

PROBING THE PHYTOTOXIC EFFECTS OF ARSENIC ON GROWTH AND PHOTOSYNTHESIS OF *SOLANUM LYCOPERSICUM* L.

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Being ubiquitous in nature arsenic (As) plays significant role as environmental pollutant and phytotoxic element which adversely affects growth and development of plants. In the present study, the phytotoxic effects of As on the growth of tomato plants (*Solanum lycopersicum* L.) was investigated with optimized 5 ppm As treatment and found reduction in number of leaves, plant height, root length, number of lateral roots and fresh and dry mass of tomato plants. Results indicate that reduction in various growth parameters could possibly due to high As accumulation in plant tissues as they may have to utilise their energy to minimize the effects of high As concentration. Adverse effects of As treatment on the normal physiological functions of the plants was assessed in terms of photosynthetic activities. Results clearly show that arsenic exposure severely affected the growth and photosynthetic machinery of plants resulting into structural and functional damage leading to death of tomato plants.

Keywords: Arsenic, Growth, Photosynthesis, Productivity, Phytotoxicity

Arsenicals were used as pesticides, fungicides, herbicides and soil sterilizers from the late nineteenth century to middle the twentieth century (Lepp 1981). Arsenic (As) is ubiquitous in nature and plays significant role in environmental pollution. Because of its solubility, mobility, bioavailability and toxicity which depend on its oxidation state, As is proved to be a phytotoxic metalloid (Masscheleyn *et al.* 1991). As phytotoxicity is influenced more by its oxidation state as trivalent form (arsenite) more phytotoxic than the pentavalent form (arsenate) while both are much more phytotoxic than their organic forms (Woolson *et al.* 1971). At low concentration As appears not to be involved in specific metabolic reactions. As has been reported to interfere with metabolic processes and inhibit plant growth and development sometimes leading to death (Marine *et al.* 1993). Uptake of phosphate and arsenate is same so the supply of phosphate to plants may be compromised (Meharg *et al.* 1994) if high concentration of As in the soil solution is found. Inside plants, As can affect growth, development and productivity due to structural and functional alteration (Chandrakar *et al.* 2016, Singh *et al.* 2017, Begum *et al.* 2016, Anjum *et al.* 2017, Srivastava *et al.* 2017). As at the sub-cellular

level shows production of the most dangerous biochemical effect in the form of the reactive oxygen species (ROS) such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) (Rafiq *et al.* 2017, Rafiq *et al.* 2017, Shahid *et al.* 2017). Previous research have indicated that As in trace amount shows stimulatory effect on plant growth, but high concentrations exerts harmful effect and may begin to outweigh beneficial ones (Azam *et al.* 2015). As exposure has also been shown to suppress the number of leaves, leaf area, number of roots, plant height and fresh and dry weight of plants (Nath *et al.* 2014). As is widely reported to inhibit photosynthetic process of plants (Nagajyoti *et al.* 2010, Gusman *et al.* 2013). After As uptake, light harvesting apparatus of plants can be affected with a reduction in chlorophyll concentration and activity of photosystem- II (Anjum *et al.* 2011). A remarkable inhibition of chlorophyll pigment synthesis was reported due to shortage of the adaptive adjustments of photosystem-I and -II as a result of high As concentration. As caused degradation of chloroplast membrane and disorganised the function of fundamental photosynthesis process (Rafiq *et al.* 2017, Pandey *et al.* 2015). Thus present study was carried out to probe the phytotoxic effect of As

on various growth parameters and photosynthesis of tomato plants.

MATERIALS AND METHODS

Plant material and growth conditions :

Tomato (*Solanum lycopersicum* L.) is a well known model vegetable plant to study plant-stress interactions. It belongs to Solanaceae family of Angiosperm. In present research, the tomato seeds were obtained from Indian Institute of Vegetable Research, Varanasi for proposed experiments. Tomato plants were grown in controlled green house condition of 14 h light and 10 h dark at $27 \pm 0.5^\circ\text{C}$.

Hydroponic culture development: Firstly, tomato seeds were germinated in Petri dishes containing moist blotting paper in a growth chamber under controlled condition. Tomato seedlings were then placed in a container of 2 L capacity having Knop's medium prepared by 6 mmol L⁻¹ KNO₃, 4 mmol L⁻¹ KH₂PO₄, 500 µmol L⁻¹ MgSO₄.7H₂O, 500 µmol L⁻¹ Na₃C₆H₅O₇.2H₂O, 100 µmol L⁻¹ FeCl₃, 1 mmol L⁻¹ CaCl₂.2H₂O, 50 µmol L⁻¹ H₃BO₃, 10 µmol L⁻¹ MnCl₂.4H₂O, 0.9 µmol L⁻¹ ZnSO₄.7H₂O, 2 µmol L⁻¹ NaMoO₄.2H₂O, 0.4 µmol L⁻¹ CuSO₄.5H₂O, and 0.2 µmol L⁻¹ CoCl₂.6H₂O at pH 5.5-5.8 of the solution which was adjusted using 1N NaOH and 1N HCL. After 3-4 weeks, the tomato plants were ready to use for As treatment to assess expected outcomes.

As treatment to tomato plants: Treatment of As was given directly to the roots of hydroponically grown tomato plants in triplicate manner. Different As concentrations were prepared by using NaAsO₂ (sodium arsenite) for the present study. Tomato plants were treated with various concentrations of arsenic (1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm and 7 ppm). Tomato plants without arsenic treatment were considered as control. To assess the phytotoxic effect of As the plants were harvested at 0, 12, 24, 36, 48, 72 and 96 h after treatment and cut at the root-shoot junction.

The roots and shoots were washed, weighed, and dried at 65 °C to a constant weight for estimation of As accumulation.

Plant growth and As accumulation : The phytotoxic effect of As on the growth of tomato plants was assessed. Various growth parameters, such as plant height, root length, fresh weight, dry weight, number of lateral roots and number of leaves of tomato plants exposed to 5 ppm As for 96 h were measured in triplicates and repeated two times. Fresh weight (FW) and root length and shoots were measured after 96 h of As treatment, whereas dry weight (DW) was determined after drying the samples to a constant weight at 65°C. The dried plant samples (roots and shoots) were then ground into fine powder using mortar and pestle. Addition of 5 ml conc. HNO₃ was done to 0.25 g of dried sample in a 50 ml digestion glass tube and left overnight at room temperature. The digestion tubes in a heating block at 150°C for proper digestion. Tubes were allowed to cool after 1h of digestion and 2 ml 30% H₂O₂ were added to them. The content was swirled and, heated again for 2 h at 150°C, and then allowed to cool. Finally the solution was diluted with distilled water up to 50 ml and its upper clear layer was separated for As estimation by Atomic Absorption Spectrometer (AAnalyst 800, Perkin-Elmer; Singapore). The As content accumulated in the plant parts was expressed as mg g⁻¹ DW.

Measurement of cell death after As treatment: The measurement of As-induced cell death was assessed by Evans blue staining which was used as cell death marker. The cell death was measured in terms of Evans blue uptake after 96 h of treatment with various concentrations of As. Leaves infiltrated with sterile distilled water were served as control.

Evans blue staining to assess cell death : The Evans blue staining to assess As-induced cell death was performed by gentle heating the treated and control leaves for 1 min in a freshly prepared solution of phenol, lactic acid,

glycerol and distilled water (1:1:1:1) containing 20 mg/ml Evans blue. The leaf tissues were then clarified overnight in a fresh solution of 2.5 g/l chloral hydrate on a platform shaker with 160 rpm at 27°C. The leaf tissues were then mounted on glass slides and observed under the microscope to assess the amount of cell death in treated as well as control leaf tissues.

Evans blue assay for cell death : Amount of cell death in tomato leaves induced by As treatment was estimated according to the methodology of Baker and Mock (1994). Firstly the treated and control leaf tissues were placed in beakers containing 1 ml of 0.25% Evans blue solution and put on a platform shaker with 80 rpm at 27°C for 20 min. The contents of the beaker were poured into a small buchner funnel and the tissues were rinsed well by deionized water until no more blue stain was eluted. The contents were then transferred to 1.5 ml microfuge tubes. One half ml of 1% aqueous sodium dodecyl sulphate (SDS) was added in each tube to release the trapped Evans blue from the cells. The tissues were then ground finely by using a pestle-mortar and the homogenate was diluted with 0.5 ml deionized water. Finally the tubes were centrifuged at 9000 g for 3 min. and supernatant was removed in aliquots. Optical density was measured spectrophotometrically at 600 nm.

Phytotoxic effect of As on photosynthetic machinery: The following methods were used to assess the phytotoxic effect of As on the photosynthetic machinery of tomato plant.

Determination of photosynthetic pigment contents: Total chlorophyll content was measured as per the method of Arnon (1949). In this method 100 mg leaves were crushed in 10 ml chilled solution of 80% acetone. The content was then centrifuged at 2000g for 10 min. Absorbance of the supernatant was estimated spectrophotometrically at 645 and 663 nm (UV-VIS Spectrophotometer 2202, Systronics, India). Total chlorophyll content was expressed

in terms of mg g^{-1} of fresh weight. Carotenoid content was determined according to the method of Duxbury and Yentsch (1956). For the estimation of carotenoids, absorbance of the supernatant was measured spectrophotometrically at 480 and 510 nm. Carotenoid content was expressed as mg g^{-1} of fresh weight of tissue.

Assay of photosynthesis: Net photosynthesis rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate were recorded by using Photosynthesis System LI-6400XT (LI-COR Biosciences, USA).

Statistical analyses: Experimental design was completely randomized. All the experiments were carried out in triplicates and were repeated two times. The mean, standard error, and one way analysis of variance (ANOVA) were calculated using the average data of one experiment. To analyze significance of the factors studied, ANOVA was calculated at 5% probability level according to the method described by Gomez and Gomez (1984). The mean separations were carried out using Duncan's multiple range tests (Duncan 1955) and significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

As-induced cell death in tomato root: After 96 h of As treatment significant amount of cell death was observed which was increased with increasing concentration of As (Fig. 1). It was observed that maximum cell death was induced by 5 ppm of As concentration as assessed by Evans blue staining method. At 7 ppm concentration As showed similar amount of cell death. Hence, the 5 ppm concentration of As was selected for further experiments to assess its phytotoxicity in tomato plant parts. Similar phytotoxic concentration of As was used by Mishra, Jha, and Dubey (2011) to induce oxidative stress and modulation of antioxidant defence system in rice seedlings (Mishra and Dubey 2006). Cao *et al.* (2004) also reported that greater As concentration in

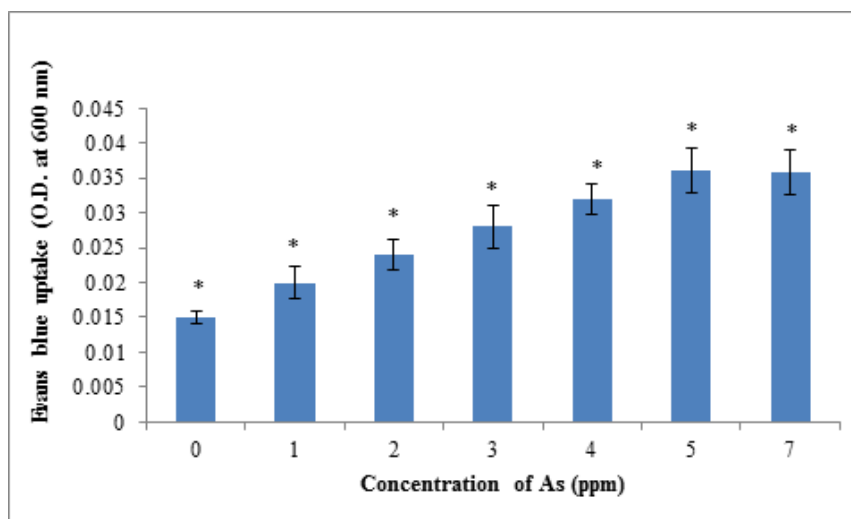


Figure 1: Cell death in the leaves of tomato plant treated with various concentrations of As after 96 h (O.D., Optical density. Bars represent \pm standard error. * indicates that values are significantly different ($p < 0.05$).

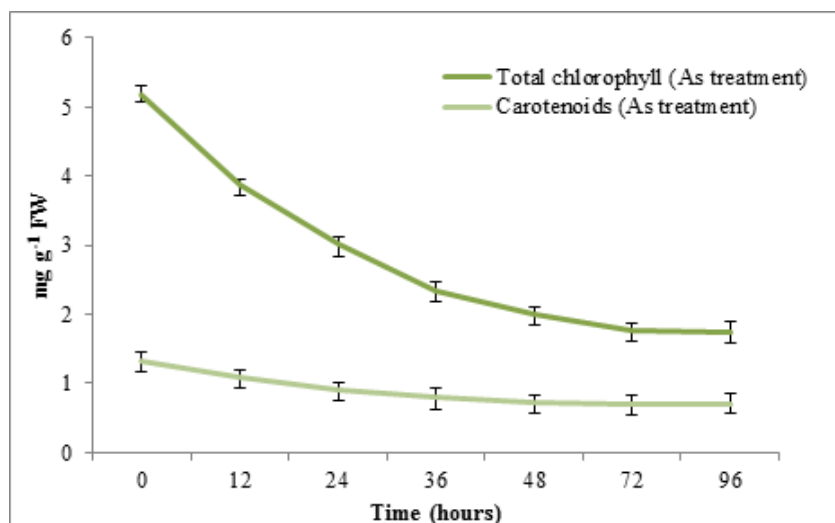


Figure 2: Effect of arsenic (5 ppm) treatment on photosynthetic pigment contents of tomato leaves. (Bars represent \pm standard error.)

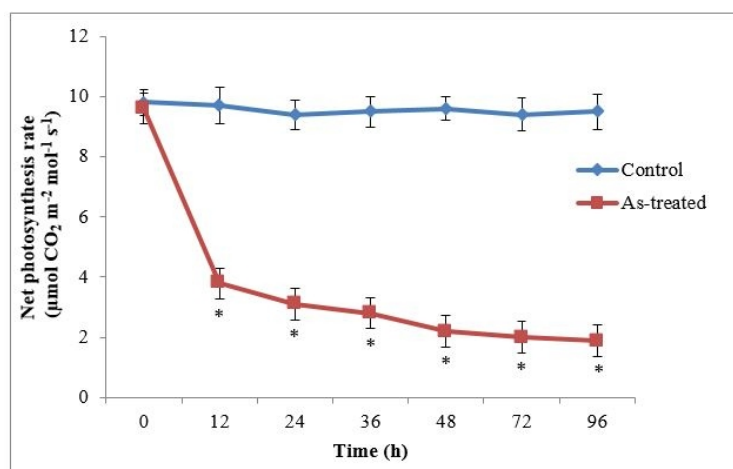


Figure 3: Effect of arsenic (5 ppm) treatment on net photosynthesis rate. (Bars represent \pm standard error. * significant ($p < 0.05$) compared to control.)

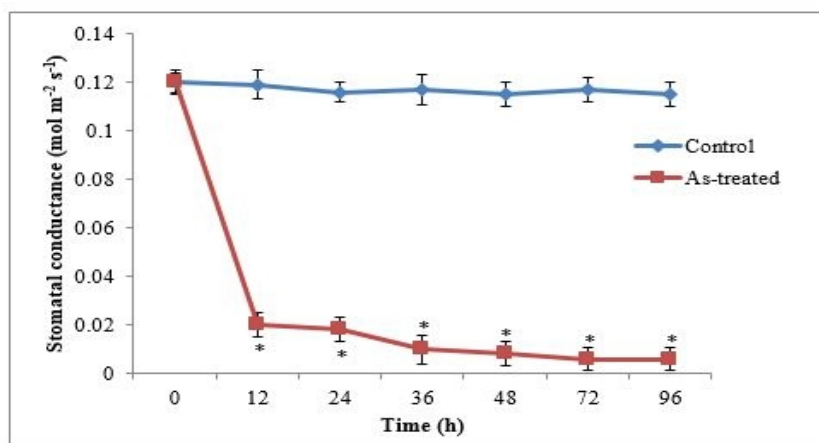


Figure 4: Effect of arsenic (5 ppm) treatment on stomatal conductance. (Bars represent \pm standard error. * significant ($p < 0.05$) compared to control.)

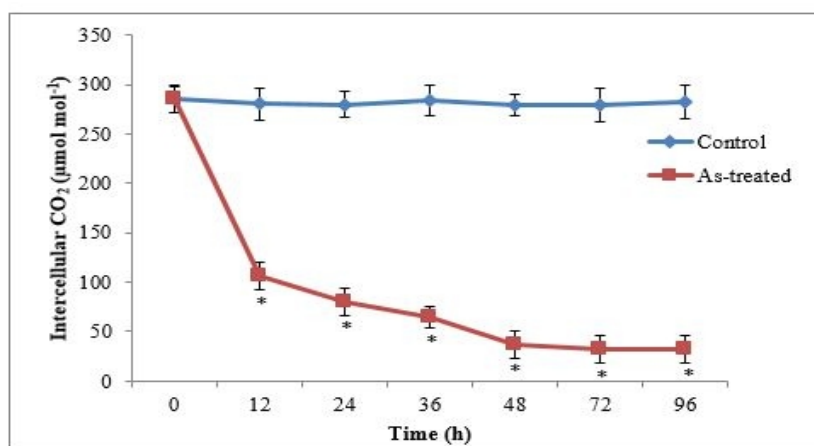


Figure 5: Effect of arsenic (5 ppm) treatment on intercellular CO_2 concentration. (Bars represent \pm standard error. * significant ($p < 0.05$) compared to control)

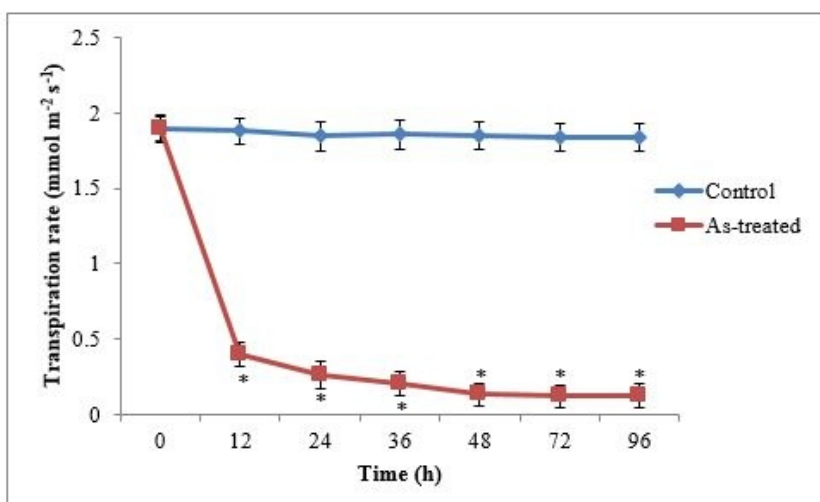


Figure 6: Effect of arsenic (5 ppm) treatment on transpiration rate. (Bars represent \pm standard error. * significant ($p < 0.05$) compared to control.)

Plant growth and arsenic accumulation: The phytotoxic effect of As on various growth parameters of tomato plants was investigated and found that plant height, fresh weight, dry weight, main root length and number of lateral roots of tomato plants treated with 5 ppm As concentration were decreased significantly after 96 h in comparison to control. As treatment caused significant ($p < 0.05$) reduction in the growth of root and shoot as measured in terms of length and biomass. The root and shoot accumulated 301.2 and 411.3 $\mu\text{g As/g DW}$, respectively, as observed after 96 h of As treatment (Table 1). It was observed that shoot accumulated more As than root, showing movement of considerable amount of As to shoot from root (Cao *et al.* 2004). In the present study, reduction in various growth parameters after As treatment have revealed that plant tissues may have to use more energy to cope with the high As accumulation in plant parts. (Greger 1999).

Effect on Photosynthetic Mibachinery

Chlorophyll and carotenoid contents of leave:

As has the potential to damage the photosynthetic pigment apparatus. Total chlorophyll and carotenoids contents were decreased with increasing time accompanied by pale green coloration of leaves after As treatment (Fig. 2). Reduction in pigment contents is due to oxidative damage in the leaf tissues after As treatment (Halloin *et al.* 1970, Wilhelmoiva *et al.* 2005).

Net photosynthesis rate: As exerted negative effect on photosynthesis rate of tomato leaves. It was observed that fast fall of net photosynthesis rate during 8-12 h, persisting decline during 12-48 h and almost the minimum reached at 72 h after treatment of As in comparison to control (Fig. 3).

Stomatal conductance: Reduction of the stomatal conductance was observed after As treatment in time-dependent manner. Sharp fall of the stomatal conductance up to 12 h, slow fall during 12-48 h and minimum at 72 h were seen after As treatment in comparison to control (Fig. 4).

Intercellular CO_2 concentration: Fall trend of intercellular CO_2 concentration in tomato

Table 1. Effect of As on growth parameters of tomato plants after 96 h of treatment.

Growth parameters	Control	As treatment (5 ppm)
Root length (cm)	8.2 \pm 0.11	*7.5 \pm 0.01
Plant height (cm)	15.3 \pm 0.21	*14.1 \pm 0.23
Root fresh weight (g plant ⁻¹)	0.14 \pm 0.02	0.12 \pm 0.01
Shoot fresh weight (g plant ⁻¹)	1.85 \pm 0.18	1.69 \pm 0.02
Root dry weight(mg plant ⁻¹)	13.5 \pm 0.32	*11.2 \pm 0.21
Shoot dry weight(mg plant ⁻¹)	70.1 \pm 0.33	*57.1 \pm 0.41
No. of lateral roots	17.0 \pm 0.51	*14.0 \pm 0.32
As content in roots ($\mu\text{g As g}^{-1}$ dry weight)	—————	*301.2 \pm 1.15
As content in shoots ($\mu\text{g As g}^{-1}$ dry weight)	—————	*411.3 \pm 1.27

Data are means \pm SE (n=3). * significant ($p < 0.05$) compared to control.

leaves was obtained during prolonged duration (0-48 h) of As treatment in comparison to control. Steep decline at early hours and smooth decline at later hours were observed (Fig. 5).

Transpiration rate: As treatment exerted negative effect on transpiration rate of tomato leaves. Dramatically fall on 12 h in treatment and then slower decline in 12-48 h were observed in comparison to control. Minimum transpiration rate was at 72 h after As treatment (Fig. 6).

The results of present study showed severe suppression of photosynthesis in leaves after As treatment. This damage was caused by the decrease in the photosynthetic pigment contents and by reduction of photosynthetic activities. Destruction of photosynthetic machinery is presumably due to the development of oxidative stress along with membrane damage in leaf tissues after As exposure (Halloin *et al.* 1970, Wilhelmova *et al.* 2005).

CONCLUSION

As is considered as a non-essential element for plants. As can adversely affect growth and development due to a plethora of morphological, physiological and biochemical alterations inside plants. As exposure causes reduction in plant growth and photosynthesis of tomato plants. The observations on certain growth parameters of tomato plant have revealed that As dose (5 ppm) proved toxic, causing significant reduction in the growth of the plants. This reduction could possibly be related to high As accumulation in plant tissues as they may have to use energy to cope with the high As concentration in the plant tissues. Shoots accumulated more As than the roots, implying that a considerable amount of As was translocated to the aerial plant parts. As has an adverse effect on the normal physiological functions of the plant which was assessed in terms of photosynthetic activities. As exposure has suppressive effect on the photosynthetic

machinery of tomato plants. Photosynthetic machinery of the plant is severely damaged after As treatment indicating their phytotoxicity. Thus, results of the present study clearly indicate that As has negative effects on tomato plants.

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