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Phytotoxicity of Arsenite on Early Seedling Growth of Mung Bean: A Threat to Potential Pulse Cultivation

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Abstract Arsenic toxicity has gained at present an alarming global importance. Pulse crop is very sensitive to arsenic contaminated groundwater. In order to determine the phytotoxicity, effects of Sodium arsenite ($NaAsO_2$) on seed germination and early development of Mung bean (*Vigna radiata* (L.) Wilczek cv. B-105) seedlings were investigated. Sodium arsenite had a toxic effect and posed a stress over germination parameters and early growth of mungbean seedlings. Considerable reduction in seed germination percentage and seedling vigour index was recorded due to arsenite. Sodium arsenite inhibited growth at very small concentrations such as 0.5µM. With the increase in concentration of sodium arsenite (0.5µM, 1µM, 2µM), significant decrease in seedling length i.e., shoot length and root length was observed. Arsenite was found to be more toxic for root growth than for shoot growth. Decrease in primary leaf area was also observed with increase in concentration of sodium arsenite. Number of stomata decreased and hence, reduction in stomatal density and stomatal index was also observed. Fresh weight and dry weight also reduced appreciably in the arsenite stressed seedlings. Treatment with 2µM concentration proved to be the most sensitive concentration for arsenite application by giving least values for seedling length and seedling vigour index.

Keywords *Vigna radiata*, Sodium Arsenite, Seed Germination, Seedling Vigour Index

1. Introduction

Arsenic a ubiquitous, carcinogenic trace metalloid [1], is found in virtually all environmental media [2]. Arsenic is known to have many toxic effects on living organism and is ranked first, in the priority list of hazardous substances compiled by the US Environmental Protection Agency (USEPA) ATSDR [3]. Arsenic exists in the environment in various organic and inorganic forms (species) [4]. The most important inorganic species are arsenate (As V) and arsenite (As III). Its toxicity to plants depends on its valence state. Due to greater cellular uptake, As III is much more soluble, mobile and toxic than As V [5].

Groundwater contamination by arsenic has been reported from many countries with the most severe problems occurring in Asia namely, Bangladesh, India, China and Taiwan. Among the numerous countries in different parts of the world affected by groundwater arsenic contamination, the largest population at risk is in Bangladesh [6] followed by our state, West Bengal in India. In West Bengal, concentration of arsenic (As) in groundwater of some places has been found to be above the maximum permissible limit (recommended value by WHO and US EPA is 0.01 mg/l) in 9 districts covering an area of 38,865 sq. km. This is regarded as the biggest arsenic calamity in the world. Crop plants are also affected when irrigated in fields by arsenic contaminated groundwater, which is expressed with reduction in growth and yield.

Seed germination is the first and most critical stage in seedling establishment, determining successful crop production [7]. Plants grown in presence of Arsenic show reduced seed germination and growth [8, 9]. There are a number of studies investigating the effect of As on different plant species including rice [10,11] but little work has been done on mung bean crops. Leguminous plants are found to be highly sensitive to arsenic [12]. Pulse crop Mung bean is also an important crop and principal source of protein in Indian diet. Yield of mung bean is bound to suffer if As toxicity becomes prevalent in the productive land where it is grown.

Therefore, the present investigation was undertaken to examine the effect of different concentrations of sodium arsenite on the germination and development of mung bean seedlings and to study the altered morphology of the seedlings due to stress induced by arsenite (As III) toxicity.

2. Material and Methods

2.1. Plant Material and Arsenic Treatments

Fresh, viable and uniform mung bean seeds (*Vigna radiata* (L.) Wilczek cv. B -105) were collected from Bidhan Chandra Krishi Vishwavidyalaya, Nadia, West Bengal. The plants were irrigated with water not contaminated with arsenite. The seeds were treated with 0.1% $HgCl_2$ solution for 2 min for surface sterilization and thereafter repeatedly washed with distilled water.

Sodium arsenite ($NaAsO_2$) (Merck) was used for arsenic treatment. Mung bean seeds were treated with arsenite at concentrations-0.5µM, 1µM and 2µM for 5 days. About 20 seeds were placed in each petriplate of 9cm diameter lined with double layered filter paper. Freshly prepared 10ml of treatment solution was applied to respective petriplates after washing the previous solution daily. The petriplates were kept in climatic room under controlled conditions of temperature (25 ±2℃) and relative humidity 65%. The set which received only distilled water for 5 days served as control. The above mentioned concentrations of As salts are comparable to soil conditions and are environmentally relevant.

2.2. Percentage of Germination and Seedling Vigour Index

Germination percentage (GP) is an estimate of the viability of a population of seeds. A seed was considered as germinated when 2 mm of radicle had emerged out of seed coat. Number of germinated seeds was counted daily and data were recorded after every 12 h for 5 days regularly to obtain Germination Percentage. It was measured according to the formula given by Tanveer *et al*. [13].

Germination percentage = (Germinated seeds ÷ Total seeds) x 100

Vigour index for seedling was calculated as the formula given by Cokkizgin and Cokkizgin [14] and ISTA [15] rules.

Seedling vigour index (SVI) = Seedling length × Germination percentage

2.3. Morphological Studies

After 5 days, arsenite induced damaging effects were observed and shoot length and root length of growing mung bean seedlings were measured in all the sets. Seedling length of 20 seedlings were determined after excising the cotyledons and averaged. Fresh and dry weight of 10 randomly selected seedlings were determined after excising the cotyledons and averaged. For estimating dry weight, the seedlings were allowed to dry in an oven at 70℃ for 3 days. After 5 days of growth, estimation of leaf area (primary leaves) of mung bean seedlings of all the sets were carried out using graph paper. Data were collected from 20 plants at a time that were selected randomly from the same set and then averaged. All the experiments were repeated 3 times and analysed statistically.

2.4. Measurement of Stomatal Density

The impression approach was used to determine leaf stomatal density, which was expressed as the number of stomata per unit leaf area [16]. The abaxial epidermis of the leaf was cleaned first using a degreased cotton ball, and then carefully smeared with nail varnish in the mid-area between the central vein and the leaf edge, for approximately 20 min. The thin film was peeled off from the leaf surface, mounted on a glass slide, immediately covered with a cover slip, and then lightly pressured with fine-point tweezers. Number of stomata and epidermal cells for each film strip were counted under a photomicroscope. Impressions were taken from the six youngest, fully expanded leaves for each treatment. Stomatal Density was expressed as stomatal density per cm^2 of primary leaf area.

2.5. Measurement of Stomatal Index

After 5 days of growth, estimation of Stomatal Index of primary leaves of mung bean seedlings of all the sets were carried out. Stomatal index (SI) was calculated as suggested by Sha Valli Khan *et al*. [17].

$$\text{Stomatal index} = \frac{\text{Total no of stomata per unit area}}{\text{no of stomata per unit area + no of epidermal cells per unit area}} \times 100$$

2.6. Statistical Analysis

The experiments were carried out in a randomized design (CRD) with 3 replicates; each replica comprising a single petridish containing an average of 20 seeds. All the experiments were analyzed statistically using ANOVA Table (and expressed as Critical Difference of 5%).

3. Result and Discussion

3.1. Effect of Sodium Arsenite on Germination Percentage and Seedling Length

From the present work, it is well established that Sodium arsenite, As (III), is very toxic for the growth of mung bean seedlings. It inhibited growth at very small concentrations such as 0.5µM. Roots were characteristically stubby and brittle and root tips gradually turned brown (Photo 1). In the present work, length of mung bean seedlings demonstrated an inverse relationship to the applied molar concentrations of sodium arsenite. Inhibition of elongation of mung bean seedlings started at a concentration of 0.5µM

and it was remarkably pronounced at 2µM (Table 1). The reduction in seedling length increased with higher doses of As (III), the effect being more pronounced on root than shoot. Present investigation reveals that Arsenite is more toxic for root growth than for shoot growth (Photo 1). Seedling Vigour Index was also found to be decreased with the increase in concentration of Sodium arsenite (Table 1). The above mentioned concentrations of Arsenite are comparable to field soil conditions and are environmentally relevant.

3.2. Effect of Sodium Arsenite on Fresh and Dry Weight and Primary Leaf Area

Treatment with sodium arsenite resulted in progressive decline of average fresh weight of 5-day old mung bean seedlings with increasing concentrations; decline being 25%, 62% and 79% with sodium arsenite due to 0.5µM, 1µM and 2µM concentrations in comparison to the control set. Dry weights of 5 day old seedlings also decreased with increasing concentrations of sodium arsenate; decline being 45%, 57% and 65% with sodium arsenite due to 0.5µM, 1µM and 2µM concentrations in comparison to the control set (Table 2).

A greater reduction of fresh weight relative to the dry weight of the germinated seedlings, induced by As toxicity, is a common observation as drastic water loss takes place as an immediate effect of most of the abiotic and biotic stresses imposed. It appears that marked dehydration of seedlings is one of the effects associated with growth inhibition. Reduction in fresh and dry weight due to As toxicity has also been reported in tomato plants [18], maize and bean plants [19]. With higher concentration of As(V) also, the water content of the mung bean seedlings declined [20].

The applied concentrations of sodium arsenite were found to have significant effect on the area of pair of primary leaves. With the increase in concentration of sodium arsenate, subsequent reduction in the area of first pair of primary leaves was noted. Reduction of leaf area with As(III) led to 35%, 53% and 67% reduction due to 0.5µM, 1µM and 2µM concentration in comparison to the control set (Table 2). Simultaneous reduction of leaf area of the stressed seedlings is naturally a consequence of inhibition of cell division and cell enlargement [21].

Photo 1. Effect of various concentrations of Sodium arsenite, As (III) on the growth of 5 day old mung bean seedlings

Table 1. Effect of various concentrations of Sodium arsenite, As(III) on germination percentage, seedling length and seedling vigour index of 5 day old mung bean seedlings. C.D. – Critical Difference

Treatment	Germination percentage (%)	Shoot length (cm)	Root length (cm)	Seedling length	Seedling Vigour Index
Control	100	12.5	4.5	17	1700
0.5µM	48	5.8	0.8	6.6	316.8
1µM	30	3.6	0.5	4.1	123
2µM	22	1.5	0.3	1.8	39.6
S.E. (mean)	2	0.31	0.1	0.4	2.4
C.D. (P=0.05)	2.8	0.46	0.2	0.51	3.5

Table 2. Effect of various concentrations of Sodium arsenite, As(III) on fresh and dry weight and primary leaf area of 5 day old Mung bean seedlings. C.D. – Critical Difference

Treatment	Fresh weight(g)	Inhibition %	Dry weight(g)	Inhibition %	Primary leaf area (sq.cm)	Inhibition %
Control	3.29	_	0.40	_	1.20	__
0.5µM	2.45	25	0.22	45	0.72	35
1µM	1.25	62	0.18	57	0.52	53
2µM	0.7	79	0.14	65	0.36	67
S.E.(mean)	0.02	_	0.02	_	0.22	_
C.D.(P=0.05)	0.28	_	0.030	_	0.76	_

3.3. Effect of Sodium Arsenite on Stomatal Density and Stomatal Index

Along with reduction in primary leaf area simultaneous decrease in total number of stomata or Stomatal density was also noticed in mung bean seedlings. Stomatal Index decreased with the increasing concentrations of Sodium arsenite (Table-3). Similar results were observed in mung bean seedlings under $CdSO_4$ treatment [22].

Table 3. Effect of various concentrations of Sodium arsenite, As(III) on Stomatal density and Stomatal Index of leaves of 5-day old mung bean seedlings. C.D. – Critical Difference

Treatment	Stomatal Density	Stomatal Index
Control	7	36.42
0.5µM	2	26.22
1µM	2	23.28
2µM	1	18.32
S.E.(mean)	0.21	1.18
C.D. (P=0.05)	0.38	3.40

4. Conclusions

Arsenite has an inhibitory, toxic effect on growth, metabolism and thereby, productivity of plants. Sodium arsenite toxicity led to reduced seed germination percentage and seedling vigour index. Arsenite also led to reduction in seedling length, especially, root development and fresh and dry weight of mung bean seedlings. The first formed primary leaf area was also reduced considerably and stomatal density and stomatal Index also decreased in respect to control. The concentration of Arsenic contaminated water in polluted sites often exceeds the concentration used in the present research work. This has practical importance and is of much concern in agricultural systems, as it hampers plants early development and leads to lesser productivity of crops.

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