DOI: 10.5958/0976-4038.2015.00035.4

Full Research Article

Role of Essential Nutrients as Tools for Reducing Arsenic Hazards in Chilli (*Capsicum annuum* L.)

Dipak Girhepuje*, P. V. Takawale, Jayashree Ugalat and P. K. Bandopadhyay

Dept. of Plant Physiology, Agricultural Biochemistry, Fruits & Orchard Management, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal (741 252), India

Article History

Manuscript No. AR807
Received in 26th October, 2014
Received in revised form 6th March, 2015
Accepted in final form 26th March, 2015

Correspondence to

*E-mail: dipakgirhepuje@gmail.com

Keywords

Arsenic, manganese, phosphate, chilli, nutrients

Abstract

Capsicum annuum L. was treated with arsenic (As) in pot culture condition to substantiate hazardous effects on growth and biosynthesis of some biomolecules with coexisting effects of essential nutrients namely, manganese (Mn) and phosphate (P). Treatments were applied to plants after 20, 50 and 80 days of transplanting as a soil application. Arsenate (5 mg As kg^{-1} soil) showed somewhat stimulatory effect on growth and biosynthesis of soluble sugar and soluble protein at the stage of 30 days but at later stages of growth, arsenate was found to be obstructive for fresh and dry shoot weights as well as root weights. Fresh and dry leaf weights and leaf area gradually decreased with increasing concentrations with more doses of As. Arsenic showed significant adverse effect on leaf length and stem diameter of chilli plant. The duration of exposure to arsenate stress was negatively correlated with partitioning of dry matter accumulation. The inhibitory effect of arsenate was less pronounced at the beginning but increased with time. It decreased soluble sugar content, protein content and finally the yield of chilli in the later stages of growth at high concentrations of arsenate. The application of manganese (1 ppm), phosphate (13 mg kg^{-1} soil) separately and together exhibited significant helpful alterations of all the growth and biochemical characteristics tested under the purview of arsenate treatment alone. In conclusion better growth and yield was observed with the application of manganese and phosphate in chilli seedlings by declining As hazards in arsenic infested soils.

1. Introduction

Capsicum is a genus of plant under the family of Solanaceae and this capsicum has varieties of names according to their location and type. The most familiar peppers names are chilli, bell, red, green or just called as pepper (Faustino et al., 2007). India is the major chilli producing country in the world, but the average productivity of chilli is low (1 t ha^{-1}) as compared to China, Taiwan, and Mexico where it yields 3 tons ha^{-1} of dry chilli (Peter, 1998). Dry chillies are extensively used as a spice in India. It is administered in the form of powder, tincture, plaster, ointment and medicated wool etc. Capsicum species have been reported to have antioxidant properties (Sharma et al., 2012). Chilli is fruit-vegetable that commonly found in multiethnic of Malaysian's daily food menu. They are extremely popular for the huge content of vitamin C and total soluble phenolics higher than other vegetables commonly recognized as a source of this substance (Marinova et al., 2005). Now a days chilli is an important vegetables crop and used world-widely as for flavour, aroma and add colour to foods (Shaha et al., 2013). Scientific research has proven that, *Capsicum annuum* is the only crop that produce alkaloid compound called capsaicinoids, which is responsible for the hot test. Capsaicinoids are alkaloids that are important in the pharmaceutical industry for their neurological effectiveness (Hayman and Kam, 2008). Pepper that are fresh is known as the very good source of vitamin C, vitamin E, provitamin A and carotenoids (Krinsky, 2001; Perucka and Masterska, 2007; Navarro et al., 2006; Chatterjee, et al., 2007; Deepa, et al., 2007; Serrano Martinez et al., 2008). The productivity of the crop generally fluctuates with the fluctuation of environmental factors and in many cases decreases the yield and quality of the crops. Underground irrigation of land which is generally man made pollutes the surface soils by arsenic. Arsenic is an element that is non essential and toxic to plants. Its accumulation in food crops may pose a health risk to humans (Zhaol et al., 2009). It is metalloid compound found in arsenite and arsenate form and very much toxic to

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animal and plant system when mixed in metabolic pathways. Among the two forms available in soil, arsenite is highly toxic to plant system, which ultimately drastically hampers the growth of the plant. The main affected part is the root system and ultimately damages to the shoot system. These arsenite/arsenate when in tissue system in substantial amount, decline the productivity of the crop and it is very difficult to get rid of (Dipak et al., 2013). The phytotoxicity of arsenic is affected considerably by the chemical form in which it occurs in the soil and its concentration. In view of the variety of reactions in plants that involve sulphydryl groups and phosphorus, arsenites and arsenates may interfere with physiological and biochemical processes which constitute growth in a number of ways (Patra et al., 2004). To overcome hazard of As toxicity, simple economical treatments were chosen to detoxify the toxicity effects of arsenic in a very small concentration during different growth phases of the chilli plants. The main purpose of the present investigation is to study the influence of some nutrients diminishing arsenic toxicity.

2. Materials and Methods

The pot experiment was carried out at University, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India during 2010 and 2011 with 4 replications in a randomized block design. The seedlings of *Capsicum annuum* were planted in earthen pots of 10 inch diameter (5 plants pot^{-1}). Before transplanting the plants in pots, they were prepared by pouring 5 kg soil in each pot; the soil was added thoroughly with standard doses of fertilizers and organic manures were fortified into the soil. The solutions of sodium arsenate, manganese chloride and single super phosphate were prepared by dissolving it in the distilled water. Quantity of salt is taken in such a way that it would contain 5 mg As kg^{-1} soil, 1 ppm Mn (200 ml kg^{-1} soil) and 13 mg P kg^{-1} soil for each time of application. Solutions were prepared properly just before the application and given as soil application after 20, 50 and 80 days of transplanting (DAT) to the same pots of chilli plants each time; data were recorded after 10 days of treatment application. Total yield of chilli in terms of fruits throughout the life cycle was calculated and expressed on fresh weight basis only. Soluble sugar was estimated with the ethanol extraction method given by Somogyi, 1952 and Krishnaveni et al., 1984. 100 mg of the sample was extracted with hot 80% ethanol twice and collected the supernatant which was evaporated by keeping on a water bath at 80 °C. 10 ml water was added and dissolved the sugars. 0.5 ml of aliquot was taken in a test tube and the volume was made up to 2 ml with distilled water. Tube containing 2 ml distilled water served as a blank. One ml of alkaline copper tartrate reagent was added to each tube and placed the tubes in boiling water for 10 minutes. After cooling the tubes, 1 ml of arsenomolybdate reagent was added and the volume in each tube was made up to 10 ml with water. The absorbance of blue colour was read at 620 nm after 10 min. From the graph drawn, the amount of soluble sugars present in the sample was calculated and expressed in terms of %. Protein was estimated with the methodology proposed by Sadasivam and Manickam (1996). 500 mg of finely cut leaves samples were grinded with addition of 10 ml buffer. Extracts were centrifuged at 5000 rpm for 10 minutes and used the supernatants for protein estimation. 0.2 ml of supernatant was taken into a test tube and the volume was made up to 1 ml in each tube including blank with distilled water. Five ml of alkaline copper solution was added to each tube, mixed well and allowed to stand for 10 minutes. After a proper mixing 0.5 ml folin-ciocalteau reagent was added, mixed well and incubated at room temperature in the dark for 30 minutes for blue colouration. The intensity of blue colour was measured in a spectrophotometer at 660 nm. From the graph prepared of standard bovine serum albumin concentration the soluble protein content of the sample was calculated and represented in terms of %.

3. Results and Discussion

3.1. Fresh and dry weights of shoot and root

Table 1 presents the fresh and dry weight of shoots and roots under different growth stages of chilli treated with As, Mn, P and their combination with each other. It was observed that fresh and dry weight of shoot increased a little during 30 days growth phase due to application of 5 mg As kg^{-1} soil, but the results were statistically not significant. All the ameliorating treatments of essential nutrients showed significant increase in fresh and dry weights of shoot over control during 30 days growth. During 60-90 days growth As substantially reduced fresh and dry weights of chilli shoot. The reduction in shoot weight due to As was recovered to some extent due to application of 1 ppm Mn, but more recovery was found with the application of P (13 mg P kg^{-1} soil). The maximum beneficial effects on shoot weight were observed with As+Mn+P treatment in all the growth stages of chilli plant. The treatment effect in case of dry shoot weight is so prominent due to Mn and P application. It happened possibly due to check in the deterioration of chloroplast along with chlorophyll molecules and there was less damage in the photosynthetic machinery. Obviously the photosynthetic efficiency of the plant restored due to the treatment of Mn and P and hence there was increase in dry weight accumulation in the plant. In the early literature of Rahman et al., 2007 on rice cultivar suggested that high concentration of As affect both chlorophyll a and b content of the leaf which in turn drastically affect the growth and yield of rice crop due to decline in photosynthetic efficiency. Similarly the measurement of fresh and dry root weights sn indicated that, in the early growth phases (30 days growth), the roots of chilli had some enhancement effect of As; however difference was not comparable with the control one. The hazardous effects of As was extended over the period of 60 to 90 days growth and the maximum effect of toxicity was reported in 90 days growth phase after third does of treatments. On the other hand the treatment with Mn and P reduced the hazardous effects of As with respect to fresh and dry root weight. The amelioration effect showed that there was an increment in fresh and dry root weights even than that of untreated control. The maximum reduction of As toxicity was observed in As+Mn+P treatment plants with fresh and dry root weights of 17.29 and 6.51 g respectively, during 90 days growth.The observed results justifies the previous results reported in sorghum by Shaibur et al., 2008 where they have shown that in the early phase, As had betterment effect on shoot and root growth but toxicity effect expressed in later growth phases.

3.2. Fresh and dry weight of leaf, leaf area, yield, leaf length and stem diameter

Table 2 presents the fresh and dry weight of leaf, leaf area and yield at different growth phases of plants in relation to As effect and its improvement by Mn and P. The fresh and dry leaf weights as well as leaf area of As treated plants did not differ significantly from the control treatment during 30 days growth, where as the treatments of essential nutrients namely, Mn, P and their combination showed somewhat encouraging results in both the cases of weight and area of leaf. The hazards of As were much more magnified from 60 days onwards and up to 90 days at high concentration. Fresh leaf weight significantly decreased to 0.26 and 0.31 g from 0.46 and 0.60 g respectively, during 60 and 90 days growth due to application of As. With respect to dry weight of leaf, the negative effect of As was found only after 90 days of transplanting. It might be due to reduced moisture content of the leaves as well as plants. It destroys the chlorophyll molecule and the chloroplast with the enhancement of enzyme chlorophyllase turning down photosynthetic activities. So substantial decrease in dry weight content of chilli leaves occur. The reduction in fresh and dry weight of leaf was recovered to some extent with the application of Mn, P and their combination treatments. The most superior effects on reduction of arsenic hazard with respect to fresh and dry leaf weight was recorded in As+Mn+P treatment followed by As+P and As+Mn treatments. The present results justified that treatment with Mn and P nullifies the hazardous effect of As by restoring the disordered mechanism of photosynthesis in order. This may be possible reason for betterment in increasing photosynthates (Chang Simin et al., 2007; Stoeva et al., 2003; Milton et al., 1989). The leaf area is one of the most important criteria in respect of its growth performance as because increase in leaf area helps directly or indirectly in better harvesting of solar energy resulting in an increment of photosynthetic efficiency. The observations confirmed that As hazard on leaf area was much pronounced in later phases of plant growth at higher concentrations of As than that of early phases. However the hazard was much more decreased when soil treated with Mn and P or their combination. The leaf area of As+Mn treatment (14.60 cm^2) and As+P treatment (15.08 cm^2) after 60 days of transplanting was statistically at par with each other but differed significantly between the mentioned treatments after 90 days of transplanting. The maximum betterment results with respect to leaf area (19.60 cm^2) were observed when treated with As+Mn+P in combination. It was interesting to note the superior recovery of leaf area due to essential nutrients, manganese and phosphate, as it exceeded in respect of untreated control. Same pattern of results was observed in yield characteristic with maximum yield of 28.53 g $plant^{-1}$ in control treatment followed by in As+Mn+P treated plants (27.67 g $plant^{-1}$) and As+P treated plants (23.46 g $plant^{-1}$). The present results indicate that As somehow delays the growing process of leaves by delaying cell division and cell elongation. It happens possibly due to imbalance in sugar/

Table 1: Hazardous effects of arsenic on fresh and dry shoot weight and root weight of chilli plant (g) and its recovery by some essential nutrients (pooled data)

Characters	Fresh shoot weight			Dry shoot weight			Fresh root weight			Dry root weight		
Treatments	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
Control	23.48	52.48	62.10	9.23	15.57	20.08	6.69	10.65	12.54	1.85	3.98	5.05
As	24.73	45.02	50.24	10.33	10.72	13.05	6.97	9.02	9.68	1.97	2.94	3.54
As+Mn	26.45	47.14	51.98	11.61	12.26	14.90	8.90	10.16	13.34	2.46	4.14	5.45
As+P	29.15	49.84	56.79	12.71	14.29	20.21	8.29	9.76	11.29	2.19	3.66	4.54
As+Mn+P	31.12	53.09	62.80	14.52	16.71	22.06	10.82	13.20	17.29	2.76	5.79	6.51
SEm±	0.83	1.01	0.97	1.66	0.71	0.97	0.34	0.36	0.45	0.19	0.21	0.22
CD (p=0.05)	2.50	3.04	2.93	1.99	2.15	2.92	1.02	1.07	1.37	0.58	0.62	0.65

starch ratio and creating hindrance in absorption behavior of K, Ca and Mg. This might be the possible reason of As delays the developmental process.

The little concentration of As showed stimulating effects on both leaf length and stem diameter of chilli as it significantly increased the values of said characteristics during 30 days growth over control which are presented in Table 2 and Fig. 1 respectively. Present results are coinciding with the results of Ma and Hong, 1998 who indicated that low concentration of As could stimulate the shoot growth of wheat seedlings. All the treatments of essential nutrients along with As also increased the leaf length significantly as compare to control one during early growth phase. The comparable observations were recorded in leaf length of all the treated plants with each other during this phase. After second dose of As, leaf length significantly decreased to 5.70 cm from 6.10 cm which was recovered by essential nutrients, Mn and P. The maximum leaf length (6.50 cm) was observed in As+Mn+P treatment during 60 days growth which was significantly higher than control and other all treatments. After 90 days of transplanting and third dose of treatments, there was a massive decline in leaf length due to application of 5 mg As kg^{-1} soil (3 doses) while manganese, phosphate and their combination got well the detrimental effects of As to some extent. The ameliorating treatment, As+Mn+P showed maximum improvement in leaf length (7.10 cm) after 90 days of transplanting which was statistically at par with control (7.20 cm). During 30 days growth, stem diameter was the maximum in As+Mn+P treatment followed by As+P and As+Mn treatment; stem diameter of As+P treatment (4.80 mm) was on par with As+Mn treatment (4.70 mm) and at par with As+Mn+P treatment. Similar to leaf length, stem diameter also decreased after application of second dose of As after 60 days of transplanting but maximum detrimental effects were observed after 90 days of growth at high concentrations of As (3rd application). It may be caused by increment in MDA content due to As stress condition (Chris et al., 1992). The reduced stem diameter was enhanced by the application of manganese and phosphate in a very small quantity during both the growth phases. As+P treatment showed more recovery of stem diameter than As+Mn treatment during both the growth phases of 60 and 90 days. The highest recovery of stem diameter was observed in As+Mn+P treatment during 60 (6.50 mm) and 90 (8.90 mm) days growth which was near to controls. These results indicate that the chilli seedlings were sensitive to As, and the growth of leaf and stem was inhibited by high concentration of As. Abedin and Meharg (2002) reported that growth of rice decreased significantly with increasing concentrations of As. Miteva and Merakchiyska (2002) reported that, the higher doses inhibited the photosynthesis; so this may be the possible reason behind the reduction of leaf length and stem diameter in the present experiment.

3.3. Soluble sugar and soluble protein

Soluble sugar is an important as it plays obviously central role in plant structure and metabolism. The results showed that there was an increase in soluble sugar (1.67%) of chilli leaves with As treatment in 30 days growth as compare to control (0.93%) but significant decline was observed in 90 days growth phase. During 30 days growth, there were no comparable differences found between soluble sugar of As treated chilli leaves and all other nutrient treated chilli leaves. In case of fruit, there was no significant differences observed in soluble sugar concentration of control, As and other treatments of essential nutrients. The hazards found during 90 days growth at high concentrations of As were reduced to some extent by the treatments of Mn and P but their combination treatment (As+Mn+P) showed maximum improvement with soluble sugar content of 1.63% over As (0.99%). The maximum soluble sugar content in fruits (1.35%) was recorded in the plants treated with As+Mn+P which was more as compare to both control (0.78%) and As (0.58%) treatment. It was reported in the earlier literature that As toxicity disturbs the carbohydrate metabolism in rice. There was an increase in soluble sugar content may be due to activity of starch phosphorylase and/or on the contrary due

Table 2: Effects of arsenic, manganese and phosphate on leaf characteristics and yield of chilli fruit (pooled data)

Characters	Fresh leaf weight (g)			Dry leaf weight (g)			Leaf area (cm^2)			Leaf length (cm)			Yield $plant^{-1}$ on FW basis (g)
Treatments	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	
Control	0.19	0.46	0.60	0.08	0.13	0.17	11.17	14.68	17.01	4.30	6.10	7.20	28.53
As	0.23	0.26	0.31	0.09	0.10	0.10	11.35	12.65	13.82	4.50	5.70	6.20	14.47
As+Mn	0.26	0.29	0.36	0.10	0.11	0.13	11.79	14.60	16.24	4.90	5.90	6.50	18.70
As+P	0.28	0.36	0.53	0.12	0.13	0.17	12.45	15.08	17.68	5.30	6.10	6.70	23.46
As+Mn+P	0.36	0.43	0.66	0.14	0.18	0.21	14.71	17.52	19.60	5.60	6.50	7.10	27.67
SEm±	0.02	0.03	0.02	0.02	0.02	0.02	0.22	0.26	0.24	0.05	0.06	0.06	0.225
CD (p=0.05)	0.08	0.08	0.07	N.S.	0.05	0.05	0.68	0.79	0.73	0.15	0.19	0.20	0.700

to decreased α-amylase activity (Choudhury et al., 2010). It was reported that arsenate had affected the activities of different enzymes of carbohydrate metabolism. In the present experimental studies, soluble sugar restored to some extent with different nutrient treatments over As. On the whole it showed marginal improvement over As treatment (Jha et al., 2004). The hazards of As on soluble protein contents of leaves and fruits were studied throughout the life cycle of chilli. It was observed that there was a tendency of increase in the protein content of leaves with As treatment in 30 days growth compare to control. The increase of soluble sugar content with low dose of As treatment is advantageous in holding water of cell or organization, and preventing the dehydration. The growth of plants was inhibited by As treatment. Therefore, the protein that was not used in cells was accumulated, which caused soluble protein content increase (Yu et al., 1995). All the treatments of essential nutrients along with individual treatment of arsenic showed certain betterment effects on soluble protein contents of leaves during 30 days growth over control; however differences were statistically not comparable. In 90 days plants, there was a drastic fall in the protein content of the leaves treated with As (2.88%) as compare to control (4.60%); but in case of fruit, not much decrease in protein content was observed compared to control. However significant increase in protein content was found with all the nutrient treatments in 90 days old plants as well as in fruits. Soluble protein concentrations of leaves and fruits in As+Mn and As+P treatments were at par with each other. The maximum protein concentration in fruits was found with As+Mn+P treatment (3.60%) which was highly significant with control (2.75%) and arsenic (2.52%) treatment. The result shows that Arsenic hazards derailed the protein synthesizing machinery by changing translation or transcription pathway, however Mn and P helps to restore the machinery and possessed the betterment effects, Table 3.

Table 3: Soluble sugar and soluble protein content of leaves and fruits of chilli plants (%) under different growth phases subjected to arsenate toxicity and its recovery by manganese and phosphate (pooled data)

Characters	Soluble sugar			Soluble protein		
Treatments	30 DAT	90 DAT	Fruit	30 DAT	90 DAT	Fruit
Control	0.93	1.88	0.78	4.31	4.60	2.75
As	1.67	0.99	0.58	5.17	2.88	2.52
As+Mn	1.84	1.16	1.48	5.72	5.38	3.68
As+P	1.62	1.27	0.80	5.38	5.16	3.45
As+Mn+P	1.22	1.63	1.35	5.84	4.77	3.60
SEm±	0.13	0.16	0.23	0.42	0.23	0.17
CD (p=0.05)	0.39	0.49	N.S.	N.S.	0.69	0.51

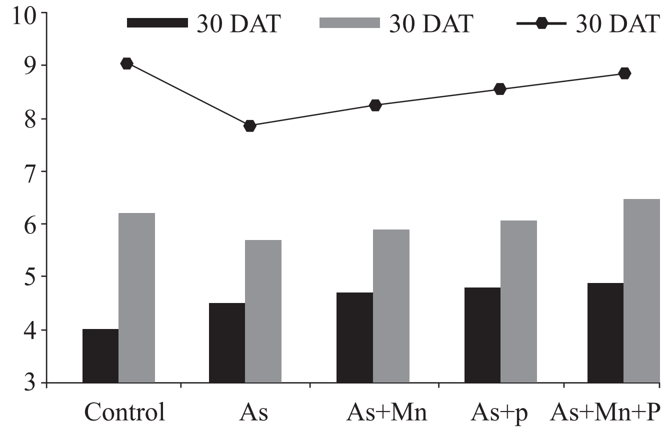

Figure 1: Effects of Arsenic on stem diameter (mm) under different growth phases of chilli plants and its recovery by essential nutrients, manganese and phosphate (pooled data)

4. Conclusion

Arsenic under moderate to high doses hampers the growth and biosynthesis of certain biomolecules which can be minimized with the application of manganese and phosphate in proper doses. The drastic fall in certain biomolecules due to arsenic can be improved with the applications of essential nutrients like manganese and phosphate in adequate quantity.

5. References

Abedin, M.J., Meharg, A.A., 2002. Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L.). Plant and Soil 243, 57-66.

Chang, S., Jia, D., Ma, X., Liu, G., 2007. Effects of arsenic toxicity on water metabolism in leaves of flue cured tobacco (*Nicotiana tabacum* L.). Journal of Northwest A and F University Natural Science Edition 35(6), 73-76.

Chatterjee, S., Niaz, Z., Gautham, S., Adhikari, S., Pasad, S.V., Sharma, A., 2007. Antioxidant activity of some penolic constituents from green pepper (*Piper nigrum*) and fresh nutmeg mace (*Myristyca fragrans*). Food Chemistry 102, 515-523.

Choudhury, B., Mitra, S., Biswas, A.K., 2010. Regulation of sugar metabolism in rice (*Oryza sativa* L.) seedlings under arsenate toxicity and its improvement by phosphate. Physiology and Molecular Biology of Plants 16(1), 59-68.

Chris, B., Marc, V.H., Dirk, I., 1992. Superoxide dismutase and stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology 42(1), 43-83.

Deepa, N., Kaur, C., George, B., Singh, B., Kapoor, H.C., 2007. Antioxidant constituents in some sweet pepper (*Capsicum annuum* L.). LWT-Food Science and Technology 40, 212-219.

Dipak, G., Bandopadhyay, P.K., Tripathi, L.K., 2013.

Amelioration of arsenic toxicity with the application of manganese and phosphate in chilli (*Capsicum annuum* L.). Journal of Interacademicia 17(4), 614-621.

Faustino, J.M.F., Barroca, M.J., Guine, R.P.F., 2007. Study of drying kinetics of green bell pepper and chemical characterization. Food Bioproduct Process 85 (3), 163-170.

Hayman, M., Kam, P.C.A., 2008. Capsaicin: A review of its pharmacology and clinical applications. Current Anaesthesia and Critical Care 19, 338-343.

Jha, A.B., Dubey, R.S., 2004. Carbohydrate metabolism in growing rice seedlings under arsenic toxicity. Journal of Plant Physiology 161(7), 867-872.

Krinsky, N.I., 2001. Carotenoids and antioxidants. Nutrition 17, 815-817.

Krishnaveni, S., Theymoli Balasubramanian, Sadasivam, S., 1984. Determination of reducing sugars. Food Chemistry 15, 229-232.

Ma, C.C., Hong, F.S., 1998. Preliminary explanation of the mechanism about effects of mercury on wheat seed germination and seedling growth. Acta Phytoecologica Sinica 22(4), 373-378.

Marinova, D., Ribarova, F., Atanassova, M., 2005. Total phenolics and total flavonoids in bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy 40(3), 255-260.

Milton, N.M., Ager, C.M., Eiswerth, B.A., Power, M.S., 1989. Arsenic and selenium induced changes in spectral reflectance and morphology of soybean plants. Remote Sensing of Environment 30(3), 263-269.

Miteva, E. Merakchiyska, M., 2002. Response of chloroplasts and photosynthetic mechanism of bean plants to excess arsenic in soil. Bulgarian Journal of Agricultural Science 8, 151-156.

Navarro, J.M., Flores, P., Garrido, C., Martinez, V., 2006. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chemistry 96, 66-73.

Patra, M., Bhowmik, N., Bandopadhyay, B., Sharma, A., 2004. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environmental and Experimental Botany 52, 199-223.

Perucka, I., Matercska, M., 2007. Antioxidant vitamin contents of *Capsicum annuum* fruits extract as affected by processing and varietal factors. ACTA Scientiarum Polonorum Technologia Alimentaria 6(4), 67-74.

Peter, K.V., 1998. Recent advances in chilli breeding. Indian Spices 35, 3-5.

Rahman, M.A., Hasegawa, H., Rahman, M.M., Islam, M.N., Miah, M.A.M., Tasmen, A., 2007. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. Chemosphere 67(6), 1072-1079.

Sadasivam, S., Manickam, A., 1996. Protein estimation by Lowry's method. Biochemical Methods (2nd edition). New Age International Private Limited Publishers, India 56-58.

Serrano, M.A., Fortea, F.M., Del, A.F.M., Nufiez, D.E., 2008. Kinetic characterization and thermal inactivation study of partially purified red pepper (*Capsicum annuum* L.) peroxidase. Food Chemistry 107, 193-199.

Shaha, R.K., Rahman, S., Asrul, A., 2013. Bioactive compounds in chilli peppers (*Capsicum annuum* L.) at various ripening (green, yellow and red) stages. Annals of Biological Research 4(8), 27-34.

Shaibur, M.R., Kitajima, N., Sugawara, R., Kondo, T., Alam, S., Huq, S.M.I., Kawai, S., 2008. Critical toxicity level of arsenic and elemental composition of arsenic induced chlorosis in hydroponic sorghum. Water, Air, & Soil Pollution 191(1/4), 279-292.

Sharma, C., Singh, N., Pal, K. 2012. The effect of salt stress on biochemicals of chili at seedling level. International Journal of Pharma Professional Research 3(3) 665-670.

Somogyi, M., 1952. Notes on sugar estimation. Journal of Biological Chemistry 200, 245.

Stoeva, N., Berova, M., Zlatev, Z., 2003. Physiological response of maize to arsenic contamination. Biologia Plantarum 47(3), 449-452.

Yu, T.Q., Chai, L.N., Liu, Z.P., 1995. Expression of the soluble protein in water-stressed wheat seedlings and the drought-resistant proteins. Journal of Beijing Agricultural College 10(1), 26-31.

Zhao1, F.J., Ma, J.F., Meharg, A.A., Mcgrath, S.P., 2009. Arsenic uptake and metabolism in plants. New Phytologist 181, 777-794.