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Effect of arsenic on growth, lipid peroxidation and antioxidant defence system in cowpea

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ABSTRACT

Arsenic (As) is a toxic ubiquitous metalloid. Exposure of plants to As can result in various morphological, physiological and biochemical variations. Hydroponic experiment was conducted to study the growth response, lipid peroxidation, proline and antioxidant defence system under arsenic (As) stress in the cowpea. Ten day old seedlings of cowpea grown hydroponically, were treated with 0, 25, 50, 75 and 100 µM (Na_2HAsO_4) sodium arsenate for 7 days and analysed for morphological and biochemical traits under As stress. Significant decline in plant root and shoot length along with biomass was recorded with increased arsenic doses, as compared to control. The As treatment resulted in increased proline and decrease in malondialdehyde (MDA) content in seedlings of cowpea with increased concentration. Enzymatic antioxidants like catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) showed increased activity in dose dependant manner over control under As stress. Results indicated lower content of MDA and enhanced activities of the enzymatic antioxidant perform a significant role in As tolerance in cowpea.

Key words: Antioxidant enzymes, Arsenic, Cowpea, MDA.

INTRODUCTION

Arsenic (As) is the most toxic metalloid known for centuries. Arsenic is omnipresent in environments and highly lethal to all forms of life. As is a food chain contaminant and considered as a class I carcinogen (Zhao *et al.,* 2010). The Comprehensive environmental response, compensation and liability act permanently listed As as no. 1 in its significant list of hazardous materials (ATSDR, 2007). Plants reportedly represent the initial path for arsenic to enters the food web (Hartley *et al*., 2001). Arsenic is known to inhibit the plant growth together with fresh and dry biomass (Stoeva *et al*., 2003) and causes physiological disorders (Wells *et al*., 1997) as well as decrease the crop productivity. (Stepanak *et al*., 1998). Plants usually take up arsenic predominantly in trivalent (As III) and pentavalent (As V) forms, which are well identified to hinder several metabolic pathways like reacting with sulfhydryl groups and substitution of phosphate from ATP (Shri *et al.,* 2009). As have been reported to stimulate the production of free radicals, leading to oxidative stress (Singh *et al*., 2007). According to Mishra *et al*. (2008) the generated free radicals may induce peroxidation of unsaturated fatty acids in the cell membrane resulting in formation of lipid peroxidation products like MDA. The generated Reactive oxygen species (ROS) as a consequence of As toxicity need to be scavenged for plants development. Reportedly, a natural detoxification mechanism in plants is the upregulation of enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate peroxidase (APX) (Dave *et al.,* 2013; Sobrino-Plata *et al.,* 2014). Under abiotic stress conditions, plant frequently accumulate osmoprotectant compound such as proline which helps in direct scavenging of free radicals and also act as membrane plasticizer (Yadu *et al.,* 2016).

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Among the most important cultivated legumes, cowpea [*Vigna unguiculata* (L.) Walp] has shown numerous environmental, agronomic and economic advantages. (Singh *et al.,* 2014). Under the present changing climatic and ecological conditions cowpea is predictable to fulfil the growing requirements of dietary proteins for humans (Goncalves *et al.,* 2016). However, the physiological and biochemical responses of cowpea to toxic As (V) are very scanty. With the above background the objective of this experiment was to inspect the effect of As (V) on the growth, lipid peroxidation, proline accumulation and antioxidant response on the cowpea cultivar grown in Saudi Arabia.

MATERIALS AND METHODS

The seeds of cowpea were obtained from the local market of Gizan from western Saudi Arabia. The seeds were surface sterilized and sown in soilrite. Three days after germination, the seedlings of cowpea were shifted to Hoagland's solution (Hoagland and Arnon, 1950). Ten-day-old seedlings were exposed to five levels of As (V) in the form of (Na_2HAsO_4) Sodium arsenate *viz*., T1 = 0 µM (Na_2HAsO_4), T2=25 µM (Na_2HAsO_4), T3=50 µM (Na_2HAsO_4), T4=75 µM (Na_2HAsO_4),

T5=100 µM (Na_2HAsO_4). Growth performance, enzymatic antioxidants and non-enzymatic antioxidants were studied after 7-days of As V treatment. All the experiments were conducted in three replicates.

Plant shoot and root length were assessed on an electronic balance (Model EBL-120-S, Shimadzu, Japan). Samples were oven dried for dry weight (DW) determination at 65°C ± 2°C for 72 h and independently weighed. Plant DW and FW were expressed in g. Plant length was determined by using metric scale and recorded in centimeter.

Malondialdehyde (MDA) content in the leaves was determined by the method of Heath and Packer (1968). The level of lipid peroxidation as MDA concentration was evaluated using an extinction (ε) of 155 $\mu M^{-1} cm^{-1}$.

Free proline content was determined by the method of Bates *et al*. (1973). The concentration of proline was estimated using calibration curve and denoted as mmol proline g^{-1} FW.

For assays of CAT, SOD, APX and GR, leaf samples was homogenised in ice cold 50 mM sodium phosphate buffer (pH 6.8) containing 1mM EDTA and 2% (w/v) PVPP. The homogenate was centrifuged at 13,000 x *g* for 40 min at 4°C and the supernatant was used for enzyme assays.

SOD activity in the leaf sample was estimated by observing its ability to inhibit photochemical reduction of NBT at 560 nm (Beauchamp and Fridovich, 1971). The activity of SOD was expressed in enzyme unit (EU) mg^{-1} protein h^{-1}.

Enzymatic activity of CAT was studied by using the method of Aebi (1984). The activity of CAT was estimated spectrophotometrically by observing the dissolution of H_2O_2, recording a decline in the absorbance at 240 nm and expressed as EU mg^{-1} protein.

APX activity was measured by the method described by Nakano and Asada (1981). Activity of APX was determined by recording the reduction in ascorbate at *A*290 ($\varepsilon = 2.8\ mM^{-1} cm^{-1}$). One enzyme unit, measures the quantity of enzyme required to degrade 1 µmol ascorbate per mg of protein per min. The activity of APX was expressed as enzyme unit (EU) mg^{-1} protein.

GR activity in the leaf samples was estimated by utilising the method of Foyer and Halliwell (1976). GR activity was measured by extinction coefficient of 6.2 $mM^{-1} cm^{-1}$ and expressed as EU mg^{-1} protein. Protein concentration was measured by using the method of Bradford (1976).

All analyses were done on a completely randomized design. All the data were subjected to one-way analyses of variance (ANOVAS). All the data point was the mean of three replicates. Comparisons with P values < 0.05 were considered significantly different.

RESULTS AND DISCUSSION

Growth parameters

Arsenic is reported to have phytotoxic effects on some plant species (Talukdar 2013). Data of plant shoot length, root length, plant fresh weight and dry weight of cowpea as influenced by various doses of As (V) treatments are presented in Table 1. In the present study As (V) proved toxic, in a dose dependant manner, causing significant reduction in the plant growth. Plant shoot length and root length was reduced up to 20-58% and 24-63% over control. Whereas reduction in plant fresh weight and dry weight was 24-62% and 30-83% respectively when compare to control. Corresponding to result of this study significant decrease in root and shoot length under arsenic stress has been reported in other plants also by many investigators (Liu *et al*., 2005; Shri *et al*., 2009; Talukdar, 2013).

Lipid peroxidation

A significant change was observed in the MDA content at all level of As (V) treatment. Maximum amount of the MDA (98%) was reported at T2 (25 µM) while the minimum level of MDA content (21%) was observed at T5 (100 µM) As (V) treatment (Fig 1) Yadav *et al*. (2015) also observed same trend in *Zea mays* under As stress. As lipid peroxidation is the indicator mostly ascribed to oxidative damage high level of MDA content indicates toxicity, whereas a lower MDA content indicates improved antioxidative system as reported by various authors (Rai *et al*., 2013; Upadhyay *et al*., 2016).

Proline accumulation

Among non-enzymatic antioxidants, proline is a well-documented osmolytic compound which accumulates in plants under stress condition. (Chandrakar *et al*., 2016, Begum *et al*., 2016). In the present study proline accumulation in the cowpea increased significantly with increasing levels of As (V) treatments, The percent increase in the proline accumulation was (52-161%) when compared to control (Fig 2). An increase in proline content upon As (V)

Table 1: The effect of different As (V) concentration on plant length, plant fresh weight (g) and plant dry weight (g).

(Na_2HAsO_4) (µM)	Plant length (cm)		Plant fresh weight (g)		Plant dry weight (g)	
	Root	Shoot	Root	Shoot	Root	Shoot
0	11.0[a]	16.3[a]	0.410[a]	0.680[a]	0.067[a]	0.071[a]
25	10.2[a]	15.5[a]	0.401[a]	0.662[b]	0.053[b]	0.060[b]
50	9.7[b]	14.2[b]	0.356[b]	0.536[b]	0.049[b]	0.055[c]
75	8.3[b]	12.3[b]	0.295[c]	0.423[c]	0.040[c]	0.042[c]
100	6.4[c]	10.2[c]	0.201[d]	0.316[d]	0.028[d]	0.039[d]

Data are means of three replications (n =3).

Data (within each column) followed by same superscript letters are not significantly different according to the LSD test at P = 0.05.

exposure has also been reported in earlier works on *Vigna mungo* (Srivastava and Sharma 2013) and rice (Kumar *et al.,* 2014) Soybean (Kamran *et al.,* 2018).

Antioxidant defence system

Under As stress, plants can induce oxidative stress, which causes an imbalance between ROS generation and ROS scavenging (Abbas *et al*., 2018). Among the antioxidant enzymes, SOD is a major enzyme that constitutes the first line of defence against ROS in plants (Abbas *et al*., 2014). In the present study SOD activity showed variation at different levels of As (V) treatments (Fig 3). The highest activity of SOD was recorded at T5 (100 µM) (31-86%) when compared to control. As-induced SOD activities have been observed by many other investigators (Begum *et al*., 2016, Singh *et al.,* 2017).

In the present study As (V) treatment induced the CAT activity in cowpea seedlings in dose dependant manner. Maximum induction (36-89%) was observed at T5 (100 µM) when compared to control (Fig 4). Similar to our findings increased CAT activity under As stress was also observed in *P. vittata* by Tiwari *et al*. (2017).

The vital role of APX in mitigating the harmful effects of H_2O_2 under As (V) stress, has been well documented (Singh *et al*., 2017). The activity of APX showed similar trend to that of SOD and CAT. The maximum APX activity (27-94%) was observed at T5 (100 µM) level of As (V) treatment when compared to control (Fig 5). Similar results of increased APX

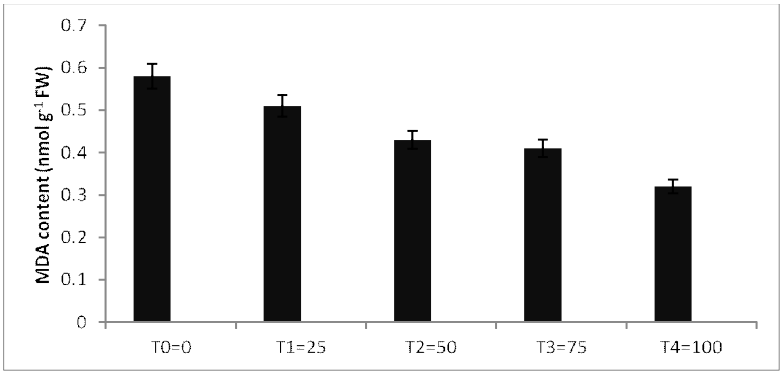

Fig 1: MDA content of cowpea exposed to various levels of As (V) treatment (25µM-100µM) at 7 DAT.

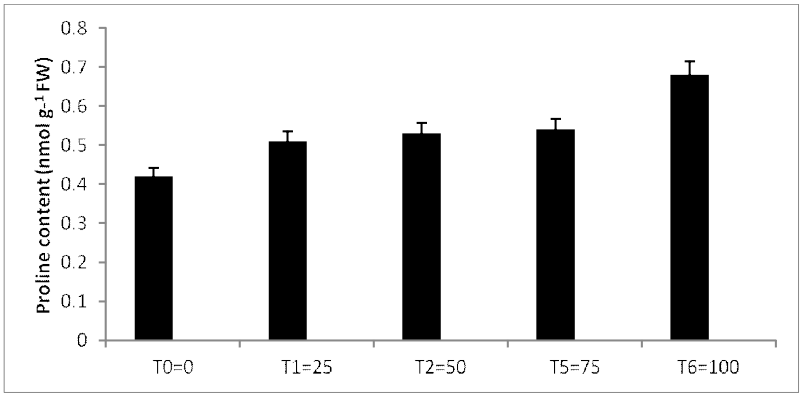

Fig 2: Proline content of cowpea exposed to various levels of As (V) treatment (25µM-100µM) at 7 DAT.

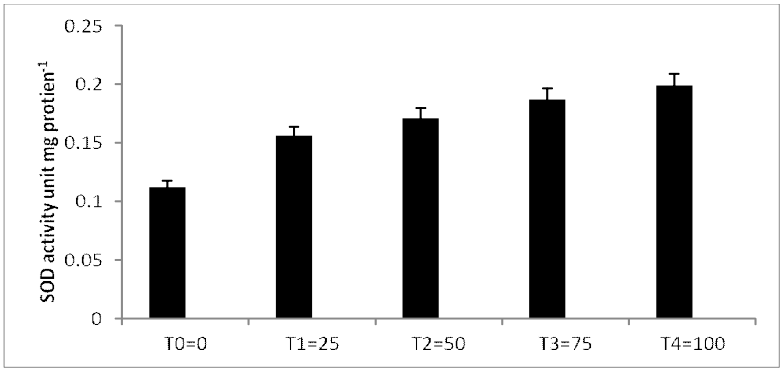

Fig 3: SOD activity of cowpea exposed to various levels of As (V) treatment (25µM-100µM) at 7 DAT.

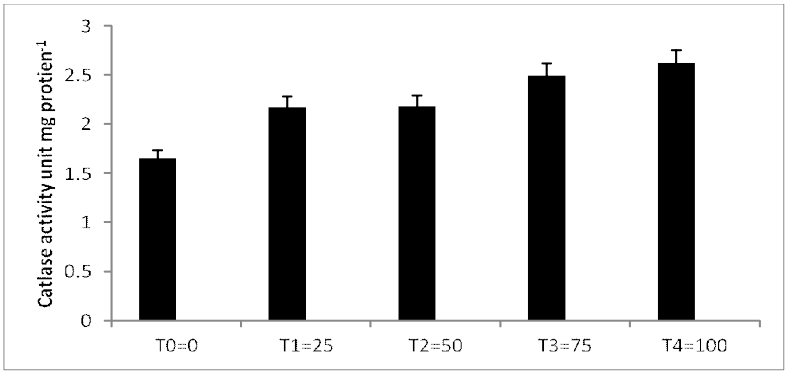

Fig 4: CAT activity of cowpea exposed to various levels of As (V) treatment (25µM-100µM) at 7 DAT.

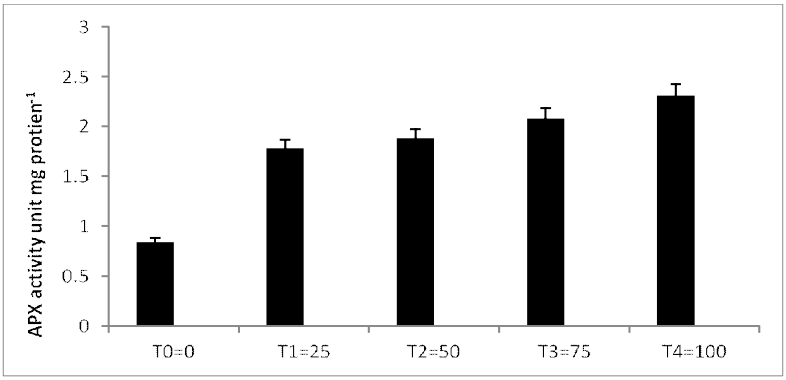

Fig 5: APX activity of cowpea exposed to various levels of As (V) treatment (25µM-100µM) at 7 DAT.

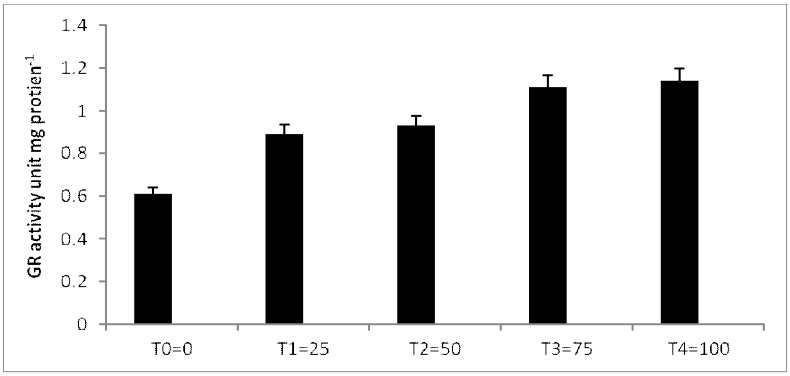

Fig 6: GR activity of cowpea exposed to various levels of As (V) treatment (25µM-100µM) at 7 DAT.

activity in response to As stress was reported in *Zea maize* and *Oryza sativa* by Shri *et al*. (2009).

Glutathione is an essential antioxidant involved in cellular defense mechanism against toxicants (Goldsbrough, 2000). In our study GR activity showed significant variation at different levels of As (V) treatment but the maximum GR activity (34-79%) was observed at T5 (100 µM) over control. (Fig 6). Overproduction of GR, under As stress, has been reported by number of other investigators (Degola *et al*., 2015, Jozefczak *et al*., 2012.).

CONCLUSION

Arsenic is considered as one of the most toxic metalloid threatening the plant productivity. The study showed that As (V) causes significant reduction in growth rate of cowpea in terms of fresh and dry biomass. As toxicity induced the oxidative stress in cowpea which is related to higher proline production. To combat arsenic-induced oxidative damage in cowpea enzymatic antioxidant proved to play a major role. Thus, the present study contributes to the understanding of biochemical strategies adapted by cowpea to avoid As toxicity. Further investigation on a molecular level is essential to comprehend the mechanism of free radicals induced by As stress.

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REFERENCES

Abbas, G., Murtaza, B., Bibi, I., Shahid, M., Khan, Niazi, N.K. Khan, M.I., Amjad, M., Hussain, M., Natasha. (2018). International Journal of Environmental Research and Public Health. 1: 59.

Abbas, M., Cheema, K.J. (2014). Correlation Studies of Arsenic Level in Drinking Water and Hair Samples of Females in District Sheikhupura, Pakistan. International Journal of Current. Microbiology and Applied Sciences. 3: 1077-1085.

Aebi, H. (1984). Catalase in vitro. Methods in Enzymology. 105: 121-126.

ATSDR (2007). U.S. Toxicological Profile for Cadmium. Centers for Disease Control and Prevention. Atlanta, GA, USA:

Bates, L., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil. 39: 205-207.

Beauchamp, C., Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 44: 276-277.

Begum, M.C., Islam, M.S., Islam, M., Amin, R., Parvez, M.S., Kabir, A.H. (2016). Biochemical and molecular responses underlying differential arsenic tolerance in rice (*Oryza sativa* L.) Plant Physiology and Biochemistry. 104: 266-277.

Bradford, M.A. (1976). Rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein- dye binding. Analytical Biochemistry. 7: 248-254.

Chandrakar, V., Dubey, A., Keshavkant, S. (2016). Modulation of antioxidant enzymes by salicylic acid in arsenic exposed Glycine max L. Journal of Soil Science and Plant Nutrition. 16: 662-676.

Dave, R., Tripathi, R.D., Dwivedi, S., Tripathi, P., Dixit, G., Sharma, Y.K., Trivedi, P.K., *et al* (2013). Arsenate and arsenite exposure modulate antioxidants and amino acids in contrasting arsenic accumulating rice (*Oryza sativa* L.) genotypes. Journal of Hazardous Materials. 262: 1123-1131.

Degola, F., Fattorini, L., Bona, E., Sprimuto, C.T., Argese, E., Berta, G., di Toppi, L.S. (2015). The symbiosis between Nicotiana tabacum and the endomycorrhizal fungus *Funneliformis mosseae* increases the plant glutathione level and decreases leaf cadmium and root arsenic contents. Plant Physiology and Biochemistry. 92: 11-18.

Foyer, C.H. and Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism. Planta. 133: 21-25.

Goldsbrough, P. (2000). Metal tolerance in plants: the role of phytochelatins and metallothioneins. In: phytoremediation of Contaminated Soil and Water, [Edited by: Terry, N and Banuelos, G.] CRC Press LLC. Boca Raton, FL: 221-233.

Gonçalves, A., Goufo, P., Barros, A., Dominguez-Perles, R., Trindade, H., Rosa, E. A. S. (2016). Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. Journal of the Science of Food and Agriculture. 96: 2941-2951.

Hartley-Whitaker, J., Ainsworth, G., Meharg, A. A. (2001). Copper- and arsenate-induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. Plant, Cell and Environment. 24: 713-722.

Heath, R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 125: 180-198.

Hoagland, D.R. and Arnon, D.S. (1950). The water culture method for growing plants without soil. California Agricultural Experiment Station Circular. 347: 1-32.

Jozefczak, M., Remans, T., Vangronsveld, J., Cuypers, A. (2012). Glutathione is a key player in metal-induced oxidative stress defenses. The International Journal of Molecular Sciences. 13: 3145–3175.

Kamran M.A., Xu, R.K., Li, J.Y., Jiang, J., Nkoh, J.N. (2018). Effect of different phosphorus sources on soybean growth and arsenic uptake under arsenic stress conditions in an acidic ultisol. Ecotoxicology and Environmental Safety. 165: 11-18.

Kumar, A., Dwivedi, S., Singh, R.P., Chakrabarty, D., Mallick, S., Trivedi, P.K., Adhikari, B., Tripathi, R.D. (2014). Evaluation of amino acid profile in contrasting arsenic accumulating rice genotypes under arsenic stress. Biologia Plantarum. 58:733-742

Liu, X., Zhang, S., Shan, X., Zhu, Y.G. (2005). Toxicity of arsenate and arsenite on germination, seedling growth and amylolytic activity of wheat. Chemosphere. 61: 293-301.

Mishra, S., Sudhakar, S., Tripathi, R.D., Trivedi, P.K. (2008). Thiol metabolism and antioxidant systems complement each other during arsenate detoxification in *Ceratophyllum demersum* L. Aquatic Toxicology. 86: 205-215.

Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology. 22: 867-880.

Rai, U.N., Singh, N.K., Upadhyay, A.K., Verma, S. (2013). Chromate tolerance and accumulation in *Chlorella vulgaris* L. role of antioxidant enzymes and biochemical changes in detoxification of metals. Bioresource Technology. 136: 604-609.

Shri, M., Kumar, S., Chakrabarty, D., Trivedi, P.K., Malick, S., Mishra, P., Shukla, *et al* (2009). Effect of arsenic on growth, oxidative stress and antioxidant system in rice seedling. Ecotoxicology and Environmental Safety. 72: 1102-1110

Singh, A.P., Dixit, G., Kumar, A., Mishra, S., Kumar, N., Dixit, S., Singh, P.K., Dwivedi, S., Trivedi, P.K., Pandey, V. (2017). A protective role for nitric oxide and salicylic acid for arsenite phytotoxicity in rice (*Oryza sativa* L.) Plant Physiology and Biochemistry. 115:163-173.

Kumar, B.B. (2014). Cowpea: The Food Legume of the 21st Century. CSSA, Madison, WI.

Singh, S.K., Juwarkar, A.A., Kumar, S., Meshram, S., Fan, M., (2007). Effect of amendment on phytoextraction of arsenic by *Vetiveria zizanioides* from soil. International Journal of Environmental Science and Technology. 4: 339-344.

Sobrino-Plata, J., Carrasco-Gil, S., Abadía, J., Escobar, C., Álvarez-Fernández, A., Hernández, L.E. (2014). The role of glutathione in mercury tolerance resembles its function under cadmium stress in Arabidopsis. Metallomics. 6: 356-366.

Srivastava, S., Sharma, Y.K. (2013) Arsenic phytotoxicity in black gram (*Vigna mungo* L. Var. PU19) and its possible amelioration by phosphate application. Journal of Plant Physiology and Pathology. 1:3.

Stepanak, V. (1998). The effect of arsenic on the yield and elemental composition of agricultural crops. Agrokhimiya. 12:57-63.

Stoeva, N., Bineva, T. (2003). Oxidative changes and photosynthesis in oat plants grown in as contaminated soil. Bulgarian Journal of Plant Physiology. 29: 87-95.

Talukdar, D. (2103). Arsenic-induced changes in growth and antioxidant metabolism of fenugreek. Russian Journal of Plant Physiology. 60: 652-660.

Tiwari, S., Sarangi, B.K. (2017). Comparative analysis of antioxidant response by *Pteris vittata* and *Vetiveria zizanioides* towards arsenic stress. Ecological Engineering. 100: 211-218.

Upadhyay, A.K., Singh, N.K., Singh, R., Rai, U.N. (2016). Amelioration of arsenic toxicity in rice: Comparative effect of inoculation of *Chlorella vulgaris* and *Nannochloropsis* sp. on growth, biochemical changes and arsenic uptake. Ecotoxicology and Environmental Safety. 124:68-73.

Wells, B.R., Gilmor, J. (1997). Sterility in rice cultivars as influenced by MSMA rate and water management. Agronomy Journal. 69:451-454.

Yadav, R.K., Srivastava, S.K. (2015). Effect of Arsenite and Arsenate on Lipid Peroxidation, Enzymatic and Non-Enzymatic Antioxidants in *Zea mays* Linn. Biochemistry and Physiology. 4: 186.

Yadu, B., Chandrakar, V., Keshavkant, S. (2016). Responses of plants to fluoride: An overview of oxidative stress and defense mechanisms. Fluoride. 49: 293.

Zhao, F.J., McGrath, S.P., Meharg, A.A. (2010). Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Annual Review of Plant Biology. 61:7.1-7.25.