 0.080 g, 0.071 and 0.069 at 50 µM, which was decreased up to 0.03 g, 0.025 and 0.032 g at 500 µM of Cu, Pb and Ni, respectively. Similar results were reported by Fathalla *et al*. (2011) in *Catharanthus roseus* (L.) callus cultures that were treated with mercury (Hg).

A significant decrease in fresh weight and dry weight of shoot and root derived callus with increasing concentrations of Hg was observed during their study. Similarly, decrease in the fresh weight of callus tissue was reported as 87% using zinc, 97% using nickel, 98% using copper treatments in the tobacco plant and as 69% using zinc, 88% using nickel, 90% using copper treatments in rue plant at the highest concentrations used (Maroti and Bognor, 1988). Abdel-Wahab*et al.,* 2019 documented a significant decrease in fresh and dry weight of callus cells due to the impact of CuO nanoparticles. Yan *et al*. (2008) also reported decreased fresh weight of cotyledons, while Pompeu *et al.* (2008) reported a decline in tobacco cell suspension cultures. Ghanavatifard *et al*.(2018) investigated the response of two chamomile species to different levels of heavy metal (nickel) under *in vitro* conditions and reported that the *Matricaria chamomilla* leaf explants had callogenesis and its callogenesis indices showed a significant decrease at high levels of nickel when compared with the control In *M. aurea*, the callii did not grow sufficiently, suggesting the sensitivity of *M. aurea*'s callogenesis to the presence of different levels of nickel.

In the present results, dry matter content was decreased from 4.748 to 2.517 % at 50 µM and 500 µM, respectively, for Cu treatment, 4.518 to 2.607 % at 50 µM and 500 µM, respectively for Pb treatment, 4.35 to 2.59 % at 50 µM and 500 µM, respectively for Ni treatment (Table 3).The fresh weight of the callus was decreased with increasing concentrations of Ni and Pb in the callus induced from internodes of Iraqi *Cynodon dactylon* L (Abdulhameed *et al.,* 2019). Fayiga *et al.* (2004) reported decreased plant biomass with Ni treatments in *Pteris vittata*. The copper treatments inhibited callus growth of pith explants in *Nicotiana glauca* resulting in reduced biomass. The extent of inhibition of bio-

mass was directly correlated to metal concentration in a study by Taddei *et al.* (2007). Rehman *et al.* (2020) reported that the addition of Pb to the culture media resulted in a drastic decline in callus fresh weight and quality. The lowest fresh weight was recorded in callus exposed to 0.3 mg/L of Pb while red spots appeared in all callus clumps treated with Pb at all concentrations in *Lantana camara*

Conclusion

The present study indicated adverse effects of copper, lead and nickel on different callus growth dynamics of *S. lycopersicum* in terms of reduction in percent callus survival, percentage callus multiplication, fresh weight and dry weight of callus, root and shoots generation. Among all three heavy metals studied, the order of toxicity in terms of percent callus survival was Pb > Ni > Cu. It is the first study to evaluate the effects of heavy metals in induced callus cultures of *S. lycopersicum* variety Punjab Kesar Cherry, which revealed that callus cultures were more sensitive towards lead as compared to nickel and copper for their survival rate while copper exhibited maximum toxicity by inhibiting root and shoot regenerations. Apart from this, the current study focuses on developing advanced *in vitro* protocols to understand better and formulate abiotic stress combating measures in different food crops.

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Conflict of interest

The authors declare that they have no conflict of interest.

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