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MITIGATION OF HEAVY METALS FROM THE SOIL THROUGH *IN-VITRO* AND *IN-VIVO* PRODUCED PLANTS OF *IMPATIENTS BALSAMINA* L.

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ABSTRACT:

In this research Heavy Metal extraction capacity or phytoremediation capacity of *Impatiens balsamina* L. was assessed. Here, two different approaches *In-vitro* and *In-vivo* were used for the production of plantlets. *In-vitro* approach involved tissue culture approach and *In-vivo* direct through media (soil, cocopeat, mosses). Seeds were used for the production of plantlets. After 30 days of seedlings development all the plantlets which are produced through *In-vitro* and *In-vivo* approaches and plants were transplanted in the pots and treated with two metals Lead and Cadmium in the form of Pb $(NO_3)_2$ and Cd $(NO_3)_2$. Different concentrations were selected for Lead 200mg, 400mg, 600mg, 800mg/Kg and for Cadmium 5mg, 10mg, 15mg, and 20mg/Kg. Each pot was filled with 5Kg of soil. The metals were given directly through root zone of plants in solution form. After incubation time of 75 days mature and treated plants were collected and each and every part of every concentration treated plants were collected and AAS (Atomic Absorption Spectroscopy) was assessed. As compare to In-vivo produced plants In-vitro produced plants has more capacity to accumulate Lead and Cadmium.

Keywords: *In-vitro* approach, *In-vivo* approach, Lead, Cadmium, *Impatiens balsamina L.*, Phytoremediatiion.

INTRODUCTION:

Impatiens balsamina L. belongs to Balsaminaceae family. It is also known as Garden Balsam or Rose Balsam. It is also cultivated in China and Myanmar as an ornamental herb. This is annual ornamental herb and cultivated as seasonal ornamental in India. The plant is generally growing up to 20-75cm length with succulent type of branched stem. The leaves are spirally arranged 2.5-9.0 cm long and 1-2.5cm broad and it has toothed margin. The flowers are pink, red, lilac or white and 2.5-5.0cm diameter. Generally, it is bees or insect pollinated plant. It has short lifecycle, large number of flowers and various colors of flowers. The plant is not directly used as fodder plant by animal or directly used by humans. So many researchers worked on this plant and it also has different pharmacological activities like Anti-cancer, Anti-ulcer, Antioxidant, Anti-malarial, Antibacterial, Anti-fungal etc. it also has the weedicide potential. Even one researcher also assessed the naphthalene tolerance and remediate capacity of *Impatiens balsamina* L.

MATERIALS AND METHODOLOGY:

The seeds were selected for the propagation of plants for both the *In-vitro* and *In-vivo* approaches. The experimental work was completed at (Plant Biotechnology Laboratory and Botanical Garden) Botany Department, Gujarat University.

***In-vitro* production of plantlets:**

Sources of Explant:

Seeds were purchased of Fine Grow Company from Alpesh Nursery, Gandhinagar. So, seeds were used as an explant for the production of plantlets. All the seeds were sterilized with the help of 0.1%$HgCl_2$ solution and 70% methanol and rewashed with Grade-1 Distil water.

Aseptic Conditions for Production:

Culture room and the laboratory or transfer room were sterilized through Fumigation technique (Potassium iodide and Formaldehyde were used for it with 2:4 ratio). All the glassware and miscellaneous agents were washed with soap solution and rapped with papers and then sterilized through Autoclave (121°C for 20 min). Laminar Air flow hood, weighing scale and all the other small equipment like micropipette were sterilized with 0.1% mercuric chloride solution and 70% methanol.

Preparation of M. S. Media for the production of plantlets:

Here for the practical work most widely used media Murashige and Skoog's media (1962) was used. For the preparation first all the Major, Minor, Iron and Vitamin stalk solutions were prepared as per the Table-1. PGRs were not used because in seeds generally we use to avoid PGRs in *In-vitro* condition and production of plantlets. Here all the chemicals used for the preparation of stalk solution were Hi Media and SRL company.

Different stalk solutions were prepared in the amount of 500ml (Major, Minor and Iorn) and 100ml (Vitamin) and then for the preparation of 1 litter M. S. Media 50ml from Major, 50ml from Minor, 50ml from Iron and 10ml from Vitamin stalk were taken and sequentially dissolved and other chemicals which were separately weighed like Myo Inositol, Agar-Agar, Glycine and Sucrose were added for the preparation of media. (Here Grade-1 Purified water was used for the preparation of media with the help of Genie Direct Pure (Rephile) Instrument was used for the preparation of Purified water). After the preparation of media, it was sterilized with the help of autoclave at 121°C temperature for 20 min. under the Laminar Air Flow Hood in all the sterilized culture flasks and Glass jars media was poured about 50ml in each vessel. And all the vessels with media were transferred in Culture room where 25±1°C temperature and sterilized conditions were maintained. After 24 hrs media was ready for the Inoculation process.

Inoculation of Explant:

All the sterilized seeds were inoculated separately in the jars or culture flasks under the sterilized conditions of Laminar Air flow hood. Different small equipments were used like forceps and scalpels for the inoculation process. After the inoculation of the seeds in the media all the jars and flasks were again transferred carefully at Culture room where 25±1°C temperature and 16hrs light and 8hrs darkness was maintained (In seed culture total darkness provided to all the cultures for first 3 days). Incubation time was of 30 days.

***In-vivo* production of Plantlets:**

By same way sterilized seeds were directly sawed in the media (soil, cocopeat and mosses) in separate pots and regular irrigation process was maintained and up to 15 days the plantlets were produced. The production was carried out at Botanical Garden, Gujarat University.

Now same conditions were provided to all the *In-vitro* and *In-vivo* produced plantlets. 15 days all

the plantlets were transferred for the hardening process in the net house of Botanical Garden, Gujarat University where 60% moisture was maintained. Here same media soil, cocopeat were applied for all the *In-vitro* and *In-vivo* produced platelets. After 15 days in the Net house all the mature plants with 8-12 leaves they were transplanted in different pots separately with 5kg of soil in each pot. *In-vitro* and *In-vivo* produced plants were segregated and potted individually in triplicate sets.

Treatment of Heavy Metal to the plants:

Lead and Cadmium metals were used for the treatment in the form of Lead nitrate and Cadmium nitrate. For the treatment lead the concentrations were selected 200mg/kg, 400mg/kg, 600mg/kg, 800mg/kg of soil. And for cadmium the concentrations were selected 5mg/kg, 10mg/kg, 15mg/kg, 20mg/kg of soil. One set was kept as control both the series and both the approaches. Lead nitrate and Cadmium nitrate solution series were prepared and the treatment was provided to individual directly through rootzone via digging the soil near by the roots.

Incubation time of the plants:

After the treatment to all the *In-vitro* and *In-vivo* produced plantlets all the plants were placed at Botanical Garden for 75 days incubation period. Regular irrigation was done to all the plantlets.

Collection, Drying of the plant parts:

After the incubation time of 75 days all the plants were collected individually and the parts roots, stem and leaves were segregated and dried in the oven at 80°C for 45 minutes. Dried each plant material crushed and weighed 1gm.

Quantitative Estimation of Lead and Cadmium in Plant part:

All the collected and segregated plant parts of each concentration crushed segregate. 1gm dry powder of each sample was weighed and taken in to conical flask and 10ml of concentrated HNO_3 was added. The mixture was boiled at constant temperature for 10min. After cooling 5ml of 70% $HClO_4$ was added and the mixture was further boiled until the realise the dense white fumes. After cooling, 20ml distilled water was added and heated until a clear solution was obtained. The mixture was filtered after cooling with the help of Whatman filter paper no. 44 and transferred quantitatively to a 50ml volumetric flask by adding de-ionized and double distilled water. Samples were analysed through AAS (Atomic Absorption Spectroscopy) for quantification of Cd and Pb. Results were expressed as mg/kg metal content in dry powdered material of respective plant part. The results were collected as Mean ± S.D. (Standard Deviation).

RESULTS AND DISCUSSION:

As the results data showed that every plant has some amount of heavy metal with different concentration and as the concentration of the metal increases the amount of phytoextraction rate also increases of both the metals. Roots has maximum accumulation of lead and cadmium metals as compared to stem and leaves. Sekar Kumaran *et al* in 2013 assesed the lead accumulation capacity of balsam with 100mg highest concentration treatment. Here in this research 800mg/kg metal was provided to the plants so, it's very higher concentration than 100mg. William and Daniel in 2006 described the remediation capacity of one variety of balsam (*Populus balsamifera* L.) and here in this research *Impatiens balsamina* L. was analysed with two approaches of plant production. Satashiya *et al* in

2017 conducted the experiments in which 9mg/kg Cd and 450mg/kg Pb was provided to the *Impatiens balsamina* L. plants and in this research 20mg/kg Cd and 800mg/kg highest concentration were selected for the treatment. Mohd Zaini Nawahwi *et al* in 2014 described with experiments Nephthalene accumulation capacity of *Impatiens balsamina* L. Hiromi *et al* in 2016 explained the total petroleum hydrocarbon accumulation capacity of *Impatiens balsamina* L. In future the proteins or the phytochemicals can be identified which are responsible for the extraction of metal and its binding capacity with the help of *In-silico* analysis.

On the basis of *In-vitro* and *In-vivo* plantlet's extraction or uptake capacity this can be concluded that In-vitro produced plants has more capacity to accumulate or uptake heavy metal as compared to In-vivo produced plants. It means the phytochemical or the proteins which are responsible for metal up take produced more in *In-vitro* produced plants.

CONCLUSION:

Impatiens balsamina L. has phytoremediation capacity and its hyperaccumulator plant for specific lead and cadmium metals. For on site pollution deduction from the soil *In-vitro* plantlets can be used because they can accumulate or uptake or extract more amount of heavy metal from the soil. So, it's one application that can be added of tissue cultured plant. After the treatment the plant material which is obtained that can be utilised for the production of "Biochar" which can be used as fuel in different industries and specially used as secondary component for the preparation of Tier and Tube Industry.

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LIST OF TABLES:

Table:1 Showing the Composition and Components of M. S. Media (1962) preparation

Stock	Constituents	Quantity		Stock medium
		1 liter (gm)	10 liter (gm)	
A.	**Major Stock (gm)**			
	Ammonium Nitrate (NH_4NO_3)	1.65	16.5	
	Potassium Nitrate (KNO_3)	1.9	19	500 ml
	Calcium Chloride ($CaCl_2.2H_2O$)	0.44	4.4	
	Magnesium Sulphate ($MgSO_4.7H_2O$)	0.37	3.7	
	Monobasic Potassium (KH_2PO_4)	0.17	1.7	
B.	**Minor Stock (mg)**	(mg)	(mg)	
	Potassium Iodide (KI)	0.83	8.3	
	Boric Acid (H_3BO_3)	6.2	62	
	Manganese Sulphate ($MnSO_4.4H_2O$)	22.3	223	
	Cobalt Chloride ($CoCl_2.6H_2O$)	0.025	0.25	500 ml
	Zinc Sulphate ($ZnSO_4.7H_2O$)	8.6	86	
	Sodium Molybdate ($Na_2MoO_4.2H_2O$)	0.25	2.5	
	Copper Sulphate ($CuSO_4.5H_2O$)	0.025	0.25	
C.	**Iron Stock**	(mg)	(mg)	
	Sodium EDTA (Na_2 EDTA.$2H_2O$)	37.3	373	500 ml
	Ferric Sulphate ($FeSO_4.7H_2O$)	27.8	278	
D.	**Vitamin Stock**	(mg)	(mg)	
	Nicotinic Acid	0.5	5	
	Pyridoxine HCl	0.5	5	100 ml
	Thymine HCl	0.1	1	
E.	**Myo Inositol**	**100mg**	After the combination of all the required stocks for 1 litter all these weighed chemicals were added in that combination of solution for the preparation of media.	
F.	**Glyine**	**2mg**		
G.	**Agar-Agar**	**8mg**		
H.	**Sucrose**	**30gm**		

x

Table:2 Results table showing accumulation of Lead in different parts of *In-vitro* produced plants.

***In-vitro* produced Balsam**			
Treatment	**Pb in roots mg/kg**	**Pb in Stem mg/kg**	**Pb in leaves mg/kg**
Control	11.23±1.15	6.29±0.30	2.42±0.22
400mg/kg	1510±10.40	909±6.42	769±5.90
800mg/kg	1840±4.20	1430±7.80	905±4.20

Table:3 Result Table showing accumulation of Lead in different parts of *In-vivo* produced plants.

***In-vivo* produced Balsam**			
Treatment	**Pb in roots mg/kg**	**Pb in Stem mg/kg**	**Pb in Leaves mg/kg**
Control	8.09±0.90	4.2±0.25	1.02±0.08
400mg/kg	1489±9.40	858±8.09	601±4.02
800mg/kg	1742±8.82	1389±4.80	915±2.41

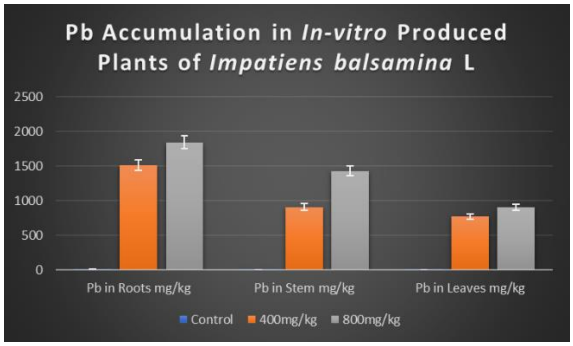
Table:4 Results Table showing accumulation of Cadmium in different parts of *In-vitro* produced plants.

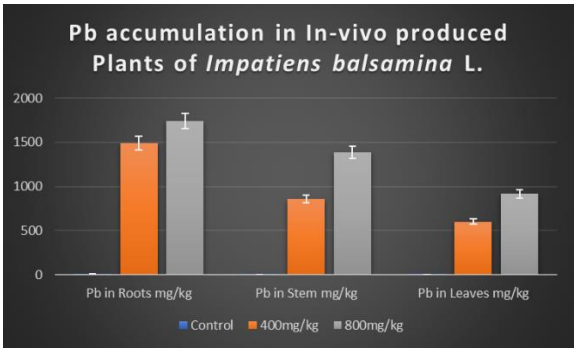
***In-vitro* produced Balsam**			
Treatment	**Cd in Roots mg/kg**	**Cd in Stem mg/kg**	**Cd in Leaves mg/kg**
Control	0.85±0.9	0.58±0.09	0.25±0.04
10mg/kg	80.60±6.20	40.02±2.10	25.40±1.32
20mg/kg	89.00±9.40	54.00±3.90	30±1.89

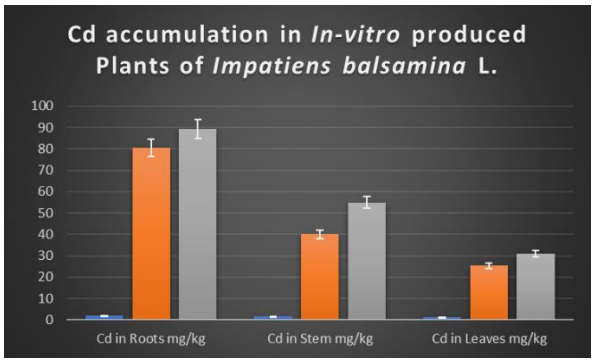
Table:5 Result Table showing accumulation of Cadmium in different parts of *In-vivo* produced plants.

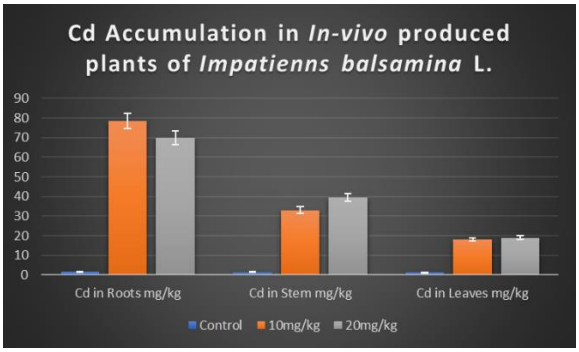
***In-vivo* produced Balsam**			
Treatment	**Cd in Roots mg/kg**	**Cd in Stem mg/kg**	**Cd in Leaves mg/kg**
Control	0.79±0.05	0.49±0.09	0.16±0.03
10mg/kg	78.40±5.90	33.00±4.02	18.02±1.37
20mg/kg	70.02±6.04	39.0±4.40	19.0±2.21

LIST OF GRAPHS









LIST OF FIGURES:

In-vitro production of Balsam

In-vivo production of Balsam

Preparation of Mature plants for Transplantation

Transplanted all the plantlets in pots with 5kg of soil

Treatment of heavy metals to the plants

After Incubation Collection of plants

Collected Treated plants with control series

Separation of all the parts of the plants as per different Concentrations

Acid digestion 1st step (concentrated Nitric Acid)

Acid digestion 2nd step (70% Perchloric Acid)

Acid Digestion 3rd Step (Distilled water)

Filtration after cooling (Filter paper-44)