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In vitro evaluation of arsenic accumulation and tolerance in some agricultural crops growing adjacent to the Ganga River

Dheeraj Pandey[1]*, Harbans Kaur Kehri[1], Ifra Zoomi[1], Shweta Chaturvedi[2], Kanhaiya Lal Chaudhary[1]

[1]Sadasivan Mycopathology Laboratory, Department of Botany, University of Allahabad, Prayagraj, India.
[2]K. Banerjee Center of Atmospheric and Ocean Studies, University of Allahabad, Prayagraj, India.

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ABSTRACT

The presence of arsenic in water is linked not only to health concerns, but also to the socio-economic conditions of a huge population in poor countries. The severity of As-poisoning might be accelerated by poor health and nutritional status. Many people suffer from pre-cancerous skin keratosis, Bowen's disease, and Arsenicosis, among other conditions. Long-term exposure can cause cancer. For *in vitro* screening of As tolerant plant, four plants viz., *Triticum aestivum*, *Lycopersicon esculentum*, *Solanum melongena*, and *Capsicum annuum*, were raised in As amended triple sterilized soil and sand mixture (1:1 ratio). *L. esculentum* and *S. melongena* could survive up to 100 ppm but extremely poor growth and biomass were recorded. The maximum tolerance was recorded in *T. aestivum* up to 150 ppm, whereas least survival was recorded for *C. annuum*.

1. INTRODUCTION

Arsenic (As) compounds are highly toxic and non-essential for the growth and development of living organisms. It has toxic effects on metabolic processes of plants, mitotic abnormalities, leaf chlorosis, growth inhibition, reduced photosynthesis, DNA mutations, and inhibition of enzyme activities. In general, plants growing in natural soil contain low level of As (<3.6 mg/kg) [1,2]. Arsenate is the dominant form in which arsenic is present in soil and its similarities with phosphate allow it to compete for the same uptake carriers in the root plasma lemma [3]. It interferes with the metabolic processes and inhibits plant growth and development through arsenic-induced phytotoxicity. The contamination of As in South Asian groundwater aquifers was first reported in the mid-1990s and since then a lot of work has been conducted for the last two decades [4]. The utilization of these groundwater sources for irrigation and drinking badly affected human beings, cattle and crops. Historically, As had been used as a drug to treat skin infection and beautification. It was also used as a homicidal drug and named as "king of poison" [5]. In the 20th century, useful applications were also started as wood preservation, rodents poisoning, and fungicides, etc. but in modern times it was noted as carcinogenic and toxic heavy material [6,7]. In the current situation, arsenic pollution happens globally, affecting more than twenty countries such as Argentina, Bangladesh, Chile, China, France, Germany, India, the Soviet Union, Peru, Namibia, Mexico, Sweden, and the United States. Southeast Asia and the plains of the Ganga-Meghna-Brahmaputra are heavily contaminated by As [8,9]. The presence of arsenic in water is linked not only to health concerns, but also to the socio-economic conditions of a huge population in poor countries. The severity of As-poisoning might be accelerated by poor health and nutritional status. Many people suffer from pre-cancerous skin keratosis, Bowen's disease, and Arsenicosis, among other conditions. Long-term exposure can cause cancer [10].

Metal tolerant plants have recently received great attention for the establishment of vegetation in heavy metal contaminated soils. Such plants have developed several mechanisms for the detoxification of heavy metals. Exploration of such plants and microbes for sustainable agriculture and improvement of degrading habitats is a new approach toward the modern steps. An agro-climate of India is rich in food grain as well as vegetable diversity and their production. In the present scenario population and rapid increased pollution provides more burden to produce much amount of food

*Corresponding Author
Dheeraj Pandey, Sadasivan Mycopathology Laboratory, Department of Botany, University of Allahabad, Prayagraj, India.
E-mail: dheerajpandey817@gmail.com

crops that should be safe and free from health hazards. In an era of rapidly urbanization when agricultural fields are reduced day by day. It is a great challenge to serve humankind and their health. Therefore, there is a need for the selection of more tolerant and resistant crops from traditional growing areas for the production of foodstuff in such contaminated areas. For the above study purpose in this experiment selection of four important crops from natural agricultural fields, i.e., *Triticum aestivum*, *Lycopersicon esculentum*, *Solanum melongena*, and *Capsicum annuum*.

Triticum aestivum (spring wheat), main cereal crop of India belongs to the family Poaceae (Monocot). Plant body is hexaploid [$2n = 6X = 42$, allopolyploid (ABD)] and well adapted to temperate climatic and needed 30–90 cm rainfall. Plant is monocotyledon anatomy with hollow node and solid internode, long sheathing leaves with parallel venation. An average wheat grain contains carbohydrate, protein, fat, minerals, fiber, thiamin, riboflavin, niacin, and rest of water. *Lycopersicon esculentum* is commonly known as tomato, which belongs to the family Solanaceae. The species originated traced central and South America. Tomato fruits are rich in fibers and a good source of vitamins such as A, K1, C, B2, folate, and other antioxidant compounds like beta-carotene, chlorogenic acid, lycopene, etc. *Solanum melongena* commonly known as eggplant belongs to family Solanaceae. Fruit is berry, globose to cylindrical in shape, and has many seeds and used as vegetables. Fruit contains many antioxidants, antimicrobial phytonutrients, phenolics, chlorogenic acid, nasunin (anthocyanin), etc. [11]. *Capsicum annuum* commonly known as chili belongs to family Solanaceae. It is a source of vitamins C (ascorbic acid), A and E, and other antioxidant compound. The pungent taste of the chili is due to the presence of capsaicin and dihydrocapsaicin occurs in quantities >80% with strong physiological and pharmacological properties [12]. In this experiment, raising these important field crops and vegetables in As-amended soil for the evaluation of their As-tolerance potential under greenhouse conditions.

2. MATERIAL AND METHODS

2.1. Preparation for Selection of As-Tolerant Plant Raised in Different As-Concentrations Under Greenhouse Conditions

In this screening experiment, four plants were selected, viz., *T. aestivum*, *S. melongena*, *L. esculentum*, and *C. annuum*. All the four plants were raised in triplicate in finely crushed proper sterilized normal soil mixed with $NaAsO_2$ compound in different concentrations such as 0, 25, 50, 100, 150, 300, 500 ppm. All plants were grown for 4–5 months under greenhouse conditions. Plants were also raised without any amendment/treatment served as control. At harvest period of each plant, data on biomass production, length, and As-accumulation in root/shoot were collected.

2.2. Seedlings Preparation and Transplantation

The seeds of *T. aestivum*, *L. esculentum*, *C. annuum*, and *S. melongena* were procured from the certified seed agencies. *T. aestivum* from Indian Agricultural Research Institute (IARI), New Delhi, *L. esculentum* from Indian Institute of Vegetable Research (IIVR) Varanasi and other two, *C. annuum* and *S. melongena* from the certified seed agency from Prayagraj, Uttar Pradesh, India. Seeds surface was sterilized in 4% NaOCl for 10 minutes and washed thoroughly with double distilled water several times. Surface sterilized seeds (40–50) were taken as ready seed samples for sowing and transferred to plastic tray (45 × 32 × 8 cm dimension with small holes at four corners) containing autoclaved coarse sand. Seedlings were watered at regular intervals of time and half strength of Hoagland's nutrient solution was given weekly [13]. Healthy seedlings of same size (25 days old) were transplanted to the pots of respective series.

2.3. Preparation of Synthetic Mode of Substrate

Soil (substrate) was collected from the Roxburg Botanical Garden, Department of Botany, University of Allahabad, for experimental purposes. Properly crushed, dried, and three times sterilized soil was used for screening experiment. In the screening experiment, the substrate was amended with inorganic arsenic, e.g., sodium arsenite ($NaAsO_2$). The experimental soil was not heavy metal contaminated before, viz., 0.13 mg/kg Zn, 0.003 mg/kg Cd, 3.02 mg/kg of Cu, 2.31 mg/kg of Ni, 0.26 mg/kg Cr, and (As) was not detectible. In experiment, the soil substrate was artificially As-amended 25 to 500 ppm/kg soil in all experimental series.

2.4. Length and Biomass of Plant

Lengths of root and shoot of plant were recorded at harvest. For dry weight (DW), the plant's samples were oven-dried at 80ºC for 48 hours.

2.5. Arsenic Accumulations

Arsenic content in soil sample analyzed by high performance, double-focusing magnetic sector field Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) (S.A.I.F. Laboratory, IIT Mumbai). Aerosol of sample is used for desolvated and ionized at very high temperature (6,000°C–10,000°C) into the core of the inductively coupled argon plasma. These ions are separated and collected by mass to charge ratio. The sewage-contaminated Ganga water sample was analyzed by Atomic Absorption Spectrometer (ICE 3000 Series, Model 3500 AAS, Thermo Scientific, UK) from C.I.L., Botany Department, University of Allahabad.

2.6. Statistical Analysis

The parameters were presented as the means of three replicates. A single-way analysis of variance (ANOVA) was used to assess all of the data gathered (ANOVA). Duncan's multiple range test of homogeneity was used to separate the means at the $p = 0.05$ level. The statistical study was carried out using Microsoft 365 Excel and SPSS 16.0.

3. RESULTS

3.1. Rate of Mortality

Effect of Sodium arsenite ($NaAsO_2$) (in different concentrations such as 0, 25, 50, 100, 150, 300, and 500 ppm) on the rate of mortality of *T. aestivum, S. melongena*, *L. esculentum*, and *C. annuum* under pot conditions have been presented in Tables 1

Table 1: Evaluation of As-tolerance of different plants raised with different As-concentrations (ppm).

Plants	Arsenic concentrations (ppm)						
	0	25	50	100	150	300	500
Triticum aestivum L.	+	+	+	+	+	−	−
Lycopersicon esculentum Mill.	+	+	+	+	−	−	−
Solanum melongena L.	+	+	+	+	−	−	−
Capsicum annuum L.	+	+	−	−	−	−	−

and 2. The rate of mortality ranged from 0% to 100% and complete mortality was recorded in all the four plant species in series where the soil was amended with 300 and 500 ppm of $NaAsO_2$ compound. At 150 ppm, only *T. aestivum* could survive but showed 60% mortality of the plants. At 100 ppm, 100% survival of *T. aestivum* plants was recorded while 66.67% mortality was recorded in *L. esculentum*, 33.3% in *S. melongena*, and 100% in *C. annuum*. At 25 and 50 ppm, all the plant species showed 100% survival but for *C. annuum*, it was recorded only survive up to 25 ppm of concentration.

3.2. Root/Shoot Length

Effect of Sodium arsenite ($NaAsO_2$) (in different concentrations on the root/shoot length of *T. aestivum*, *S. melongena*, *L. esculentum*, and *C. annuum*) under pot conditions has been presented in Tables 3 and 4. Minimum root length was recorded in 150 ppm series in *T. aestivum* (66.35% decrease in root and 69.73% in shoot). In comparison to control (where the soil was without amendment of Sodium arsenite), all other series showed a significant decrease in the root length of root/shoot; however, the magnitude of decrease varied with the As-concentration and crops. In comparison to other three crops, *T. aestivum* always showed improvement in root/shoot length irrespective of the As-concentration. At 150 ppm, only *T. aestivum* survived but the performance was very poor, hence minimum root/shoot length was recorded in these plants. Out of four crops, only three crops survived at 100 ppm of As-concentration series and *S. melongena* was severely affected, while *T. aestivum* showed tolerance and better root/shoot length.

3.3. Fresh/DW of Plants

Effect of Sodium arsenite ($NaAsO_2$) on the fresh/DW of plants of *T. aestivum*, *S. melongena*, *L. esculentum*, and *C. annuum* under pot conditions have been presented in (Tables 5 and 6**)**. The fresh weight (FW) of the plants varied from 14.17 to 152.93 g in control series, whereas 8.32 to 49.67 g in 100 ppm series. The maximum

Table 2: Mortality of the plant raised in different As-concentrations (ppm).

Plants	Mortality percent of plants under different Arsenic concentrations (ppm)						
	0 ppm	25 ppm	50 ppm	100 ppm	150 ppm	300 ppm	500 ppm
Triticum aestivum L.	0	0	0	0	60	100	100
Lycopersicon esculentum Mill.	0	0	0	66.67	100	100	100
Solanum melongena L.	0	0	0	33.33	100	100	100
Capsicum annuum L.	0	66.67	100	100	100	100	100

Table 3: Root length of plants raised with different As-concentrations (ppm).

Plant	Root length of plant* (cm plant^{-1})						
	0 ppm	25 ppm	50 ppm	100 ppm	150 ppm	300 ppm	500 ppm
Triticum aestivum L.	26.07 ± 3.57^{b}	25 ± 5.03^{b}	24.0 ± 3.6^{b}	17.267 ± 2.35^{b}	8.95 ± 3.6^{a}	-	-
Lycopersicon esculentum Mill.	22.17 ± 2.75^{ab}	21.0 ± 4.4^{ab}	20.67 ± 1.5^{b}	10.17 ± 1.26^{a}	-	-	-
Solanum melongena L.	24.17 ± 4.31^{ab}	23.67 ± 2.5^{ab}	20.0 ± 3.0^{b}	9.3 ± 2.79^{a}	-	-	-
Capsicum annuum L.	19.13 ± 2.95^{a}	15.33 ± 4.9^{a}	-	-	-	-	-

*Means ± standard deviation with same letter in the column are not significantly different at $p = 0.05$ ($n = 3$).

Table 4: Shoot length of plants raised in different As-concentrations (ppm).

Plant	Shoot length of plant* (cm plant^{-1})						
	0 ppm	25 ppm	50 ppm	100 ppm	150 ppm	300 ppm	500 ppm
Triticum aestivum L.	56.17 ± 3.88^{c}	55.33 ± 4.16^{b}	53.33 ± 7.5^{b}	38.93 ± 2.75^{c}	17.5 ± 4.9^{a}	-	-
Lycopersicon esculentum Mill.	41.20 ± 3.96^{b}	31.33 ± 4.04^{a}	28.33 ± 4.9^{a}	23.00 ± 1.41^{b}	-	-	-
Solanum melongena L.	51.50 ± 2.66^{c}	51.33 ± 3.6^{b}	39.0 ± 3.0^{ab}	19.57 ± 1.65^{a}	-	-	-
Capsicum annuum L.	30.18 ± 3.37^{a}	25.67 ± 67^{a}	-	-	-	-	-

*Means ± standard deviation with same letter in the column are not significantly different at $p = 0.05$ ($n = 3$).

Table 5: FW of plants raised in different As-concentrations (ppm).

Plant	FW of plant* (g plant^{-1})						
	0 ppm	25 ppm	50 ppm	100 ppm	150 ppm	300 ppm	500 ppm
Triticum aestivum L.	14.17 ± 2.82^a	14.10 ± 2.85^a	13.51 ± 2.3^a	8.32 ± 0.61^a	4.33 ± 4.0^a	-	-
Lycopersicon esculentum Mill.	117.22 ± 4.45^c	115.33 ± 3.5^c	83.0 ± 4.35^b	35.93 ± 1.2^b	-	-	-
Solanum melongena L.	152.93 ± 4.76^d	149.67 ± 4.7^d	80.0 ± 5.0^b	49.67 ± 2.93^c	-	-	-
Capsicum annuum L.	108.93 ± 4.13^b	76.80 ± 8.6^b	-	-	-	-	-

*Means ± standard deviation with same letter in the column are not significantly different at $p = 0.05$ ($n = 3$).

Table 6: DW of plants raised in different As-concentrations (ppm).

Plant	FW of plant* (g plant^{-1})						
	0 ppm	25 ppm	50 ppm	100 ppm	150 ppm	300ppm	500 ppm
Triticum aestivum L.	2.90 ± 0.29^a	2.84 ± 0.41^a	2.56 ± 0.22^a	1.49 ± 0.28^a	0.62 ± 0.6	-	-
Lycopersicon esculentum Mill.	12.30 ± 1.02^b	11.53 ± 1.03^b	9.07 ± 1.21^b	6.38 ± 0.81^b	-	-	-
Solanum melongena L.	16.40 ± 2.30^c	15.47 ± 3.13^c	9.41 ± 1.07^b	8.30 ± 0.89^c	-	-	-
Capsicum annuum L.	11.77 ± 0.55^b	8.63 ± 1.27^b	-	-	-	-	-

*Means ± standard deviation with same letter in the column are not significantly different at $p = 0.05$ ($n = 3$).

Table 7: As-accumulation in root and shoot of the plants raised with different As-concentrations (ppm).

Plant	As accumulation* (ppm)	
	Root (100 ppm series)	Shoot (100 ppm series)
Triticum aestivum L.	31.868 ± 1.74^a	23.938 ± 1.0^a
Lycopersicon esculentum Mill.	45.177 ± 1.13^b	35.410 ± 1.15^b
Solanum melongena L.	47.830 ± 1.01^c	38.800 ± 0.45^c
Capsicum annuum L.	-	-

*Means ± standard deviation with same letter in the column are not significantly different at $p = 0.05$ ($n = 3$).

FW of plants in treated series was recorded in *S. melongena* (152.93 g) and minimum in *T. aestivum* (8.32 g). The DW of plant varies from 2.90 to 16.40 g in control, whereas 1.49 to 8.30 g in 100 ppm series. The maximum DW of treated series was recorded in *S. melongena* (8.30 g) (49.39% decrease over control) and minimum in *T. aestivum* (1.49 g) with 48.62% decrease over control at 100 ppm. The variation in plant FW and DW among the four plants were significant at $p \leq 0.05$.

3.4. As-Accumulation

Arsenic was not detected in the plants of the control series. As accumulation in the roots of plants varies from 47.83 to 31.868 ppm. The maximum As-accumulation in root was in *S. melongena* (47.83), followed by *L. esculentum* (45.177 ppm) and minimum in *T. aestivum* (31.868). As accumulation in root of *T. aestivum* was significantly different (at $p \leq 0.05$) and lowest from other three plants. The level of As in shoot of screened plant data was represented in Table 7. As concentration in shoot of plants ranged from 38.80 to 23.938 ppm. The maximum As-accumulation was recorded in *S. melongena* (38.80), followed by *L. esculentum* (35.41) and minimum in *T. aestivum* (23.938). There is no data of *C. annuum* available up to 100 ppm because of not survivable up to this concentration. As accumulation in the shoots of all plants was significantly different (at $p \leq 0.05$).

4. DISCUSSIONS

This experiment idea was taken from the western bank of the Ganga river from Prayagraj, India where agricultural fields are available, on which variety of crops were grown. Because this natural site was contaminated with a moderate amount of arsenic (6.67 ppm in soil and 0.0032 ppm in water) as well as sewage water, similar crops, and vegetables were chosen for the study which were sowing there. For *in vitro* screening of As tolerant plant, four plants, viz., *T. aestivum*, *L. esculentum*, *S. melongena*, *C. annuum*, raised in As amended triple sterilized soil and sand mixture (1:1 ratio). *L. esculentum* and *S. melongena* could survive up to 100 ppm but extremely poor growth and biomass were recorded. The maximum tolerance was recorded in *T. aestivum* up to 150 ppm (Fig. 1). Root, shoot length, and biomass of all the four plants show that the minimum reduction in length was recorded in *T. aestivum* (root 33.77% and shoot 30.7%), while maximum reduction recorded in *S. melongena*, root (61.52%) and shoot (62%) at 100 ppm concentration over

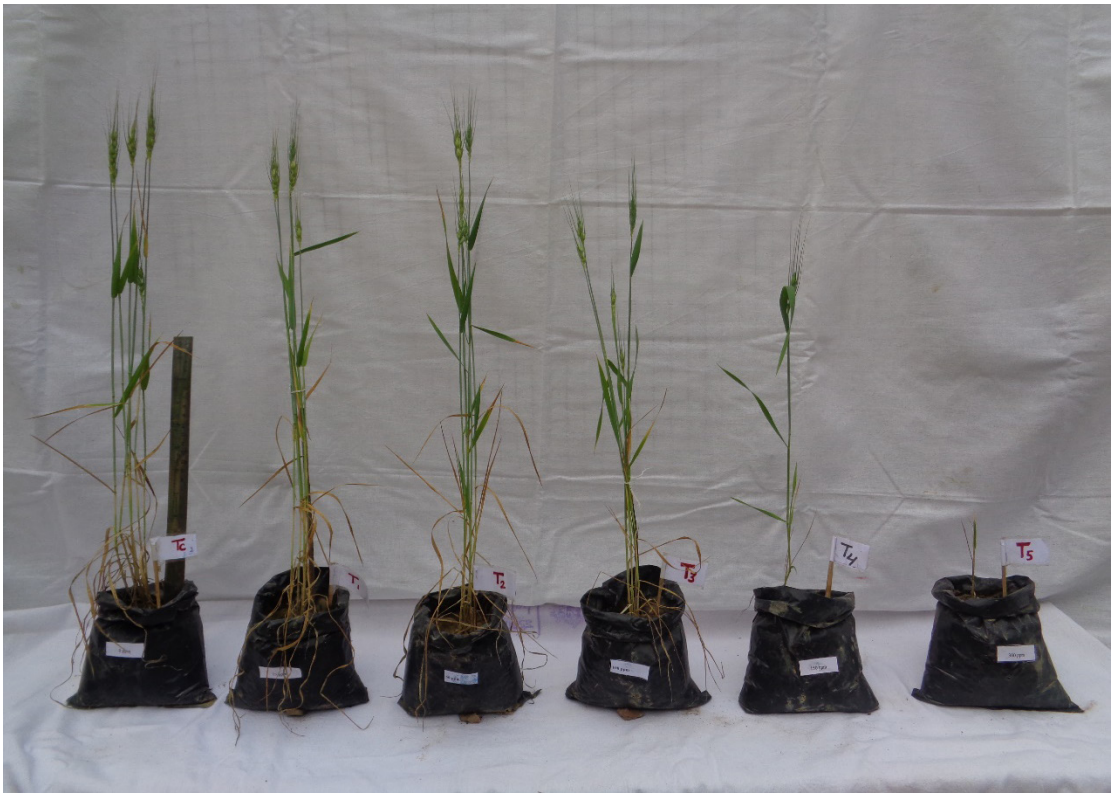

Figure 1: Photograph showing *T. aestivum* growing in different As-amended series (0, 25, 50, 100, 150, and 300 ppm) in greenhouse conditions.

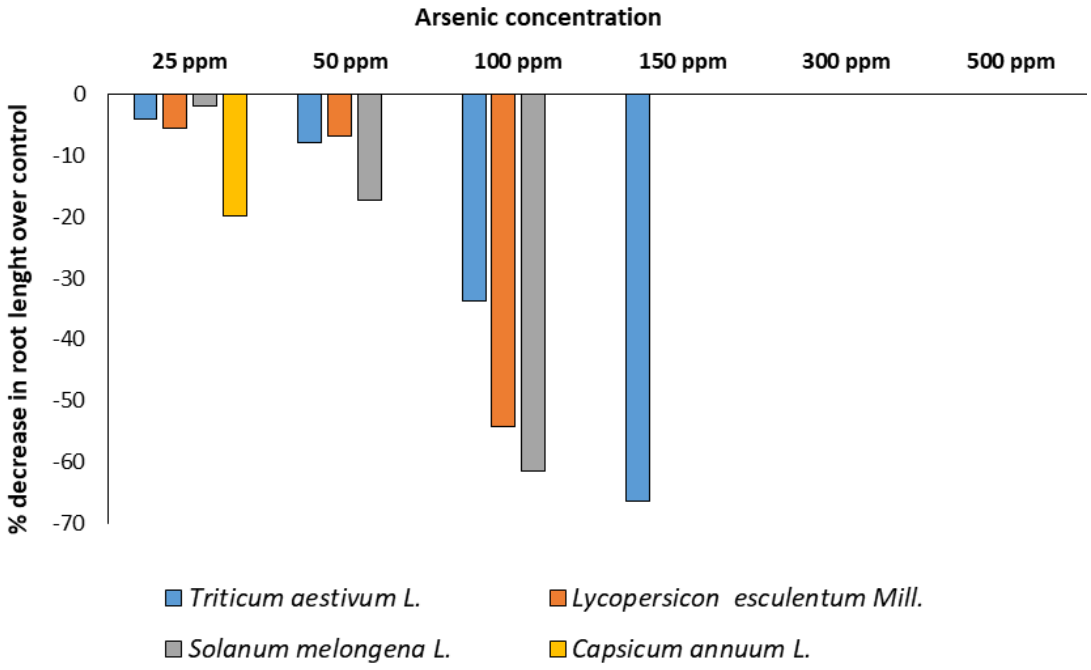

Figure 2: Column diagram showing percent decrease over control in root length of grown plants under various As-concentrations.

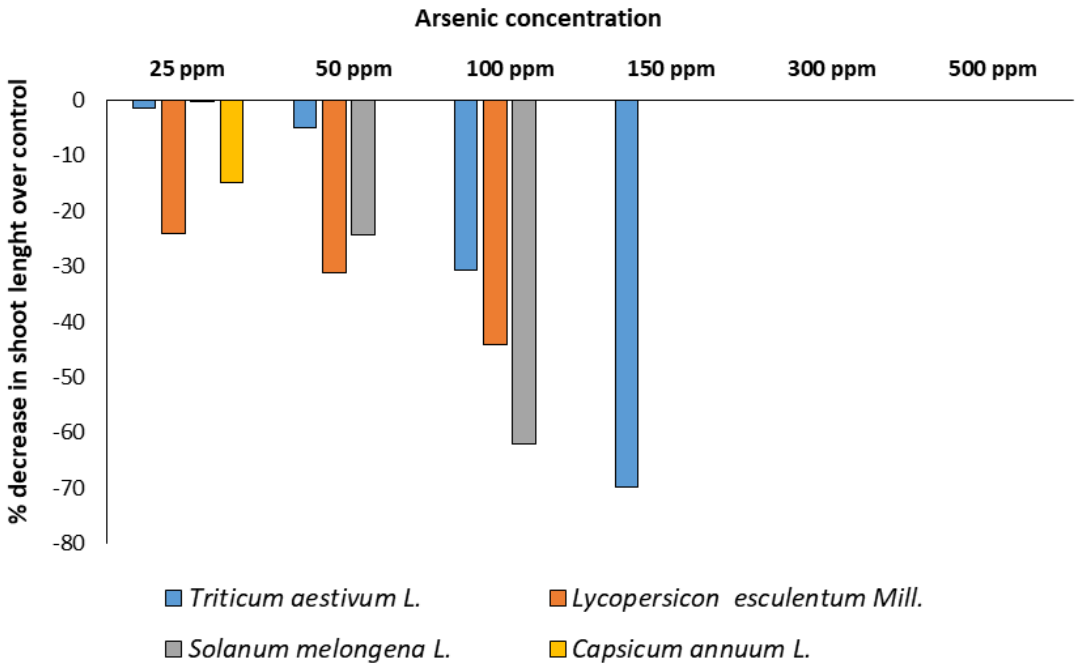

Figure 3: Column diagram showing percent decrease over control in shoot length of grown plants under various As-concentrations.

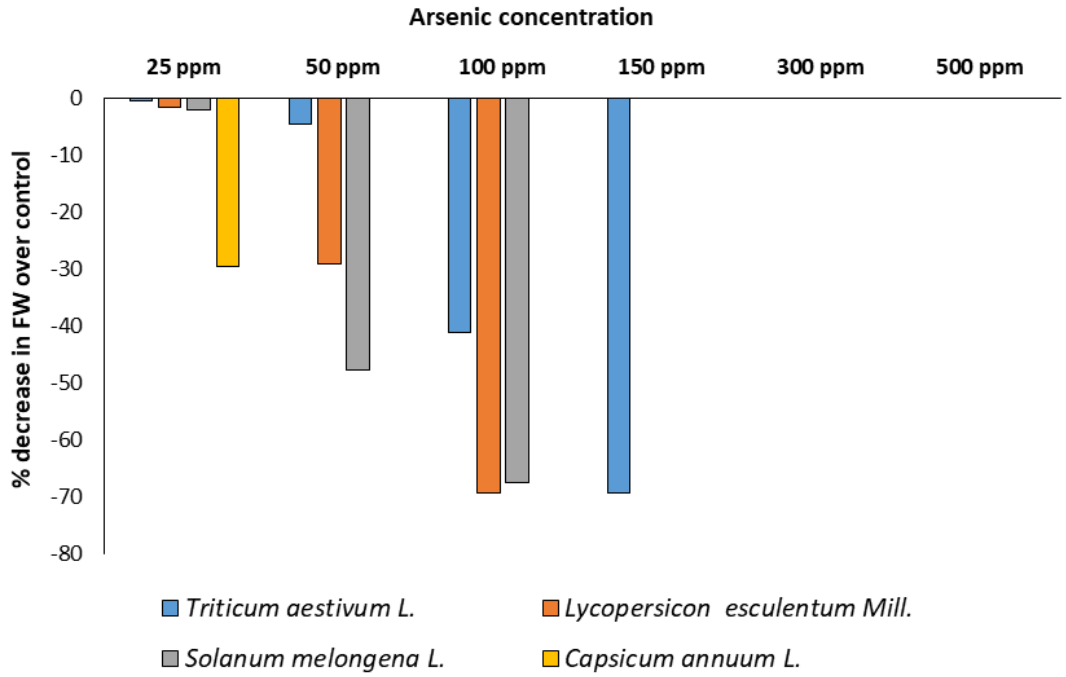

Figure 4: Column diagram showing percent decrease over control in FW of grown plants under various As-concentrations.

control (Figs. 2 and 3). Fresh and DW reduction also showed a similar trend for *T. aestivum* (58.72% FW) and (51.38% DW) at 100 ppm concentration series (Figs. 4 and 5). The minimum As-accumulation was recorded in shoot of *T. aestivum*. Overall, roots showed more As-accumulation than shoot and maximum As-content was recorded in root of *S. melongena* which survived at 100 ppm. *T. aestivum* was only found had for less amount of As-translocation (66.74%) from root to shoot parts.

As toxicity is well known for plants and As-tolerant plants adopt some mechanisms such as accumulation, phytostabilization, phytovolatilization, and compartmentation and the translocation

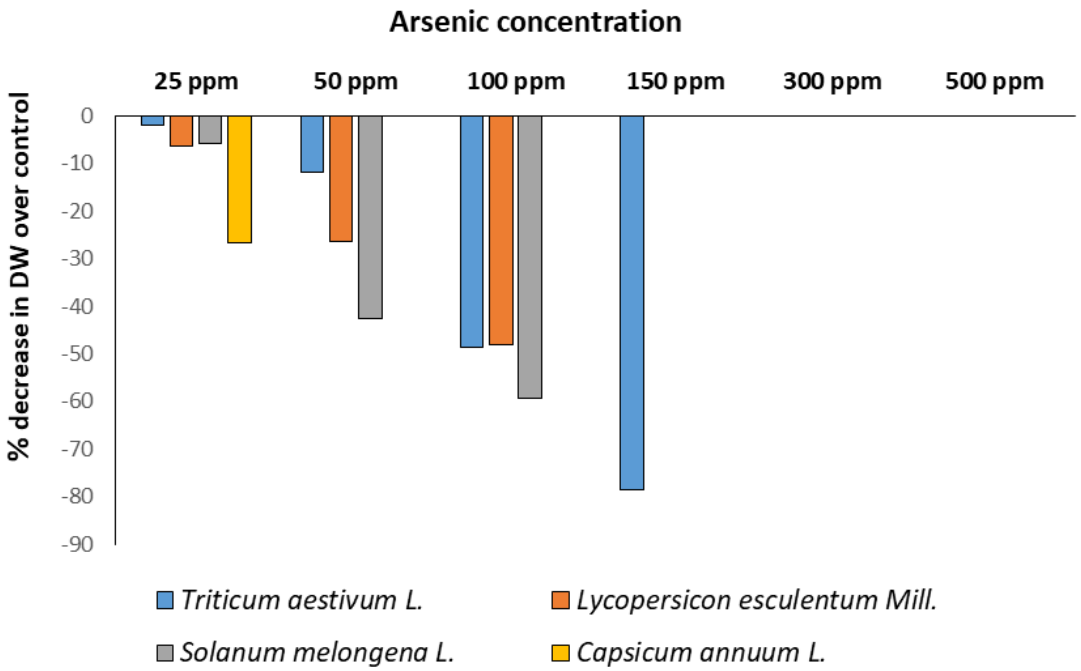

Figure 5: Column diagram showing percent decrease over control in DW of grown plants under various As-concentrations.

of As [14,15]. Uptake of As is known to obstruct various physiological and biochemical metabolisms, inhibited plant growth and biomass accumulation [16,17]. It may be the possible reason for the reduction in plant growth. Several studies reported that As-concentration is generally greater in root than shoot when plants grow in As- contaminated medium/soil [18,19]. *T. aestivum* showed maximum tolerance against As-stress, possibly adopting because of certain mechanisms such as phosphorus (P) accumulation. The presence of "P" in root decreases As (V) influx rate [20]. Phytostabilization and compartmentalization of As in roots help in the reduction of As toxicity, whereas root exudes help in the stimulation of microbe. [21,22]. Earlier, Liu et al. [23] reported that 0–60 mg/kg of As-concentration may not affect wheat and started declining when soil As concentration is more than 80 mg/kg. Similar studies also reported by Carbonell-Barrachina et al. [18] and Quanji et al. [24]. The plant may reduce the mobility of As into groundwater and also check it to reach other parts of plant. These tolerant plant root exudates stimulate microbial activity for the stabilization of heavy metals (immobilizing forms) in soil [25,26]. Phosphate and arsenate both aquaporin transport channels are provided path to As entry in plant system [27–31]. Miteva [32] reported similar results in *L. esculentum* grown in artificially As amended soil and noted the 50–100 mg/kg As concentration which leads to a decrease in growth parameters and plant health and he concluded that 25 mg/kg As amendment was threshold value. Wheat is one of the most important cereals in the world as well as in Indian subcontinents. A large population of humans and chattels is dependent on it for food and fodder. Therefore, the production of safe grains and fodder in As-contaminated areas is very necessary to feed a large population. In this prospective, this study provides a light for other several studies in this field. However, for the establishment of this fact, more research studies are also needed.

5. CONCLUSIONS

The maximum As-tolerant plant, *T. aestivum* showed maximum tolerance (150 ppm) and less As-translocation from root to shoot than the other three plants. This study opens the door of field trial experiments in such As-contaminated fields as well as the possibility of further research into the under-soil rhizospheric mechanism and microbial interactions that could aid in the remediation of As-toxic effects from growing plants.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

8. AUTHOR CONTRIBUTION

All authors contributed significantly to participate in the drafting of the paper or critically revised it for key intellectual content material and agreed to submit to the present journal.

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