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EFFECT OF VARIED LEVELS OF ARSENIC TOXICITY ON CERTAIN PHYSIO – CHEMICAL AND BIOCHEMICAL PARAMETERS IN WHEAT GENOTYPES (*Triticum aestivum* L.)

Vedant Patil[1], Md. Afjal Ahmad[2], K. Dujeshwer[3] and Manju Jat[4]

ABSTRACT

Arsenic (As) is a toxic metalloid classified as a group 1 carcinogen. The presence of arsenic in high concentrations in soil and irrigation water results into high arsenic accumulation in food grains posing a threat to the health of millions of people worldwide. The main reason for arsenic contamination is the biogeochemical weathering of rocks and the release of bound arsenic into groundwater. Human interventions through intensive agricultural practices and excessive groundwater consumption have contributed greatly to the prevailing arensec contamination. An experiment was carried out in the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, BHU, Varanasi during *rabi* season of 2022 to find the effect of different arsenic concentrations on physio-chemical and biochemical traits of wheat genotypes. The four wheat genotypes used in this experiment were Sonalika, HUW-234, HUW-669, and DBW-187 along with four different concentrations of sodium arsenate (source of arsenic toxicity) i.e.T1–50 µM, T2–100 µM, T3-150 µM, and T4-200 µM. Each genotype experienced a decline in performance for physiological parameters like Metal tolerance index except root toxicity and shoot toxicity and for biochemical parameters like á - Amylase activity, total protein content, and total soluble sugar content when the concentration of arsenic increased.

However, HUW-234 and Sonalika showed a substantial decline in each parameter, although DBW-187 and HUW-669 genotypes did not. The wheat genotypes DBW-187 and HUW-669 were shown to be more tolerant to the harmful effects of arsenic than HUW-234 and Sonalika in all treatments. The experimental outcomes suggested that arsenic toxicity significantly affected the physio-chemical and biochemical parameters in wheat and in such case the productivity and quality can also be adversely affected. Hence, arsenic tolerant wheat varieties (*viz*., DBW-187 and HUW-669) under high arsenic concentration can be recommended or such arsenic infected irrigation water should be minimized to a safe level.

(Key words: Wheat, heavy metal stress, arsenic, α– amylase activity)

INTRODUCTION

Arsenic (As), a naturally occurring metalloid, is widely present as an environmental contaminant and enters the food chain mainly from contaminated water and several widely consumed foodstuffs. Seafood has been identified as the main source of organic arsenic (e.g., arsenobetaine and arsenosugars) and is believed to be non-toxic (Taylor *et al.*, 2017). Arsenic (As) contamination is an important environmental problem due to its worldwide distribution and its high toxicity to all organisms. Presence and mobilization of arsenic occur due to natural biogeochemical reactions, but anthropogenic activities like fossil combustion, mining and the use of pesticides and herbicides containing arsenic, increases the concentration and mobilization of arsenic in the environment. This metalloid can occur in soil and water predominantly in its inorganic forms, such as arsenate (AsV) and arsenite (AsIII). Arsenic can enter plant cells by channels of essential elements and cause adverse effects in several metabolic processes, resulting in reduced germination, growth and yield of some crops (Sharma *et al.*, 2021). Arsenic adversely affects the growth and development of plants resulting in various biochemical and physiological disorders such as growth

1. P. G. Student, Dept. of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, BHU, Varanasi, Uttar Pradesh, 221005
2. Asstt. Professor, Dept. of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, BHU, Varanasi, Uttar Pradesh, 221005
3. Asstt.t Professor, Dept. of Rural Development & Agricultural Production, North Eastern Hill University, Tura campus, Meghalaya, India- 794002
4. Res. Scholar, Dept. of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, BHU, Varanasi, Uttar Pradesh, 221005

inhibition, disruption of photosynthesis and respiratory system of plant (Garg and Singla, 2011). Morphologically excess arsenic causes stunted growth, leaf chlorosis and necrosis and reduction in leaf area. At the cellular level, arsenic induces oxidative stress, evidenced by enhanced lipid peroxidation, H_2O_2 production and ion leakage. Arsenic is easily taken up by roots and transported to other parts of the plant, being toxic to living cells at very low concentrations (Finnegan and Chen, 2012). These features make arsenic a serious problem as the arsenic-enriched plants can be transferred to the food chain. Presence of arsenic in irrigation water affects plant metabolism and leads to various physiological and structural disorders. Recently, it is becoming more apparent that the consumption of cereal crops, seafood, fruits, and vegetables is the second potential source of arsenic exposure to humans (Suman *et al.,* 2020). Several physio – chemical and biochemical factors in crop plants have been found to alter as a result of arsenic toxicity, significantly reducing the crop plant's potential production. Keeping above fact, an experiment was carried out to observe the different levels of arsenic toxicity on physio-chemical and biochemical parameters in wheat genotype in Indo- Gangetic plains of Uttar Pradesh, India.

MATERIALS AND METHODS

The experiment was conducted in the Laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University during *rabi* season of 2022. Healthy seeds of wheat genotypes i.e., HUW-669, HUW-234 and DBW-187 were collected from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University and Sonalika genotype was procured from Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University. Different concentrations of sodium arsenate ($Na_2HAsO_4.7H_2O$) were used to induce the heavy metal stress to selected wheat genotypes. Sodium arsenate was dissolved in double distilled water and four different concentrations i.e., T1 - 50 µM, T2 - 100 µM, T3 - 150 µM, T4 - 200 µM of salts were prepared. Fresh, clean, air-dried Petri plates (10 cm diameter) were taken and filter paper according to base size was carved out and placed. The filter paper discs were spiked with different treatments of sodium arsenate solution. Each petri plate contained 10 seeds. 15 Petri plates of each variety *viz*., Sonalika, HUW-669, HUW-234, and DBW-187 were prepared in replicated form. All physio – chemical and biochemical parameters were measured following standard protocols. á – amylase activity, total protein content and total soluble sugar content was analyzed by Bernfeld (1955), Bradford protein assay and Nelson's and Somogyi's reagent method (1944), respectively.

Estimation of α – Amylase activity [µM maltose min^{-1} g^{-1} Fresh weight (FW)]was done with Bernfeld (1955) method to measure the enzyme's activity in wheat endosperm.There were three replications of each treatment, each containing 100 mg of endosperm. Then, endosperm was removed from the embryo section and 100 mg of it was crushed with mortar - pestle in 5 ml of 0.02 M phosphate buffer (pH 6.9). This mixture was then centrifuged at 5000 rpm for 10 minutes to obtain a supernatant i.e. enzyme extract. The volume of supernatant was finally raised up to 10 ml by adding a phosphate buffer. 1 ml of the substrate i.e. 1 % starch solution prepared in 0.02 M phosphate buffer, and 1 ml of the enzyme extract made up the assay mixture. The mixture was heated in a water bath for 5 min after it was cooled in running tap water. The volume was finally raised up to 25 ml by adding distilled water. Then absorbance was taken at 540 nm in the spectrophotometer. The known concentration of maltose used to prepare the standard curve for á - amylase activity. An absorbance measurement was performed, and the activity was measured in terms of µM maltose released g^{-1} FW min^{-1}.

The sample used to estimate the amount of protein content was collected seven days after germination. Each treatment contained three replications that were being examined. 200 mg of plant samples were grind with the help of 5 ml extraction buffer (0.2 M Tris – HCl), into the mortar – pestle which were kept into the ice bucket. Then fine slurry transferred into the centrifuged tubes followed by centrifugation for 5 min at 10,000 rpm. Supernatants were collected in fresh test tubes. 1 ml of this supernatant was mixed with 3 ml of diluted Bradford dye (Prepared by addition of 100 mg of coomassie brilliant blue G 250 in 50 ml of 95 % ethanol and 100 ml of orthophosphoric acid) in different test tubes for each treatment. The mixture was then incubated for 20 minutes. Absorbance was taken at 595 nm in the spectrophotometer. Solution for standard curve was prepared with the help of Bovine Serum Albumin (BSA). Absorbance values were plotted against concentration to get the total protein content measured in mg g^{-1} FW.

Sample was taken for sugar estimation on the seventh day after germination. For each treatment, three replications were examined.200 mg of sample was taken and grinded in 10 ml of 80 % ethanol followed by centrifugation at 4000 rpm for 20 minutes. Supernatant was collected and residue was re-extracted with 10 ml of 80 % ethanol and again centrifuged at 4000 rpm for 20 minutes. Final volume of samples was made up to 10 ml in each test tube. Nelson's reagent or Arsenomolybdate colour reagent and Somogyi's reagents or Copper – carbonate tartrate was made by following standard protocols. Determination of reducing sugar was done by mixing of 1 ml of sugar extract to 1ml of distilled water followed by addition of 1 ml Somogyi's reagent. This mixture was then heated in a water bath for 12 minutes followed by cooling in tap water. After cooling 1 ml of Nelson's reagent was added and final volume was made up to 10 ml. Then absorbance was taken at 530 nm in spectrophotometer. The standard curve was prepared by plotting the absorbance value on Y – axis against the concentration of the sugar in solution on X – axis and sugar content was measured.

Metal Tolerance Index (MTI) was calculated at the end of 7thday after germination and the formula used to calculate was given by Tumar and Marshal (1972).

$$MTI = \frac{\text{Radicle length in seed (T)}}{\text{Radicle length in seed (C)}} \text{ X } 100$$

Where T denotes treatment and C denotes control or distilled water.

The root and shoot phytotoxicity was calculated on the 7thday after germination using the following formula given by Chou *et al.* (1976)

$$\text{Root toxicity} = \frac{\text{Radicle length in T0} - \text{Radicle length in treatment}}{\text{Radicle length T0}} \times 100$$

$$\text{Shoot toxicity} = \frac{\text{Shoot length in T0} - \text{Shoot length in treatment}}{\text{Shoot length T0}} \times 100$$

Data were analyzed using SPSS v26.0. The experimental design used for the experiment was Factorial Completely Randomized Design (FCRD). Least significant difference (LSD) of 5 per cent was accepted for significance to compare the treatments means. All the significant treatments and their interaction were compared using critical difference (CD) values, and significant treatments were marked with Duncan's multiple range test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

The harmful effect of arsenic salt on the wheat crop may also be determined by looking at biochemical characteristics. The alpha amylase activity was measured by the amount of maltose released minute^{-1} and among the genotypes, Sonalika showed maximum reduction in á - amylase activity (Figure 1). But drastic change was recorded at 200 ìM concentration, at which á - amylase activity was reduced by 57.11 per cent. However, similar but less impact of arsenic was seen in DBW-187 genotypes. Maximum á - amylase activity was recorded in control condition i.e. 616.05 ìM maltose min1 g^{-1} FW, and minimum per cent reduction was seen at 200 ìM, at which á - amylase activity was reduced by 35.49 per cent which was less similar to other genotypes. Zhang *et al.* (2016) showed that, over the whole concentration range, alpha-amylase activity declined as arsenite or arsenate concentrations increased in wheat.

Among biochemical parameters, there was a decrease in total protein content due to arsenic treatment as shown in Figure 4. The least total protein content was recorded in control condition in all genotypes. Effect of arsenic on total protein content was more prominent in the Sonalika genotype. At the concentration of 200 ìM, total protein content was recorded 5.8 mg g^{-1} FW, However DBW-187 did not show steep decrease, least total protein content was recorded at 200 ìM concentration which was 7.46 mg g^{-1} FW. Protein content in organisms is an essential indication of reversible and irreversible metabolic changes, and it has been shown to respond to a wide range of stressors, including natural and xenobiotic stresses (Pandey and Gupta, 2011). Several researchers contributed to the study of the impact of heavy metals on plant protein content. It was significantly reduced in cereal wheat when the amount of heavy metal was increased. Heavy metal interactions with functional -SH groups have been postulated as a method of inhibition for a variety of physiological events, including protein and chlorophyll production. Because the enzyme is thought to be restricting overall nitrate, a fall in protein level might be the result of a decrease in nitrate reductase activity, impacting total protein and plant development. As exposure adversely affects plants at biochemical and molecular levels, and influences a majority of physiological responses, such as inhibition in overall growth processes, photosynthetic efficiency, and biomass accumulation. Arsenic can induce oxidative stress via the enhanced production and/or inefficient elimination of ROS and consequently damage lipids, proteins, and nucleic acids, and interferes with various metabolic pathways, either directly, as competitive inhibitors of Pi, or indirectly, by interfering with activities of certain key enzymes (Ghosh and Biswas, 2017; Abbas *et al.,*2018).

In the experiment, when wheat genotypes were treated with sodium arsenate salt. There was a reduced trend observed in total soluble sugar content. The most drastic effect was seen in the Sonalika genotype (Fig. 3). In which at the concentration of 200 ì M sodium arsenate, total soluble sugar content was reduced by 71.57 per cent. However, remaining varieties had the same trend. DBW-187 at the concentration of 200 µM recorded decrease in total soluble sugar content by 65.98 per cent. Which elaborates that at any concentration DBW-187 was more resistant towards arsenic salt in comparison to other genotypes. Some metals toxicity may be so high that plant development is slowed before considerable amounts of the element can be translocated. It's also clear that, especially in the case of acute metal stress, many processes may be at work at the same time. Similar type of negative impact of arsenic toxicity on soluble sugar content was documented by Sil *et al.* (2019).

The data regarding effect of arsenic concentrations on metal tolerance index (MTI) in four genotypes *viz.,* Sonalika, HUW-234, HUW-669, DBW-187 are presented in Figure 2. There was a higher tolerance index observed in HUW-669 than other genotypes in all levels of concentration. Highest MTI was seen at 50 ìM concentration i.e., 84.59 per cent in case of HUW-669. However, the MTI was decreased as concentration of arsenic increases in each stage of treatment. MTI was recorded 74.4, 61.17 and 45.98 per cent at 100 ìM, 150 ìM and 200 ìM concentrations of sodium arsenate respectively. Similarly, DBW-187 and HUW-234 genotypes showed significant reduction in MTI. As arsenic concentration rises, the tolerance index of *Triticum aestivum* decreases. The reduction MTI was well-linked with declined shoot and root growth and biomass that might have been consequences of irregularity in normal physiological mechanisms of wheat seedling with increasing metal doses(Akhtar and Shoaib, 2014).

Several authors have reported on the detrimental effects of heavy metal on biological systems. Root and shoot

phytotoxicity was not severe in all genotypes analyzed, including Sonalika, HUW-669, HUW-234, and DBW-187, up to a dose of 50 ìM sodium arsenate, but above this, root and shoot toxicity was observed to be greater than 50 per cent in the experiment (Table 1). Sonalika genotype showed more phytotoxicity than other genotypes for the heavy metal arsenic (Patil and Ahmad, 2022).However, no steep increase was observed in HUW-669 genotype. When the arsenic concentration was increased to 50 ìM, 100 ìM, 150 ìM and 200 ìM, then root and shoot toxicity was increased by 8.9, 16.49, 34.01 and 43.06 per cent. respectively. Which indicates that HUW-669 was more tolerant against different concentrations of Arsenic in comparison to other genotypes. Li *et al.* (2007) showed that phytotoxicity of the shoot and root was reduced at lower concentrations (0.5 mg l^{-1}) and increased at higher concentrations (20 l^{-1}) of arsenic, which was similar to our findings.

The results of the afore- mentioned study suggest that the heavy metal arsenic significantly affected the physio–chemical as well as biochemical parameters in wheat genotypes. With increasing arsenic toxicity, α - amylase activity, total protein content, total soluble sugar content and metal tolerance index decreased, whereas root toxicity and shoot toxicity increased significantly in every genotype studied. Among all genotypes (Sonalika, HUW-234, DBW-187 and HUW-669) it has been observed that DBW-187 genotype performed better in parameter like á – amylase activity, Total protein content and total soluble sugar content in comparison to other varieties whereas, HUW-669 genotype was found to be safer towards arsenic toxicity in terms of parameter like metal tolerance index, root toxicity and shoot toxicity at each level of treatment. Based on the results of experiment, it may be concluded that DBW-187 and HUW-669 varieties of wheat are more tolerant to heavy metal arsenic in comparison to other genotypes.

Table1. Impact of different arsenic concentrations on root and shoot toxicity (%) in wheat

Treatments	Root toxicity (%)	Shoot toxicity (%)
Varieties		
V_1: Sonalika	59.3c	51.9c
V_2: HUW234	48.7b	45.3b
V_3: HUW669	33.5a	25.6a
V_4: DBW187	35.1a	26.9a
SEm±	**1.81**	**1.23**
CD (P=0.05)	**5.43**	**3.69**
Toxicity levels (Sodium arsenate)		
T_1 : 50 µM	21.3d	16.1d
T_{-2} : 100 µM	36.4c	31.8c
T_{-3} : 150 µM	53.6b	44.9b
T_4 : 200 µM	65.2a	57.0a
SEm±	**2.42**	**1.35**
CD (P≤0.05)	**7.26**	**4.05**
Interaction (P≤0.05)	**13.03**	**8.21**

Values with the same letter differ non significantly ($p > 0.05$). Different letters for each parameter show a significant difference ($p < 0.05$) under Duncan's multiple range test, NS= Not significant

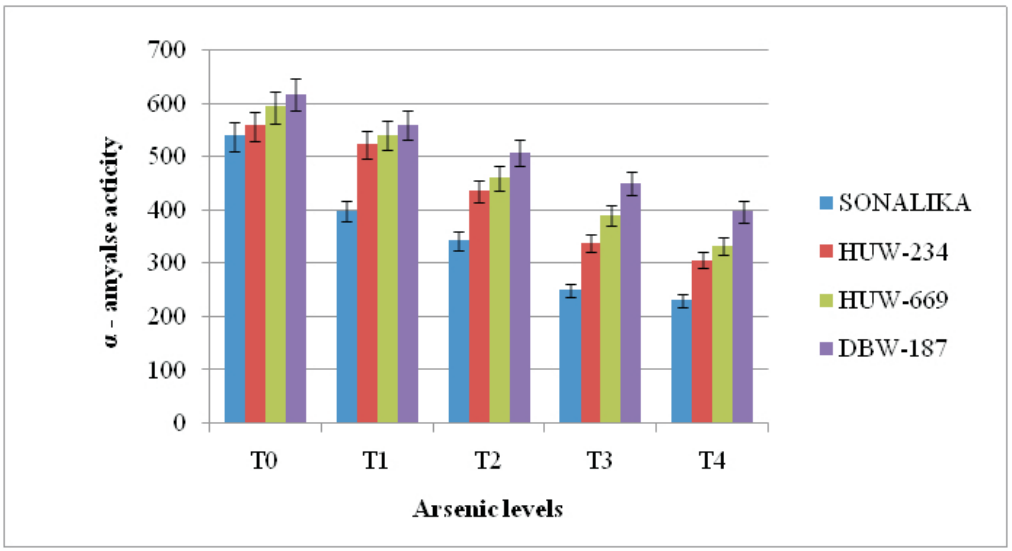

Figure 1. Impact of different arsenic concentrations on á – amylase activity (µM maltose min^{-1} g^{-1} FW), Arsenic levels (T0 – Control/Distilled water, T_1 – 50 µ M, T_2 – 100 µ M, T_3 – 150 µ M, T_4 – 200 µM)

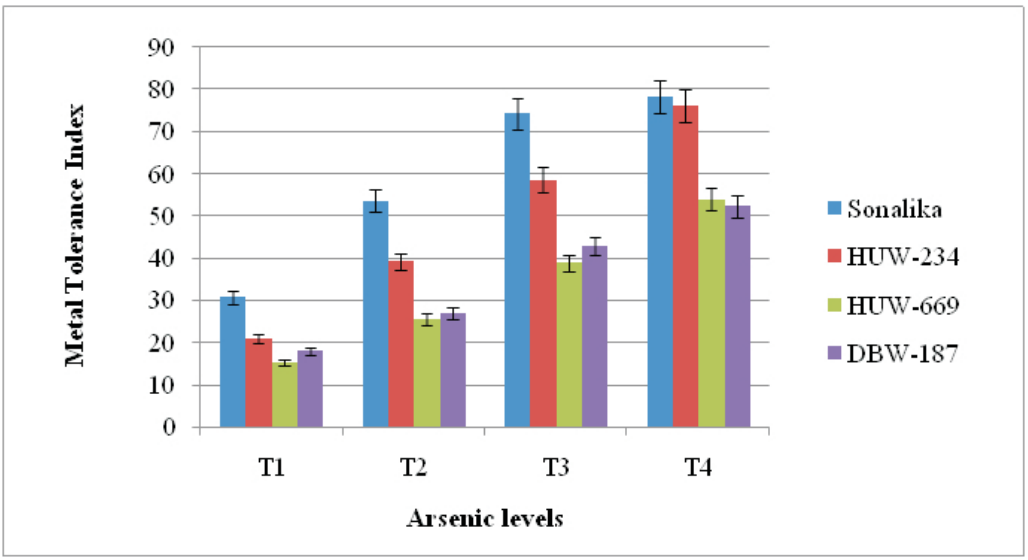

Figure 2. Impact of different arsenic concentrations on Metal tolerance index (%) in wheat genotypes, Arsenic levels (T0 – Control/Distilled water, T_1 – 50 µ M, T_2 – 100 µM, T_3 – 150 µM, T_4 – 200 µM)

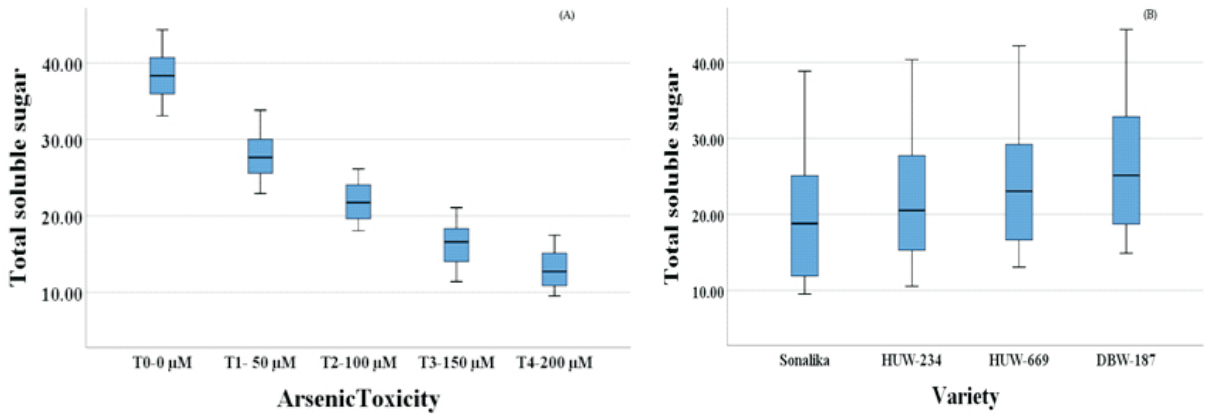

Figure 3. Box plot for total soluble sugar content (mg g^{-1} fresh weight): A) Total soluble sugar by arsenic levels. B) Total soluble sugar by varieties

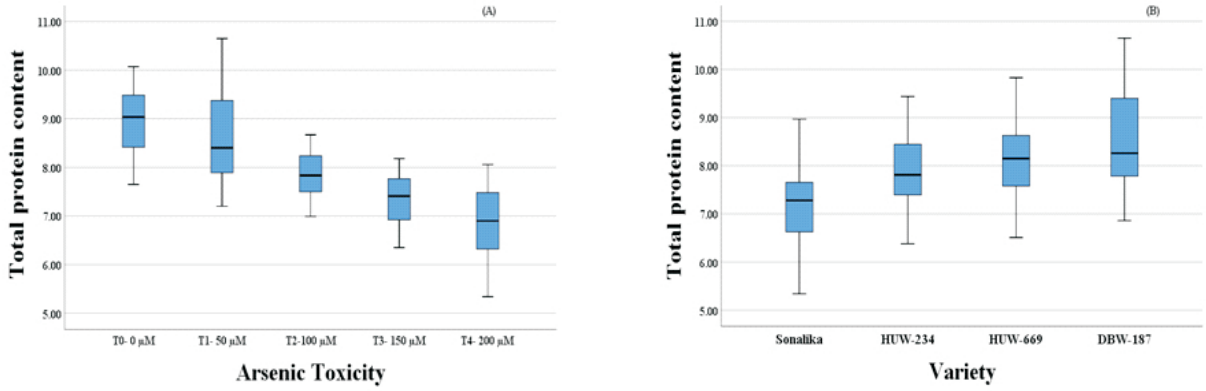

Figure 4. Box plot for total protein content (mg g^{-1} fresh weight): A) Total protein content by arsenic levels. B) Total protein content by varieties

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