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Effect of Sodium Arsenate on *Momordica charantia* L. Seeds *In Vitro*

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Abstract

Momordica charantia (bitter melon) is a vegetable crop plant of the family Cucurbitaceae. *Momordica* is a medicinally important plant species and widely used as foodstuff. Arsenic (As) is one of the toxic environmental pollutants, which has recently attracted mass attention because of its chronic and epidemic effects on human health. The absorption of As by land plants is influenced by the concentration of As in the soil. With this perspective the present investigation was undertaken to carry out a comparative analysis on fruit yield and *in vitro* efficiency of *Momordica* to establish callus culture from seeds collected from geographically arsenic contaminated and arsenic free two places in West Bengal, India. Calli established *in vitro* were subjected to Scanning Electron Microscopy (SEM) to determine effect of arsenic on morphological alteration of calli. Seedlings grown in arsenic containing media were used for Atomic Absorption Spectroscopic (AAS) analysis to get a clear picture of the content of eight metals under excess arsenic treatment.

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Introduction

Momordica charantia (bitter melon) is a vegetable crop plant of the family Cucurbitaceae (Toyama et al., 2008; Schaefer and Renner, 2010). It is widely grown in India, China, South Asia, Africa and the Caribbean islands (Bakare et al. 2010). *Momordica* is a medicinally important plant species and widely used as food (Tanaka et al., 2009) in Asian countries.

Momordica has been used extensively in folk medicine as a remedy for diabetes. It is a very common herb having various medicinal properties, viz. antifungal (Santos et al., 2012), wound healing (Prasad et al., 2006), antiviral (Balasubramanian et al., 2007) and antidiabetic (Denyer et al., 2004). Bitter melon is an important nutrient rich plant composed of variety of beneficial compounds. These include vitamins, minerals and antioxidants.

Arsenic (As) is one of the toxic environmental pollutants. Arsenic has recently attracted mass attention because of its chronic and epidemic effects on human health. Arsenic contamination of ground water has been documented from time to time by various authors from many Asian countries like Bangladesh, India, China, Taiwan, Vietnam etc. (Bhattacharya et al., 2012). The Bengal basin is regarded to be the most critically affected area of As pollution. Such contamination has been reported from some districts of the state of West Bengal in India also (Chakraborti et al., 2002). This contaminated water is

a source of irrigation and deposits As in the soil of cultivated lands (Mehrag and Rahman, 2003). The absorption of As by land plants is influenced by the concentration of As in the soil. The As concentration also depends upon the availability of soil As and the accumulation and translocation ability of a plant (Huang et al., 2006).

Large areas of Bangladesh, West Bengal and other states in India and Vietnam rely on As-contaminated ground-water for irrigation (Abedin et al., 2002). Arsenic is released into the environment in both inorganic and organic forms. Arsenic enters from soil into plants and subsequently the food chain (Rahman et al., 2007).

With this perspective the present investigation was undertaken to carry out a comparative analysis on fruit yield and *in vitro* efficiency of *Momordica* to establish callus culture from seeds collected from Burdwan town, District Purba Burdwan, West Bengal (Geographical identification 23°14′18″N 87°51′39″E) (no arsenic contamination) and Bashirhat, District North 24 parganas, West Bengal (Geographical identification 22°39′26″N 88°53′39″E) (arsenic contaminated area).

Same study was further extended to study the effect of sodium arsenate *in vitro* on callus culture from seeds collected from Burdwan where no arsenic contamination was reported. Calli established *in vitro* were subjected to Scanning Electron Microscopy (SEM) study to determine effect of arsenic on morphological alteration of calli.

Seedlings grown in arsenic containing media were used for Atomic Absorption Spectroscopic (AAS) analysis to get a clear picture of the content of eight metals under excess arsenic treatment. Therefore the present study will clearly bring about *in vitro* effect of sodium arsenate on seeds of *Momordica charantia* procured from Burdwan, West Bengal with regard to fruit yield and callus formation efficiency. A comparative study will be included to determine the effect of variation in plant trace elemental content to detect antagonism and synergism. This effect was also illustrated by SEM pictures taken on callus culture.

Materials and methods

Seeds of *Momordica charantia* were procured from Burdwan and Basirhat. Plantlets were propagated by tissue culture techniques (Paul and Raychaudhuri, 2010). Seeds were germinated in agar (0.9%) sucrose (3%) media and grown into seedlings. Seeds were germinated in sodium arsenate ($Na_2HAsO_4.7H_2O$) treated agar sucrose media. Different concentration of sodium arsenate i.e. 10µM, 25 µM, 50 µM, 75 µM, 100 µM, 125 µM, 150 µM, 175 µM and 200 µM were chosen for the experiment along with control one (no treatment).

Different concentration of sodium arsenate was used to determine LD_{50} of sodium arsenate on *M. charantia.* Leaf discs from aseptically germinated seedlings were taken as the source of explants and used for callus induction. Murashige and Skoog (MS) medium supplemented with four combinations (Table 1) of PGRs were used for establishment of calli. All cultures were grown in proper laboratory condition (at a temperature of 22–25°C and a relative humidity of 55–60% under Philips fluorescent daylight tubes emitting 32×108 mol $s^{-1}m^{-2}$ for 16/8-hrs duration in light/dark periods).

Table 1. Four different combinations of PGRs and sucrose.

Combination	Auxin (mg/L)		Cytokinin (mg/L)		Sucrose (3%)
	Name	Concentration	Name	Concentration	
C1	2,4-D	1.0	KIN	0.5	3 (Passage 1) and 2 (Passage 2)
C2	2,4-D	0.5	KIN	1.0	2 (Passage 1) and 2 (Passage 2)
C3	BAP	0.5	NAA	0.5	3 (Passage 1) and 3 (Passage 2)
C4	2,4-D	1.0	KIN	0.5	3 (Passage 1) and 3 (Passage 2)

We have determined FW and DW of the callus of established from seeds procured from Burdwan and Basirhat. FW was determined by weighing the callus grown in laboratory and after drying in hot air oven weights were measured to calculate DW.

Seedling height was determined. Roots and shoots length of the fifteen day old seedlings were measured.

AAS study was carried out using callus samples (established from seedlings) and sodium arsenate treated samples from Burdwan and Basirhat using the methods of Rahman et al. (2007). About 0.5 g of each sample was taken in clean dry digestion tubes and 5 ml of concentrated HNO_3 was added to it. The mixtures were allowed to stand overnight in fume hood. Following day, the digestion tubes were placed on a heating block and

heated at 60°C for 2 hrs. After cooling, perchloric acid ($HClO_4$) was added and heated at 160°C for about 4-5 hrs. Calibration was carried out using arsenate as standard (Bhattacharya et al., 2009). Eight elements namely arsenic, calcium, copper, zinc, iron, manganese, lead and cadmium were measured.

Calli were treated with different doses (above mentioned) of sodium arsenate along with control. Treated callus and control (no treatment) were fixed in 2.5% glutaraldehyde for 4 hrs. At first sample pieces were immersed in 50% alcohol for 5mins and then in 70% alcohol for 30 minutes, thereafter in 90% and in absolute alcohol respectively for 30 mins. Then they were immersed in the mixture of different ratios of absolute alcohol and amyl acetate [3:1, 2:2 and 1:3 respectively]. In each solution the sample pieces were immersed for 30 minutes. Samples were then taken for drying using Critical Point Dryer and followed by gold coating in Sputter Gold-Coating Machine. Samples were imaged by Scanning Electron Microscopy.

Results and discussion

Seeds were germinated in the above mentioned laboratory condition. In control set all seeds were germinated. We found LD_{50} of sodium arsenate on *Momordica charantia* var. *muricata* was 200µM. We have found that fruit yield was affected in case of seeds collected from arsenic affected zone (i.e., Basirhat). 33% fruit yield reduction was found with respect to control one (Fig. 1).

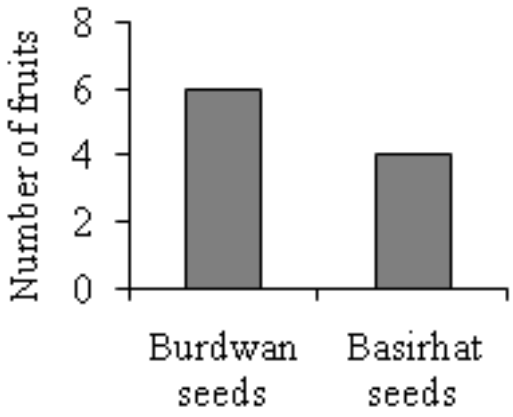

Fig 1: Histogram of number of fruits produced by *M. charantia*.

Seeds were germinated in the above mentioned tissue culture laboratory conditions in three different experimental sets containing ten seeds each. In control set all seeds were germinated. Germination rate was found to decrease as the concentration of sodium arsenate was increased in plant tissue culture media. Results are given in Fig. 2.

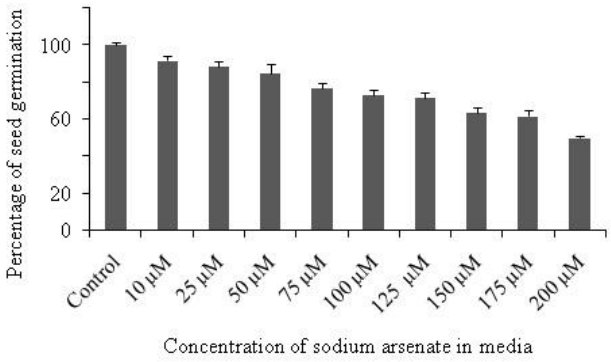

Fig 2: Percentage of seed germination in sodium arsenate treated media *in vitro*.

Arsenic showed a negative impact on growth of the seedlings especially on shoot length. In sodium arsenate treated seedlings maximum 40% reduction was observed at 200 µM treatment as compared to untreated control plants (Fig. 3). Similar decreasing trend was observed in root length also.

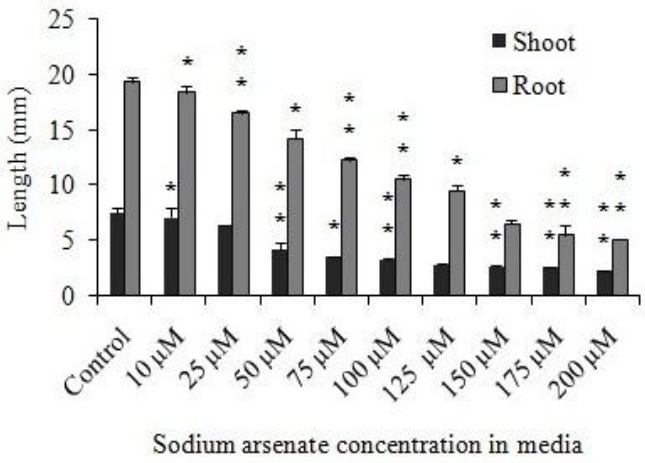

Fig 3: Histogram of root and shoot length of *M. charantia* grown in different concentration of sodium arsenate in media *in vitro*. The data were presented as the mean ± standard error mean (SE). The experiments were performed with three replicates. The statistical analysis was carried out by using one-way analysis of variance (ANOVA). Data represent the mean ± SE. Asterisks indicate significant differences at $p<0.05$ (*) or $p<0.01$ (**) or $p<0.001$ (***) compared to respective controls.

It has been clearly observed that arsenic induced alteration in biomass (FW and DW) in our experimental

plant system (Fig. 4). For the determination of biomass we determined FW and DW of arsenic contaminated and control samples. Highest FW and DW were recorded in case of control callus and it was 1.05 g and 0.21 g respectively. Callus induced from Basirhat seeds have shown reduced DW and FW with compare to control sample (Fig. 5).

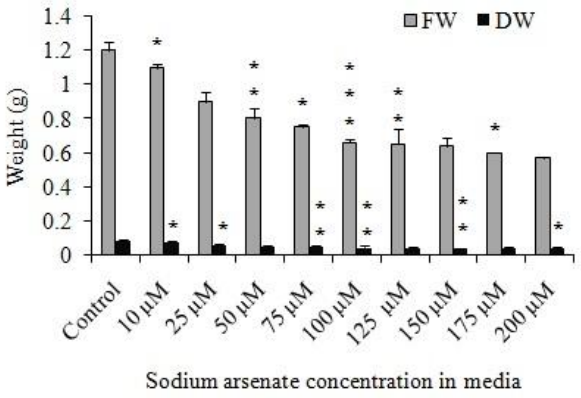

Fig 4: Histogram of DW and FW of *M. charantia* grown in different concentration of sodium arsenate in media *in vitro*.

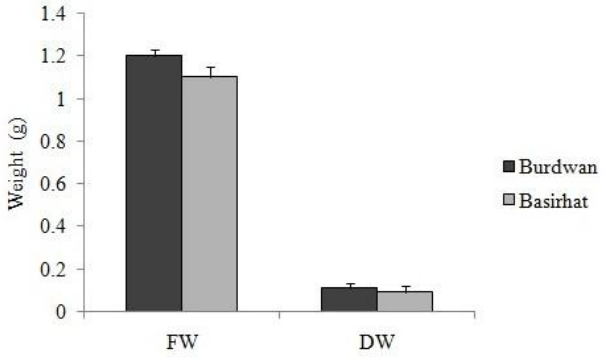

Fig 5: Histogram of DW and FW of *Momordica*.

Effect of different concentration of sucrose and PGRs of *in vitro* callus culture were studied in our present investigation. Different combination and concentration of PGRs are important for the *in vitro* induction of callus. In our experiment we have tried to establish best callus formation using different PGRs concentration and combination also with sucrose concentration. Manye et al. (2004) reported production of three types of callus from *M. charantia* namely green, yellow green and fragile yellow callus. We have also found greenish and yellowish type of callus which is similar to their findings. Proportion and quantity of PGRs and explants play a role in the formation of callus and the morphology of callus. The seedlings of *M. charantia* cultured *in vitro* for about 12-15 d were optimal for taking leaf disc as explants. Malik et al. (2007) analyzed the effect of PGRs on caulogenesis of *M. charantia* and reported callus formation from leaf explants. They used different auxin and cytokinin concentrations and combinations in MS medium.

Callus culture was established using four different combinations of PGRs and it was found that combination C4 [i.e., 2, 4-D (1 mg/L), KIN (0.5 mg/L) and 3% sucrose] showed best result. Thus this combination was further used for induction of callus in both control and arsenic contaminated samples (Fig. 6).

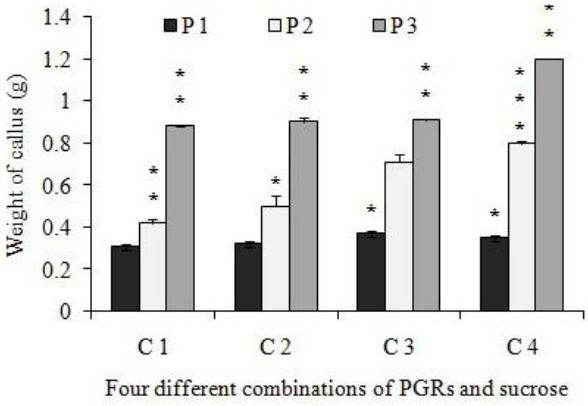

Fig 6: Weight of callus induced by different concentration of PGR and sucrose combination.

In our experiment best result obtained from using the PGRs concentration 2-4-D (1mg/L) and kinetin (0.5mg/L) with 3% sucrose and 0.9% agar. Sucrose is the most preferred carbon source for plant tissue culture. Researchers were found that leaf explants showed maximum callus percentage and caulogenic response when tested on MS medium supplemented with BAP, NAA, 2, 4-D and KIN. The callus produced at these PGRs concentrations including 2, 4-D, were of different texture and morphology depending upon nature of PGRs. Thiruvengadam et al. (2006) stated that MS medium containing 1.0 mg/L 2, 4-D approximately 90% of leaf explants of *M. charantia* gave rise to a well organized calli. We have found the similar result. This concentration in 2, 4-D gave the best callus among our four combinations. Auxin plays many roles in controlling and maintaining physiological status. Auxins influence cell enlargement, bud formation and root initiation. Thus in our experiment we have also seen that concentration of auxin was important for the morphology and health of the callus. Callus culture was established using four different combinations of PGRs and it was found that combination C4 [i.e., 2, 4-D (1 mg/L), KIN (0.5 mg/L) and 3% sucrose] showed best

result. Thus this combination was further used for induction of callus in both control and arsenic contaminated samples.

Sultana and Bari Miah (2003) were used different combinations and concentrations of cytokinins and auxins on MS media for the regeneration from shoot tip. They found multiple shoot regeneration was better using BAP and NAA. In our experimental system we have given attention to make callus for the further work. Callus induction was achieved by Sultana et al. (2003) from the leaf segments on MS media containing 1.0mg/L 2, 4-D. But we got better result using 2, 4-D and KIN. In present study, we have maintained culture for three passages (21 days for each passage). According to Hartmann et al. (2002) *in vitro* growth are regulated by interaction and balance between PGRs supplied within a medium and growth substances induced endogenously.

After arsenic exposure, morphological changes were verified, mainly in the callus of *M. charantia* at different doses of arsenic. The damage worsened gradually as sodium arsenate dose in calli was increased (Fig. 7). From the AAS study of callus samples it was found that callus established from Burdwan sample containing no detectable arsenic and high calcium content (52.39 mg/kg). Callus developed from arsenic contaminated area (Basirhat) contain low calcium content and high arsenic content (Fig. 8).

Heavy metals are conventionally known as elements with high atomic mass and with metallic properties such as conductivity, stability as cations, ligand specificity etc and they are toxic in nature and cannot be processed by living organism. Metal contamination occurs due to industrial activities such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy production, fuel production, fertilizer application, pesticide application and generation of municipal waste. Metals are natural components in soil. In soil, metals are associated with free metal ions, soluble metal complexes and soil organic matter or embedded in structure of the silicate minerals.

During life processes plant not only acquire macronutrients but also acquire essential micronutrients such as Fe, Zn, Mn, Cu, etc. Plants have evolved very specific mechanisms to use this nutrient for translocation, physiological use and storage. Metal movement across biological membranes is mediated by proteins with transport functions or specific ion channel.

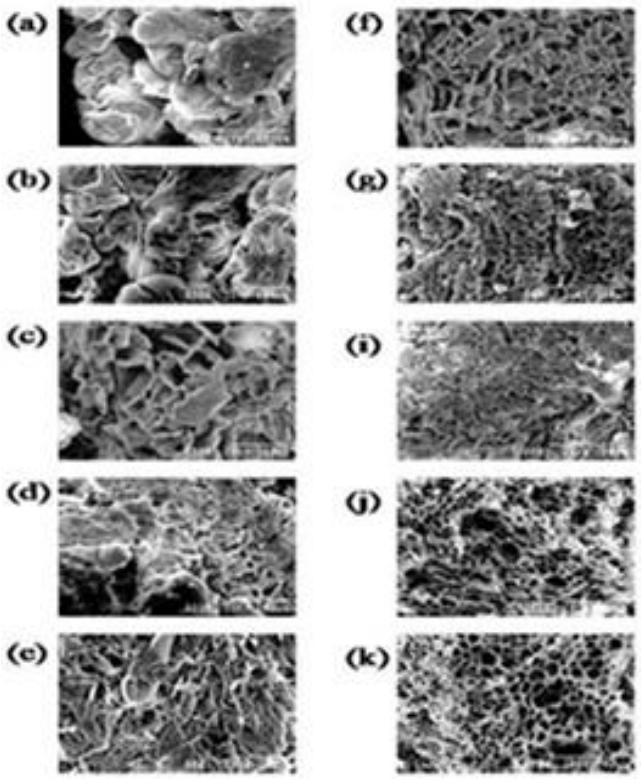

Fig 7: Scanning electron micrographs of *Momordica* callus. (a) control setup (b) 10 µM sodium arsenate treated calli sample (c) 25 µM sodium arsenate treated calli sample (d) 50 µM sodium arsenate treated calli sample (e) 75 µM sodium arsenate treated calli sample (f) 100 µM sodium arsenate treated calli sample (g) 125 µM sodium arsenate treated calli sample (h) 150 µM sodium arsenate treated calli sample and (i) 175 µM sodium arsenate treated calli sample (j) 200 µM sodium arsenate treated calli sample.

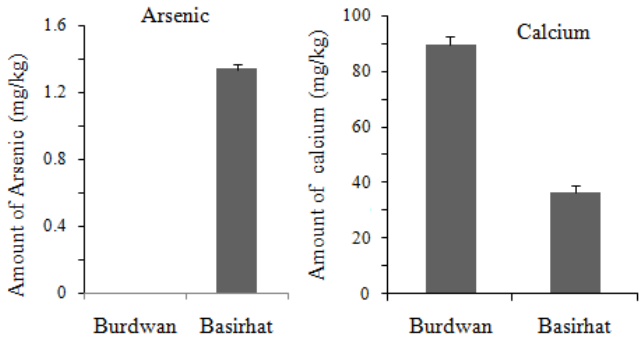

Fig 8: Concentrations of arsenic and calcium in *M. charantia* callus.

In plants the uptake mechanism is selective and there might be some metal-metal interaction or relation among the ions to each other. Ion uptake selectivity depends upon the structure and properties of membrane transporters. In plants metals transported from the root

to the shoot. Movement of metal-containing sap from the root to the shoot, termed translocation, is primarily controlled by two processes: root pressure and leaf transpiration. Micronutrients such as Zn, Mn, Fe, Cu, etc. are essential for plant growth and development but high intracellular concentrations of these ions can be toxic. To deal with metal stress, common nonaccumulator plants have evolved several mechanisms to control the homeostasis of intracellular ions. Such mechanisms are regulation of ion influx and extrusion of intracellular ions back into the external solution etc. Metal hyperaccumulator species, capable of taking up metals in the thousands of ppm, possess specific detoxification mechanisms.

We have analysed eight different elements in our experimental plant system. Two macromolecules (nutrients required 0.02–6.0% of plant nutrient, Katalin, 2011) were namely calcium and nitrogen was measured. Nitrogen has been discussed in next chapter. Four microelements (elements required in 0.01–500 mg/kg, Katalin, 2011) were measured by this techniques namely zinc, iron, manganese and copper. We also measured three elements which are known to be toxic in plants namely arsenic, lead and cadmium.

A balance of inorganic nutrients is required by plants for maximum growth and development under optimal and stressful environments. Mineral deficiencies or imbalances and depression of plant growth can result from excessive As toxicity that affects the rate of uptake and distribution of certain nutrients in plants. To reverse the negative effect of As stress, plants need to either inhibit its accumulation or enhance its tolerance capacity to As for survival. The severity of As toxicity, however, can be reduced through the optimization of these nutrients. Sufficient availability of nutrients may reduce or enhance the accumulation of a metal in plants and decrease its toxicity by inducing several physiological processes. Zinc is an important group of metal transporters family. They are thought to be involved in Cd uptake from soil into the root cell and also in transport of Cd from root-to-shoot. Enhanced root metal uptake mediated by transporters seems to be a factor necessary for hyperaccumulation. To maintain a proper physiological condition plant requires very tight homeostasis of each nutrient as well as a balanced macro and micro-nutrient composition. In this study, we further explored the relationships and interaction among some macro and micro nutrient in normal and arsenic stress condition. Iron is an integral part of chlorophyll that is involved in photosynthesis (Marschner, 1995). Chlorophyll synthesis, including the activation of several enzymes and oxidoreduction reactions requires Zn. Zinc deficiencies are associated with low total concentrations of zinc in soils featuring highly weathered parental material and low pH such as in tropical areas (Alloways, 2008).

Arsenic is not essential for plants (Marin et al., 1993) and has no known metabolic function. Arsenic toxicity had a varying effect on Ca, Zn, Fe and Mn concentration. Ca concentration decreased with exposure to As in *Momordica*. The effect of As toxicity on plant Ca levels is generally thought to result from reduced transpiration affecting transport of Ca up through the plant. Uptake of Ca, was altered by the toxic effect of As on root functions. In addition, reduced growth limited the demand for Ca contributed to low uptake (Carbonell-Barrachina et al., 1994). As had little effect on Ca, and other cations transportation to the shoot at any but the highest As exposure rate. Singh et al. (2006), reported a reduction in membrane stability with increased exposure to As. Reduced tissue Ca levels found may be related to As induced membrane stability; leaky membranes may be related to the low accumulation of other cations. Singh et al. (2006) reported membrane stability decreased with increasing exposure to As.

The concentration of calcium (Fig. 9a) in both root and shoot were decreased when the concentration of sodium arsenate in media were increased. Maximum calcium content was found in control plants and the value was 88.35 mg/kg in shoots and 84.10 mg/kg in roots. Arsenic treated seedlings showed lesser content of calcium in roots and shoot with compare to control seedlings.

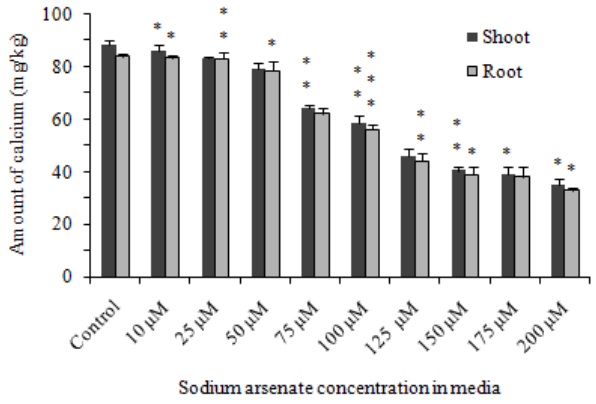

Fig 9a: Amount of calcium in *M. charantia* grown in different concentration of sodium arsenate in media *in vitro*.

Calcium acts as a second messenger in extracellular signal transduction to transfer environmental signals (Harpner et al., 2004) and is responsible for membrane stability and wall rigidity. It has also been shown that Ca^{2+} influx prompted organogenesis. Calcium is a constituent of cell walls and is involved in production of new growing points and root tips. It provides elasticity and expansion of cell walls, which keeps growing points from becoming rigid and brittle. Cell death is induced under experimental conditions not only by a rise in cytoplasmic calcium but also when cytoplasmic calcium activity drops below physiologic levels (Paschen, 2003). Cellular stimulation can lead to activation of different signal transduction mechanisms, such as alterations of the cytoplasmic levels of different ions. Among the result of eight elements analysis in HG-AAS, Ca and As gave the interesting results. In the present investigation where concentration of arsenic is more found in plant samples their Ca concentration is decreasing. Till today there is no clear evidence was found for this type of result. We are trying to give some new finding from our experiments.

Understanding the mechanisms underlying the homeostasis of the ionome and the balance of ion acquisition has been the subject of recent research (Cuypers et al., 2013). This requires knowledge of how nutrient levels are sensed by plants and how nutrients control gene expression. The Zinc- and Iron-regulated transport Protein gene family encodes transporters for divalent metal ion nutrients (Milner et al., 2013). Studies suggest that uptake, translocation, and homeostasis of Zn, Fe, and Mn is controlled by the number of active transporters embedded in cell membranes. Future research will advance our understanding of the underlying physiological mechanisms enabling plant species to thrive in native habitats characterized by soils with unusual nutrient compositions.

Content of manganese (Fig. 9b) in roots was increased as the concentration of sodium arsenate was increased in media. 22% increased in manganese in roots of 200 µM sodium arsenate treated samples were found when compared to untreated samples. Our data show increase in the concentrations of iron (group 8 element of periodic table) as compared to control and 200 µM sodium arsenate treated samples. We found 46% increase in iron content in maximum. No significant difference were found in both root and shoot iron concentration (Fig. 9c).

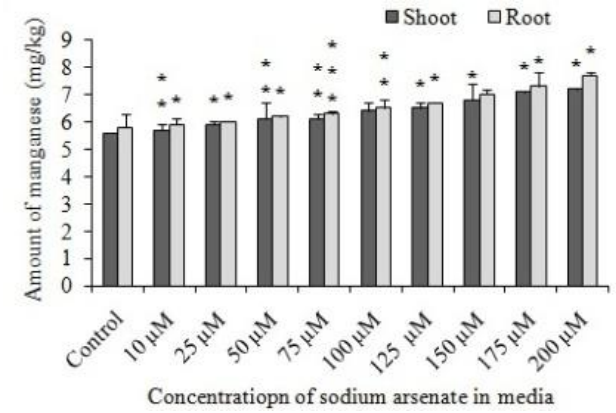

Fig 9b: Amount of manganese in *M. charantia* grown in different concentration of sodium arsenate in media *in vitro*.

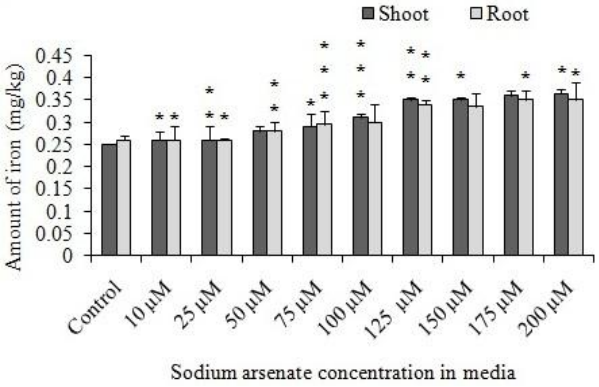

Fig 9c: Amount of iron in *M. charantia* grown in different concentration of sodium arsenate in media *in vitro*.

Concentrations of copper (Fig. 9d) and zinc (Fig. 9e) increased as the sodium arsenate concentration was increased in the test samples. We observed increase in copper concentration accompanied by increase in arsenic concentrations in seedlings but no such significant difference between root and shoot portion was found. Shoots of the seedlings contain more zinc than root. 19.7% increase of zinc content was found in highest treatment of arsenic (200 µM) than untreated control. Result showed that there might be a synergistic effect between zinc and copper.

Arsenic concentration was found more in shoot than root. Highest arsenic uptake was found in 200 µM sodium arsenate treated samples and the value was 1.35 mg/kg in case of shoot and 1.22 mg/kg in case of root (Fig. 9f). Concentration of cadmium (Fig. 9g) in root and shoot were decreased as the arsenic concentration increased in the tissue culture media. But concentration of cadmium in root and shoot portion in different doses of treatment was not showing any significance.

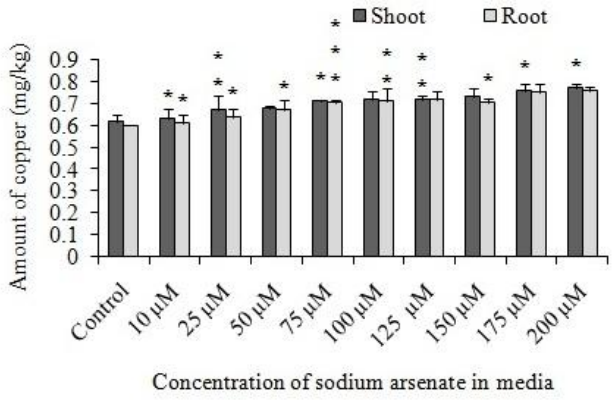

Fig 9d: Amount of copper in *M. charantia* grown in different concentration of sodium arsenate in media *in vitro.*

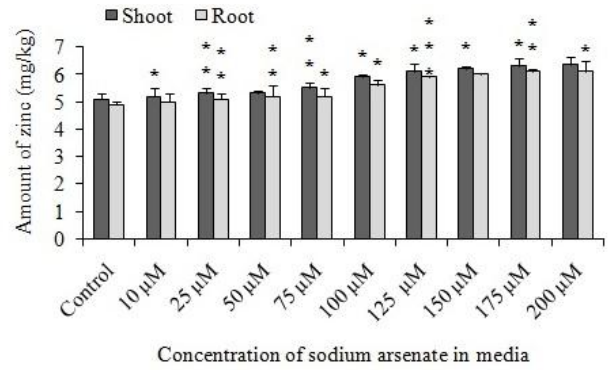

Fig 9e: Amount of zinc in *M. charantia* grown in different concentration of sodium arsenate in media *in vitro.*

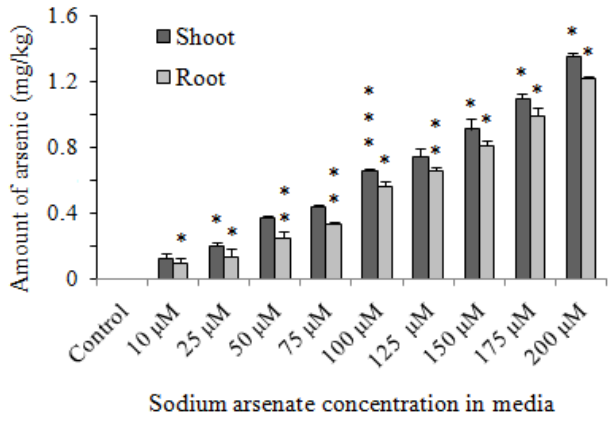

Fig 9f: Amount of arsenic in *M. charantia* grown in different concentration of sodium arsenate in media *in vitro.*

Concentrations of lead (Fig. 9h) in shoots of *Momordica* were decreased much up to 100 µM sodium arsenate stress. Lowest value was found in 200 µM sodium arsenate stress and the value was 0.29 mg/kg in case of shoot and 0.27 mg/kg in case of root samples. After arsenic exposure, anatomical changes were verified, mainly in the callus of *M charantia* at different doses of arsenic. The damage worsened gradually as sodium arsenate dose in calli was increased. In case of higher doses of sodium arsenic treated samples we observed that cells were highly damaged.

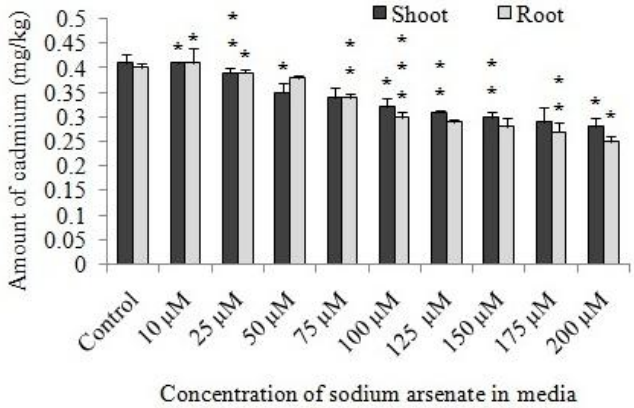

Fig 9g: Amount of cadmium in *M. charantia* grown in different concentration of sodium arsenate in media *in vitro.*

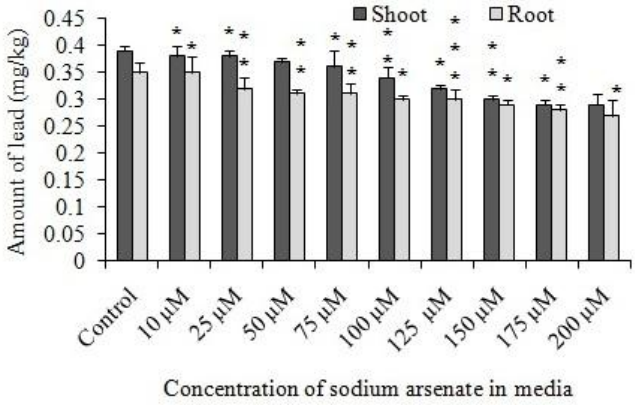

Fig 9h: Amount of lead in *M. charantia* grown in different concentration of sodium arsenate in media *in vitro.*

Iron and Mn are constituents in a variety of enzymes (Mengel and Kirkby, 1987). The effect of As on dry weight likely limited demand for Fe and Mn in sensitive species. Mn concentrations tended to increase with increasing As exposure. However, As toxicity causes a breakdown in membrane stability (Cizewski Culotta et al., 2006) and a general changes in mineral uptake. Changes in root As seemed to affect transport of Fe from root to shoot. Zinc is used in a number of enzymes and functions similarly to Mn and Mg as an enzyme activator (Mengel and Kirkby, 1987). Arsenic induced to change the uptake of Zn. Increased As exposure alters Zn translocation percent as well. Zinc is required for tryptophan synthesis as a precursor to the growth hormone auxin. Copper is used as a constituent in electron transport proteins and oxidation/reduction

reactions (Mengel and Kirkby, 1987). Most notably, Cu is used to neutralize reactive oxygen species through Cu/Zn superoxide dismutase (Cu/ZnSOD) (Benov and Fridovich, 1996). This enzyme protects the cell membrane from attack by superoxide radicals. Graham et al. (1987) reported that Cu usage in cellular functions in plant species may be very efficient. Low Cu status implies that Cu deficiency is one of the initial effects of As toxicity. Photosynthesis, respiration, lignification, and superoxide radical neutralization can all be compromised due to this interaction. Copper is a strong competitor with other metals for protein binding sites, however, very little free Cu is found in the cytoplasm (Finney and O'Halloran, 2003; Cizewski Culotta et al., 2006; Sheng et al., 2014).

Mn is one of the most important microelement and play major roles in plant tissue culture media. It is also involved in photosynthesis and respiration. Manganese is needed in plant system because it acts as an activator for some enzymes. It acts as an enzyme activator for nitrogen assimilation. It is essential for the manufacture of chlorophyll. Copper is a component of many important enzymes, which are involved in electron transport and protein and carbohydrate biosynthesis, thus it can be suggested that these enzymes might play a significant role in plant development and regeneration. Copper is involved as an enzyme activator and is thought to be involved in chlorophyll formation although its specific role is still unclear. It is also thought to be involved in protein synthesis. Copper catalyzes several plant processes. It has major function in photosynthesis and reproductive stages. It increases sugar content and improve flavor of fruits and vegetables. Zinc is essential component of more than 270 enzymes and is also responsible for stimulating growth of epidermal and epithelial cells (Kaplan et al., 2003). Plants require zinc because it activates some essential enzymes in plant systems. Zinc is necessary for chlorophyll production, carbohydrate and starch formation. It has an enzymatic system and seed formation. Zinc is an essential micronutrient for plants and is required in low concentrations to maintain life processes, in excess; this element causes toxicity in plants. This toxicity is caused by the role of Zn as a competitive inhibitor of other cofactors of metabolic pathways that are essential to plants, as in the case of Fe, which is directly linked to redox reactions and may alter cell function, causing lower growth or breaks that hamper cell maintenance.

Iron is needed in plant systems for reaction involving in cell division and growth. Iron acts as an oxygen career and play a vital role in chlorophyll formation. Iron is an essential micronutrient that plays a unique role in metabolic processes. In plant, Fe is mainly used in chloroplast, mitochondria, and peroxisomes of plants for operating oxidation-reduction reaction.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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