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Relative Toxicity of Arsenite and Arsenate on Early Seedling Growth and Photosynthetic Pigments of Rice

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Authors' contributions

This work was carried out in collaboration between both authors. Author MB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SM managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Arsenic is a potential contaminant of groundwater as well as soil in many regions of the world. Elevated soil arsenic levels resulting from long-term use of arsenic contaminated ground water for irrigation may inhibit seed germination and seedling establishment of rice, the country's main food crop. An experiment was conducted to study the effect of different forms of arsenic on growth and photosynthetic pigments of rice plants. For this purpose rice seedlings were grown hydroponically with half strength of modified Hoagland nutrient solution toxified with different doses of arsenate and arsenite namely 0, 5, 10, 15 and 20 ppm. Germination percent of rice seeds and photosynthetic pigment content of rice seedling decreased significantly with increase in concentration of arsenic of both the forms. Growth parameters affected more seriously in arsenite treated plants than arsenate treated plants.

Keywords: Arsenate; arsenite; germination; rice; seedling; toxicity; chlorophyll.

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1. INTRODUCTION

Arsenic (As) is a toxic heavy metal dispersed in the environment through a variety of anthropogenic activities like industrial, mining and agricultural etc. In the natural environment, it pollutes the soil and contaminates water, thus posing a serious threat to biota, including plants, animals and humans [1]. Groundwater contamination with arsenic is reported from many regions of the world, the most severe problems occur in Bangladesh, West Bengal, China and Taiwan [2]. Contaminated groundwater is not only the main source of drinking water but is also extensively used for irrigating crops. Rice is the main crop of India and second important crop in the world. Arsenic contaminated groundwater for irrigation increases soil arsenic levels in lands used for agriculture [3]. Both organic and inorganic forms of arsenic is present in terrestrial plants. Arsenate and arsenate is the major form of inorganic arsenic species, out of these arsenate predominates in aerobic soil, whereas arsenite in anaerobic soils. Availability of arsenic in soil is greatly influenced by its forms. Arsenical application in agriculture has introduced different arsenic compounds to soil environment. Presence of arsenic in irrigation water or in soil at an elevated level could hamper normal growth and development of plants. Plants can develop toxicity symptoms while they are exposed to excess arsenic either in soil or in solution culture such as: Inhibition of seed germination [4]; decrease in plant height [5]; reduction in root growth [6], wilting and necrosis of leaf blades [7], reduction in leaf area and photosynthesis [8]; decrease in shoot growth [9]. Arsenite and arsenate are inter convertible depending on the redox condition of the soil [10,11] with arsenite dominating in flooded paddy soils. Arsenic caused a reduction in the net photosynthesis of plants. Arsenic damaged the chloroplast by disintegrating the membrane structure and causes functional changes of photosynthetic process.

We used both of these two inorganic species in our study to see whether there is any differential toxicity effect shown by the arsenic species on rice cultivars. In this present study, we evaluated the effect of arsenite and arsenate on germination, early seedling growth and photosynthetic pigments of rice.

2. MATERIALS AND METHODS

For the present experiment rice variety IET-4786 was taken as experimental material. Rice seeds were surface sterilized with 0.1% (w/v) $Hgcl_2$ for two minutes, washed with distilled water. Seeds were than soaked with arsenic solution of different concentrations. And 10 ml of solutions of sodium arsenate ($Na_2HAs_3O_4$, 7H20) and arsenic oxide (As_2o_3) were used to soak whatman's no.1 filter paper in each of the petridishes of 9 cm diameter and 20 seeds per petriplates. Control set was prepared similarly using distilled water. Then all petriplates were placed in incubator maintaining temperatures of 28°C. The experiments were conducted in triplicate. Germination counts were obtained at eight days. Radical emergences of seeds equal to or greater than two (2) mm were considered as germination. On the eighth day, seedlings were removed from Petri dishes and length of shoot and root were measured separately and mean values were determined. On the basis of dry mass of seedlings of stressed (exposed to As) and non-stressed (not exposed to As) plants tolerance indices were calculated by adopting the formula given by Maiti et al. [12]. Promptness index (PI), germination stress index (GSI) and plant height index (PHSI) was calculated as described by Bouslama and Schapaugh [13].

$$\text{Promptness index (PI)} = nd_2\,(1.00) + nd_4\,(0.75) + nd_6\,(0.50) + nd_8\,(0.25)$$

Where, nd_2, nd_4, nd_6 and nd_8 = percentage of seeds observed to germinate on the 2^{nd}, 4^{th}, 6^{th} and 8^{th} day of observation, respectively.

$$\text{Germination stress index (GSI)} = \frac{\text{PI of stressed seeds (PIS)}}{\text{PI of control seeds (PIC)}} \times 100$$

$$\text{Plant height stress index (PHSI)} = \frac{\text{Plant height of stressed seedlings (PHS)}}{\text{Plant height of control seedling (PHC)}} \times 100$$

$$\text{Tolerance index (TI)} = \frac{\text{Dry weight of seedling under stress (g)}}{\text{Dry weight of seedling under control (g)}}$$

Pots containing 5 kg of sand used to place surface sterilized rice seeds for germination. Seedlings were grown for 14 days and after 14 days were transplanted to plastic buckets (capacity 3 litre) containing 250 ml of ½ strength modified Hogland's solution pH being 5.8- 6.0 germination. On 28th day such plants were transferred to Hoagland's solution contaminated with arsenate or arsenite. After 3 days of treatment seedling were removed. Pigments from the leaves were extracted by 80% acetone (v/v) following percolation method of [13]. After extraction was completed as indicated by discoloration of leaf samples, chlorophylls and carotenoids were determined spectro-photometrically by taking absorbance at wave length of 646, 663 and 470 nm. The amount of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids were calculated according to the [14] formulae. The data presented in the paper are average values of at least three independent experiments.

3. RESULTS AND DISCUSSION

Promptness (PI) of the seeds treated with different species and concentration of arsenical toxicant declined significantly in comparison to untreated seeds. For each species of arsenical toxicants, PI declined with increase of their concentration. Decline in PI was more striking in arsenite treated seeds than the corresponding concentration of arsenate. Seedlings of the seeds treated with arsenite concentration ≥ 15 ppm could not survive. PI is indicator of speed of germination of the seeds. These results clearly showed that germination of rice seeds was seriously affected by the presence of arsenic in the nutrient solutions at the concentrations of 5ppm and above. As a result of which germination was either delayed completely inhibited. The result also established that arsenite was more notorious than the arsenate.

Germination stress indices (GSI) also decreased with increasing concentrations of arsenite and arsenate. For both the species of arsenical toxicants, GSI declined with increase in concentration. Decline in GSI was also more prominent in arsenite treated seeds than the corresponding concentration of arsenate. GSI is the reflection of germination impairment of the seeds. These results clearly showed that germinational processes of rice seeds in the presence of arsenic in the nutrient solutions at the concentrations of 5ppm and above were either fully or partly inhibited at their operational levels. As a result of which germination was either delayed completely inhibited. The results also established that arsenite was more harmful than the arsenate.

Arsenic toxicants also had detrimental effect on post germinational growth which was sufficiently indicated in the values of tolerance index. All the values of TI except that at 5 ppm were ≤ 1 indicating lower dry matter mobilizations in the seedlings of the arsenic treated seeds. The values of TI for arsenite was lesser than that for the corresponding concentration of arsenate (Table 1) indicating again more poisonous effect of arsenite than arsenate in the processes of mobilization of stored food of seeds for seedling growth. So, it was established that arsenite causes more negative effect on growth parameters of rice than that of arsenate treatment. Lower germination of rice seed at higher concentration of arsenite than arsenate could be an important consideration for wetland rice culture because of presence of arsenite [11,15].

Shoot length and root length of the seedlings were very seriously affected by the presence of arsenic toxicants in the nutrient solution. Even in the presence of 5 ppm arsenate or arsenite in the nutrient solution the total length of root and shoot was less than 40% of that of seedlings of untreated seeds. Significant reduction in rice shoot and root length with increasing arsenic concentration suggests that rice shoot and root length can also be used as a good indicator for arsenic tolerance and toxicity. Reduced shoot length due to application of arsenic in this study also corroborates with the result of [16] who found significant reduction of rice shoot length when arsenite or mmAA was applied at a relatively lower dose of 0.8 mg As l^{-1}. The reduction of shoot height due to arsenic exposure can be an important consideration for rice cultivation as reduced shoot height will decrease rice leaf area, net photosynthesis [17], and ultimately rice yield.

The photosynthetic pigments are some of the most important internal factors, which in certain cases are able to limit the photosynthetic rate. It is believed that they are targets of the toxic As effect). According to them, the limiting step of the heavy metal effect on the plant photosynthesis is a result of the inhibition of chlorophyll (Chl) synthesis. The results from Table 2 showed that chlorophyll-a, chlorophyll-b total chlorophyll contents, carotenoid content and chlorophyll to carotenoid ratio (chl/car) in leaves decreased gradually with increase in both arsenate and arsenite in the growing media. There was a considerable decrease of total Chl contents (10,24 and 48 %) below the control at 10,15 and 20mg/L in arsenate whereas (41,54 and 73 %)below the control at same concentration in arsenite treated plants (Table 2). Carotenoids decreased by 5,14 and 27 % in arsenate treated plant, however 12,32 and 58% in arsenite

Table 1. Effect of different concentration of Arsenate and Arsenite on growth parameters of rice compared to control

Treatment	Dose	Promptness index (PI)	Germination stress index (GSI)	Tolerance index (TI)	Plant height stress index (PHSI)
Control	0 PPM	243.33±2.887	-	-	-
ASV	5 PPM	235.42±4.389	96.75±1.074	1.11±0.035	36.49±0.918
	10 PPM	192.92±9.043	79.30±4.317	0.977±0.056	30.33±3.773
	15 PPM	166.67±15.87	68.55±7.384	0.890 ±0.04	23.71±1.031
	20 PPM	135.00±16.39	55.53±7.388	0.817±0.035	19.82±1.563
ASIII	5 PPM	115.00±18.66	47.31±8.036	0.817±0.351	32.67±2.909
	10 PPM	37.08 ±10.02	15.27±4.30	0.297±0.351	19.813±5.135
	15PPM	0.00(leaves dry)	0.00	0.00±0.00	0.00±0.00
	20PPM	0.00(leaves dry)	0.00	0.00±0.00	0.00±0.00

Values in the table indicate mean ± standard deviation of three replications

Table 2. Effect of two inorganic forms of arsenic on Chlorophyll–a, Chlorophyll–b, Total Chlorophyll, Carotenoids (mg g-1 fresh wt.) chlorophyll/carotenoids ratio (chl/car) in rice

Treatment	Dose	Chlorophyll-a	Chlorophyll-b	Total chlorophyll	Carotenoid	Total chlorophyll /carotenoid
Control	0 PPM	1.455±0.071	0.508±0.072	1.963±0.124	0.648±0.099	3.066±0.392
ASV	5 PPM	1.449±.009	0.498±0.009	1.947±0.013	0.627±0.013	3.106±0.071
	10 PPM	1.299±0.073	0.460±0.019	1.759±0.057	0.616±0.007	2.855±0.065
	15 PPM	1.088±0.028	0.402±0.021	1.49±0.048	0.558±0.015	2.670±0.014
	20 PPM	0.725±0.028	0.304±0.021	1.029±0.048	0.474±0.015	2.170±0.032
ASIII	5 PPM	1.116±0.0280	0.402±0.021	1.518±0.048	0.619±0.015	2.452±0.018
	10 PPM	0.822±0.028	0.346±0.006	1.168±0.031	0.574±0.015	2.035±0.008
	15 PPM	0.631±0.028	0.27±0.021	0.901±0.048	0.442±0.015	2.038±0.039
	20 PPM	0.347±0.034	0.192±0.024	0.539±0.040	0.271±0.013	1.988±0.093

Values in the table indicate mean ± standard deviation of three replication

treated plants at 10,15 and 20mg/l as compared to control .It was established that Carotenoid content decreased to a lesser extent than Chl content. With increasing levels of heavy metals and metalloids reduction in the pigment content was also established by Marin et al. [18] The reducing levels of photosynthetic pigments in the arsenite treated plants might be due to more toxicity of this form than that of the arsenate [5]. The lower ratio of chlorophyll to carotenoids in arsenic treated plants than in control might be due to higher degradation of chlorophyll compared to carotenoids under arsenic stress.

4. CONCLUSION

From this investigation it is clearly indicate that arsenic stress in plants caused negative impact in germination and photosynthetic pigment and the inhibitory affect of arsenite was more pronounced than that of arsenate treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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