

Effect of Arsenic (As) and its Interaction with Iron (Fe) on *Abelmoschus esculentus* (L.)

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Abstract

An experiment was carried out under the control observational status of wire house to study the effects of various concentrations of arsenic and the interaction between arsenic (As) and iron (Fe) on some specific aspects of metabolism in test plant *Abelmoschus esculentus* L. (okra), commonly known as lady's finger, belonging to the family Malvaceae. In this experiment, the germination behavior of lady's finger seeds was taken into account. The soil culture method was used for the experimental procedure and the soil for cultivation of the plants was obtained from an uncultivated area of the soil of A1 horizon. This experiment was carried out under wire-house conditions by growing the seedlings in earthen pots that had been filled with soil culture medium. Growth parameters measured included the length of shoots. The biochemical parameters included chlorophyll and carotenoid content, Catalase and Peroxidase enzymes activities, and the concentration of proteins. In the course of the experiment, it has been noted that an increase in the amount of arsenic caused an increase in protein as well as chlorophyll contents, additionally, when arsenic is mixed with iron, their levels exceed than those plants of the control levels. The activity of Peroxidase and Catalase decreased with increasing concentration of Arsenic; however, the high concentration of Arsenic is highly toxic for plant growth. The increasing amount of Arsenic showed a positive effect whereas an increased amount of Arsenic along with an iron dose also showed some encouraging results.

Keywords: Arsenic, Chlorophyll, Peroxidase, Catalase etc.

Introduction

Arsenic is a naturally occurring element found in the Earth's crust. It is present in different parts of the environments such as air, soil, and water. Although arsenic exists in several forms, the gray form is the most useful in industries because of its metallic properties.

Arsenic (chemical symbol: As, atomic number: 33), is a chemical element described by both Matschullat (2000) and Hettick et al. (2015), exists in different forms of mineral compounds but is also found in its elemental form in combination with metals and nonmetals like; Sulphur. Arsenic or compounds like arsenic trioxide are used in the production of many different chemicals, including pesticides, herbicides, insecticides and wood preservatives. Some other sources of contamination of arsenic in the environment are anthropogenic actions like use of chemically synthesized insecticides, mining, manufacturing, combustion of coal, wood preservation processes etc.

Environmental and health hazards of Arsenic overdose:

Arsenic poisoning is a worldwide health hazard. Chronic exposure to arsenic has been linked to the onset of many diseases and ailments among humans. Arsenic exposure induces generation of ROS in the cells which causes various cellular changes through alteration in signalling pathways and epigenetics or through oxidative damage to the cellular molecules. Studies indicate that antioxidants that can decrease the amount of ROS produced have been found effective in treating the lesions caused due to arsenic exposure. Current studies have suggested that, in some situations, inducing an antioxidant response and reducing the level of ROS may paradoxically be involved in developing arsenic toxicity. This review examines the effect of arsenic on redox homeostasis and recent studies concerning the use of antioxidants in treating arsenic poisoning. Plants experience various abiotic stresses such as lack of nutrients and toxicity from heavy metals that adversely impact their growth, development, and productivity. Genetic differences both within and among the populations are some of the significant factors responsible for defining interactions and responses of plants towards the environment. Recent advancements have made use of natural variation within *Arabidopsis thaliana* L. to study plant development and responses towards various environmental stresses using genetics.

Phosphorus deficiency is another stressor that has negative impacts on plant physiology. Phosphorus deficiency is another stress factor that negatively impacts plant physiology. Arsenate, As(V), an analog of Pi, is absorbed through phosphate transport mechanism in the plants. Research indicates that As(V) absorption is higher when Pi concentration is low, resulting in plant toxicity. In this study, the genetic differences in *Arabidopsis* species have been used to investigate the response of As(V) stress under limited Pi availability. The root growth in length was compared to

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examine the difference between the *Arabidopsis thaliana* L. accessions, including Col-0, Sij-1, and Slavi-1. Gene expression analysis was conducted for all genes involved in uptake, detoxification, and regulation to understand the molecular pathways involved in various responses. Genetic regulation due to variation can be the reason why different *Arabidopsis thaliana* L. natural strains show differential responses to arsenate stress under low phosphorus availability. It can be concluded from the above discussion that investigation of natural variation under different stress conditions can reveal the biological mechanisms involved in stress adaptation. Background (Tapsi Shukla et al. 2015).

From the findings of the LEfSe analysis, some groups of microbes were significantly abundant within the paddy field soils. After assessing the redundancy analysis and relative importance, TS, TOC, pH, bio-available antimony (Sb), and arsenic (As) were the dominant drivers. Within them, the main drivers for bacterial taxonomy were Sb recalcitrant (Sbrec), Astot, and Asrec. Notably, Asrec, Astot, and Sbrec positively influenced Chloroflexi and Roku bacteria, while negatively influencing Proteobacteria and Actinobacteria. From the taxonomic prediction of functional genes, the findings from the RDA, relative importance analysis, and co-occurrence network show the influence of both geochemistry and bacterial community on the Sb/As bacterial functionality. From the results of the PLS-PM analysis, Sb and As contamination fractions negatively affected ecological functions, while the bacterial community positively influenced the same ecological function. Noteworthy, there were stronger effects on ecological functions directly compared to the indirect influences of community structures. Noteworthy, arsenic fractions negatively influenced bacterial community structures but positively affected bacterial functionalities. On the other hand, antimony fractions negatively influenced bacterial community structures but positively influenced their functionalities. Therefore, this research laid the groundwork for biochemical remediation of Sb and As contaminated paddy soils (Bocong Huang et al., 2019).

Arsenic (As) should be taken out of water because arsenic is highly poisonous, and even a tiny amount can lead to serious health issues such as cancer. It is estimated that there are 150 million individuals worldwide that could be affected by the challenges of arsenic contamination in water. A lots of conventional methods such as oxidation, coagulation, and adsorption were employed for its elimination, but these methods have certain restrictions pertaining to their operational expenses;

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hence, a need for modern and cost-effective membrane systems of nano-filtration has been identified. Therefore, to tackle the problem of scarcity of fresh and drinking water due to increased demand, this review paper considers advanced nano-filtration technique for arsenic removal. This paper is based on various types of nano-filtration (NF) membranes, methods of making membranes, and their efficiency in removing As from water bodies. This study provides an overview of the current status of the NF membrane from polymers, polymer composites, and polymer nano-composites. Moreover, the future scope has been elaborated on (T.A. Siddique et al. 2020).

Variability in As concentration and its species within rice grains was examined utilizing the WRC (World Rice Core Collection), having 69 germplasm lines grown over three successive years. Further, QTL analysis was carried out in order to map QTLs responsible for DMA concentration within rice grains. The grain As concentration of WRC varied 3-fold. Total-As, inorganic As, and DMA concentrations were significantly influenced by the genotypic and year effects. The two accessions with the lowest stable total and inorganic arsenic content among all accessions were the 'Local Basmati' and 'Tima', both belong to *indica* type. Two QTLs (qDMAS6.1 and qDMAS6.2) have been discovered on the chromosome 6, and another one QTL (qDMAS8) on chromosome 8 that influence the variation of DMA content within the grain. These three QTLs are responsible for 73% of the phenotypic variance in DMA (Masato Kuramata 2013).

Materials and methods

Abelmoschus esculentus L. (okra) commonly known as Bhindi was selected for the experiment as it is grown frequently in summers.

Experimental Set-Up

Pot Culture

The effects of As were investigated based on the growth and metabolic reactions of *Abelmoschus esculentus* L. (okra) Plants were grown in earthen pots using garden soil in a wire house at room temperature. The soil used for the plant cultivation was collected from the A1

horizon. Soil was dark brown/blackish in color. Soil was sieved to ensure a uniform size of the particles. Earthen pots of medium sizes were used, each with a hole for drainage. Soil pH was 6.7.

The central drainage hole was covered by an inverted watch glass. The soil filled pots were repeatedly flushed with distilled water to ensure proper leaching of the water.

Schedule Dosage

The experiment was carried in soil culture in earthen pots. These pots were supplied with treatments nutrient and different doses of heavy metal i.e. As sequence manner. The experiment was carried out for one month during which treatments were supplied for a total of ten times.



Figure 1: Effect of As and interaction with Fe on the growth of Lady's finger (*Abelmoschus esculentus* L. (okra) after 24 days.



Figure 2: Comparative study of morphological changes and appearance of visible symptoms on leaves supplied with graded levels of As and interaction with Fe with comparison to the control on Lady's finger (*Abelmoschus esculentus* L. (okra) after 24 days.

Growth attributes and visible symptoms

Height

The height of the plant was measured from the bottom of the stem or from surface of the soil to the base of the youngest leaf, vertically by the help of a scale in centimeter and data recorded in tables.

Other visible symptoms

The morphological symptoms observed were photographed. These include, the symptoms, shown by the leaves, roots and shoots as compared to the control. Arsenic being toxic to the plants,

the okra plants showed poor growth, chlorosis and reduced leaf size when supplied with high dose in comparison to the control.

Estimation of physiological and biochemical changes-

The physiological and biochemical responses of the plants to the treatment were studied by estimating chlorophyll and protein contents. The activities of catalase and peroxidase enzyme were also measured.

Chlorophyll test

We determined the chlorophyll concentration using the method of Arnon (1949). Leaf tissue homogenized with a pinch of CaCO_3 in a pestle and mortar in 85% acetone. The homogenate was filtered using funnel through what man No 42 filter paper. The filtrate was then made to known volume. The color intensity of the extract was measured on spectrophotometer at 663.2 nm for chlorophyll - a, 646.8 nm for chlorophyll- b and 470 nm for carotenoids.

Protein test

Protein content was determined using the method of Lowry et al. (1951). Reagent A was prepared with 2% NaCO_3 and 0.1 N NaOH, while reagent B was made with 0.5% CuSO_4 solution and 1% sodium citrate. To make reagent C, 50 ml of reagent A and 1 ml of reagent B were mixed. Then, 0.1 ml of appropriately diluted leaf tissue homogenate was mixed with 1.0 ml of reagent C and left to stand for 10 minutes. After an hour, 0.1 ml of folin-ciocalteu reagent was added. The optical density was measured at 750 nm with the help of a spectrophotometer. The protein content was finally measured using the following formula.

$$0. \text{D} \times 5 = \text{C}$$

$$\text{C} \times 158.43 = \text{Final reading}$$

Catalase

Catalase was assayed by the modified method of Euler and Josephson (1927). 10 ml of reaction mixture containing 10% wt. per volume H_2O_2 and 0.1 M phosphate buffer pH 7.0 was taken in

50ml test tube and stabilized at 250 C. The reaction was initiated by the addition of 1ml suitable diluted enzyme extract to the reaction mixture and was allowed to continue for 5 minutes and stopped by the reaction mixture and was allowed to continue for 5 min and stopped by the addition 5ml 2NH₂SO₄. The corresponding zero hours' blanks were run simultaneously where H₂SO₄ was added prior the addition of enzyme extract. The amount of H₂O₂ was determined by titrating the reaction mixture against 0.1N KMnO₄ and making the blank correction. Catalase activity was expressed as mu moles H₂O₂ split/mg fresh weight.

Peroxidase

Peroxidase was measured using a modified version of Luck (1963) technique. At 25°C, the enzyme assay was conducted. One milliliter of 0.5% p-phenylene diamine was added to two milliliters of 0.1 M phosphate buffer pH 6.0 and one milliliter of 0.01 percent H₂O₂. One milliliter of enzyme extract was added to the mixture above to start the reaction, which was then left to run for five minutes. 4 N H₂SO₄ was added to stop the reaction. Blanks that had sulfuric acid added before the enzyme extract was introduced were run concurrently. After 20 minutes in the refrigerator, the reaction mixture was centrifuged at 4,000 g. At 485 nm, the color intensity was measured. The enzyme unit, which is the 0.01 difference in optical density between the blank and sample, has been used to express the results.

Results

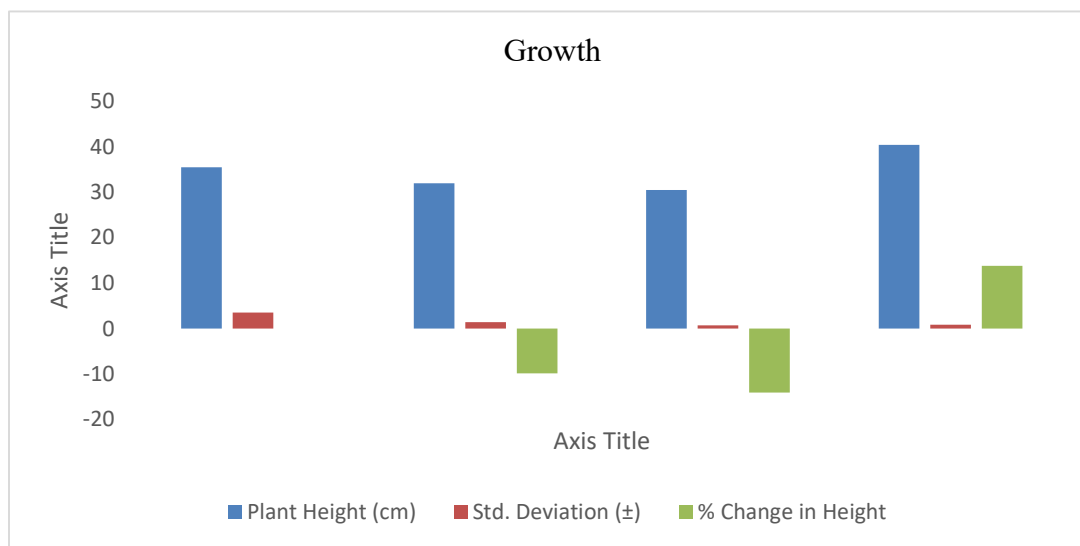
Growth-

Results indicated that the plant growth decreased on application of increasing the Arsenic concentration in plant while application of As+Fe in plant negated the decrease of plant growth. The growth in comparison with control decreases 9.85 and 14.08 percent at 0.5mm As and 1mm As respectively in (Table -1).

TABLE -1. Effect of As and As+Fe interaction on *Abelmoschus esculentus* L. (okra) plant's height.

Treatment	Plant height in (Cm)	% of Average Height
Control	35.5±3.534Vs3=9.90"yes"	35.5-35.5/35.5×100 =0%
0.5m M As	32.0±1.4144Vs2=8.40"yes"	35.5-32.5/35.5×100=-9.85%

1m M As	30.50±0.7074Vs1=4.9”No”	35.5-30.50/35.5×100=14.08%
As +Fe	40.40±0.849	35.5 -40.40/35.5×100=+13.80%



Graph-Growth

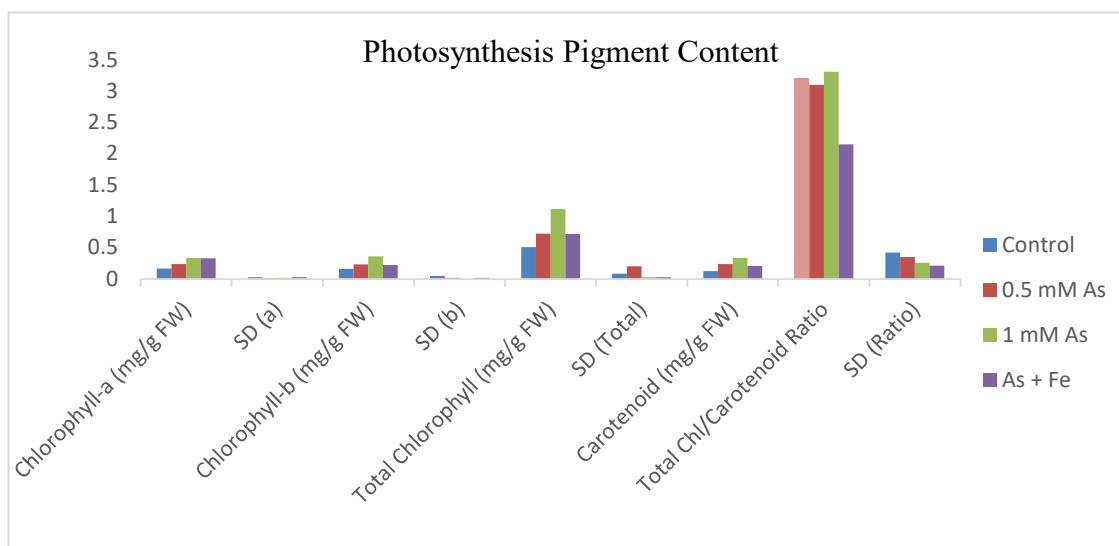
Photosynthetic Pigment content-

The photosynthetic pigment like chlorophyll a, chlorophyll b and carotenoids contents were increased on increasing the Arsenic concentration while interactive effect on application of Fe+As also enhanced these pigment in comparison to control the difference in the mean value among the treatment groups are grater. There was a significant difference in total chlorophyll and carotenoids ratio trends while interaction of As + Fe was decreased as shown in (Table -2).

TABLE -2. Effect of As and Fe interaction on Chlorophyll and Carotenoid content of *Abelmoschus esculentus* L. (okra) plant.

Treatment	Chlorophyll-‘a’ mg/gm fresh wt.	Chlorophyll-‘b’ mg/gm fresh wt.	Total chlorophyll mg/gm fresh wt.	Carotenoid mg/gm fresh wt.	Total chlorophyll carotenoid
Control	0.170±0.0283	0.165±0.0495	0.510±0.085	0.127	3.20±0.424
0.5m M As	0.240±0.0141	0.235±0.0212	0.725±0.205	0.241	3.1±0.354

1m M As	0.335 ±0.0212	0.360±0.000	1.120±0.028	0.336	3.31±0.262
As +Fe	0.330 ±0.0283	0.225±0.0212	0.720±0.028	0.210	2.15±0.212



Graph-Photosynthesis Pigment Content

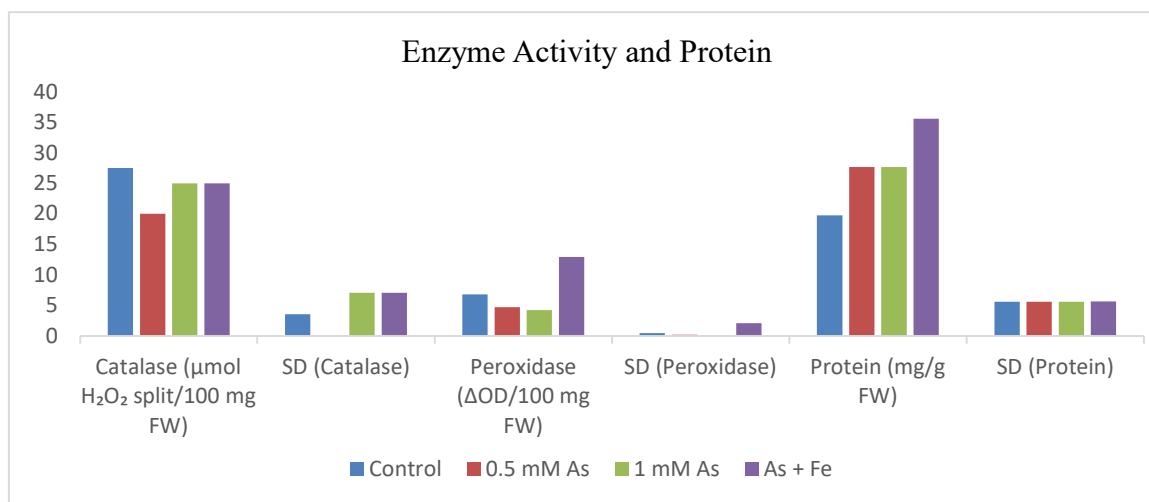
Analysis of Enzymes Activities and Protein

The activity of catalase was not found to any trend have at increasing of As concentration while activity of peroxidase was significantly decreased on increasing the concentration of As in the soil. The interactive effect of Fe with As caused significant enhancement in comparison to control. The protein concentration was significantly increased. On increasing the Arsenic concentration and interaction with Fe also enhances the protein concentration as shown in (Table - 3).

TABLE-3. Effect of As and As+Fe interaction on activities of enzymes and protein content of *Abelmoschus esculentus* L. (okra) plant.

Treatment	Catalase μ moles H ₂ O ₂ split/100mg fresh wt.	Peroxide Δ 0.D./100mg fresh wt.	Significance	Protein mg/gm fresh wt.
Control	27.500±3.536	6.8±0.438	4vs3=8.74"yes"	19.750±5.586
0.5m M As	20.00±0.000	4.68±0.170	4vs2=8.2"yes"	27.650±5.586

1m M As	25.00±7.071	4.210±0.127	4vs1=6.1”yes”	27.650±5.586
As +Fe	25.00±7.071	12.95±2.051		35.60±5.657



Graph-Enzyme Activity and Protein

Discussion

Plants exposed to arsenic show signs of nutrient deficiency, such as chlorosis (yellowing) in young and old leaves, along with significantly reduced growth. Chlorosis is associated with disturbances in iron metabolism in plants. While some of the symptoms observed were similar to iron deficiency, others were unique to particular heavy metals. As arsenic levels increased, plant growth was stunted more severely.

It was noted that photosynthesis activity decreased with an increase in heavy metal doses in plants. It was caused by too many heavy metals and other stress factors posing toxic effects on various enzymatic activities for both chlorophyll biosynthesis and its activity. Arsenic can effect photosynthesis in several ways it may affect the biosynthesis of photosynthesis pigments especially chlorophyll. It may activate enzymes by binding thiol group (SH) necessary for catalytic activity or substitute other divalent cations in metallo-enzyme and interact with electron transport in chloroplast.

Plants require protein for growth, and the amount of protein within a plant can change when it is exposed to heavy metals. This is because heavy metal stress can make some enzymes work less or more. This may have caused changes in the protein content and showed that heavy metals were involved in its metabolism. In this study, the content generally rose with higher doses of arsenic, and the combination of arsenic and iron raised protein levels. The increased protein content in the leaf tissue may have resulted from the synthesis of phytochelatin in response to heavy metal stress.

Antioxidative enzymes are those that activate and produce against heavy metal stresses, damage to the plasma membrane, and the generation of ROS and H₂O₂. In this study, the activity of antioxidative enzymes in plants increased along with higher doses of heavy metals.

Summary and conclusion

The present investigation was accomplished through analysis of six parameters of growth and metabolism. As the first parameter, the effect of different doses of arsenic on the shoot length of *Abelmoschus esculentus* L. (okra) plants was studied. In the second and third experiments chlorophyll and carotenoids contents were measured respectively. In the fourth experiments Catalase activities were measured at increasing doses of Arsenic. In the fifth and sixth parameters we analysed the activity of peroxidase and protein contents with increasing concentration of As and its interaction with Fe.

From the present investigation following conclusions can be drawn:

As was observed, the chlorophyll content was found to increase with increased dosage of arsenic, while increased dosages of arsenic coupled with iron had a higher impact on the chlorophyll content than the control. With an increase in the dosage of arsenic, the height of plants decreased, and higher dosages were more harmful.

The increase in the level of protein occurred as there was an increase in the concentration of arsenic in the soil. Moreover, the joint action of arsenic and iron resulted in a further increase in the level of protein. Peroxidase activity reduced with an increase in arsenic concentration, while

catalase activity remained unchanged. Joint activity of iron and arsenic was more pronounced than in the control group.

Thus we conclude that increasing concentration of arsenic gives positive effect, it also gave encouraging results effect, when Arsenic was combined with a dose of Iron. Here iron availability enhances the absorption of Arsenic may result in toxicity and also increases stress responses which are shown by plants by enhancing the activities of other antioxidative and heavy metal stress tolerance mechanisms involved in plants.

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Author's contribution

The corresponding author is experienced in the branch of plant nutrition and stress physiology of plants since last 18 years and by his guidance and supervision, Dr. Kanchan Awasthi has conducted the whole experiments, designed setups, analyzed and arranged all commodities essential with the help of Mr. Bilal Akhtar and Mr. Tabrez Zaki Abbas who are currently following their Ph.D. under the supervision of corresponding author in the Department of Botany, University of Lucknow, Lucknow.

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