Influence of Ascorbic Acid on Early Seedling Growth of *Cucumis sativus* L. Under Arsenic Induced Stress

Muhammad Zubair [1], Rida Nisar [2], Muhammad Ishfaq Khan [1], Fozia Bibi [3], Nida Afaqi [4], Haisam Afnan Gul [5], Imdad Ullah [6], Muhammad Sulaiman [6], Jamal Abdul Nasir [7], Nasr Ullah Khan [7*]

[1] Department of Botany, University of Science and Technology Bannu, Khyber Pakhtunkhwa, Pakistan
[2] Department of Horticulture, Faculty of Agriculture, Gomal University Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan
[3] Department of Biological Sciences, International Islamic University Islamabad (IIUI), Pakistan
[4] Attar Ur Rahman School of Applied Biosciences (ASAB), National University of Science and Technology (NUST), Islamabad, Pakistan
[5] Institute of Biological Sciences (IBS), Gomal University Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan
[6] Department of Agronomy, Faculty of Agriculture, Gomal University Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan
[7] Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan

* Corresponding Author: **Nasr Ullah Khan**

Article Info

ISSN (online): 3049-0588
Volume: 02
Issue: 03
May - June 2025
Received: 14-03-2025
Accepted: 15-04-2025
Page No: 43-48

Abstract

Among environmental toxins, arsenic is one of the most dangerous heavy metals which badly affects both flora and fauna. Ascorbic acid is an important molecule that can enhance the stress tolerance ability of plants and prevent oxidative damage. The current study was carried out to evaluate the influence of ascorbic acid on growth and physiology of *Cucumis sativus* L. under arsenic induced stress. The seeds of *Cucumis sativus* L. were soaked for two and half hours in the solution of ascorbic acid (100 ppm) before sowing. Ascorbic acid showed positive impact on physiology and phytochemical enhancement of *Cucumis sativus* L as compared to untreated samples. Amongst all the treatment, application of 100 ppm Ascorbic acid produced fruitful results related to growth of root and shoot, dry and fresh weight of shoot and root, antioxidant and phenolic contents, leaf pigments and seed germination. The physiological parameters of *Cucumis sativus* L were badly affected by the treatment of arsenic (15 ppm and 25 ppm). The mixture of ascorbic acid and arsenic showed positive effect on physiology of *Cucumis sativus* L. as low amount of arsenic boosts the antioxidants content up to some level and induces the activity of ascorbic acid. This research study recommends the application of ascorbic acid for the better growth of plants in arsenic polluted soil.

DOI: https://doi.org/10.54660/GARJ.2025.2.3.43-48

Keywords: Ascorbic Acid, Arsenic Stress, *Cucumis sativus* L., Heavy Metal Stress, Antioxidants

Introduction

Metals and metalloid having atomic density above than 5g/cm^3 are known as heavy metals. Some of the common heavy metals are Arsenic, manganese, cadmium, chromium [1]. A metal said to be toxic when it is relatively more compact metal having high potential toxicity, and can cause hazardous environmental effects.

Among environmental toxins arsenic is one of the most dangerous heavy metals which badly affects both flora and fauna. More than 500 million people are estimated to be at risk of excessive levels of arsenic exposure worldwide [2]. The metalloid enters into agricultural systems through different process like biogeochemical cycles [3].

Under typical conditions, plants sustain an ideal balance between the production and neutralization of reactive oxygen species. This balance is crucial for cellular signaling and the regulation of key physiological processes, including plant growth and development [4]. Nevertheless, the adverse environmental conditions may disturb this balance by the excessive generation of reactive oxygen species (ROS) [5].

Ascorbic acid plays a major role in protecting the body from the harmful effects of free radicals and is the most important water-soluble nutrient and plant growth regulator (PGR), also called "vitamin C". The lack of ascorbic acid leads towards different problems such as weakness of connective tissues, scurvy and capillary fragility and it regulates various essential physiological functions, including photosynthesis, respiration, and glucose metabolism, either by serving as a co-factor for metabolic enzymes or by modulating cellular redox state. Ascorbic acid's multifunctionality distinctly enables it to integrate and adjust redox-responsive transcriptional and metabolic circuits, as well as vital biological processes, in response to developmental and environmental signals [6].

Ascorbic acid also plays a major role in protecting the body from the harmful effects of free radicals. Ascorbic acid is an important molecule that can enhance the stress tolerance ability of plants and prevent oxidative damage [5]. By increasing the photosynthetic pigments, the plants enhanced their tolerance effect against the heavy metal stress while it has been studied that Ascorbic acid is implicated in and provide more clues in enhancing the environmental stress tolerance of plants [7].

The role of plant growth regulators in seed germination were observed by some researchers which stated that "Low level of ABA in seed increases the concentration of ROS which leads towards stimulation of Ascorbic Acid and it enhances the production of gibberellins which shows significant increase in germination process, as gibberellins stimulates amylase to convert the starch into sugar, resulting promotion germination of seed [8].

Cucumis sativus L. is the botanical name of cucumber. It's locally known as Kheera in Pakistan. It belongs to family Cucurbitaceae (gourd family). The family consists of about 117 genera and 727 species. Heavy metals when released into the soil via natural and anthropogenic causes at higher amount they badly affect the physiology and morphology of *Cucumis sativus* L. It is therefore necessary to protect plant from toxicity related with arsenic. Therefore, the present study was main focused to analyse the outcome of ascorbic acid on seedling performance of *Cucumis sativus* under arsenic stress & conclude the antioxidant content and effect of arsenic stress on physiology of *Cucumis sativus* along with assessment of result of ascorbic acid on physiological and biochemical mechanism of arsenic stress tolerance in *Cucumis sativus*.

Materials and Methods

Materials

Uniform-sized, vigorous seeds of *Cucumis sativus* L. were obtained from the Department of Agriculture Research Station, Pakistan, and sterilized for surface cleanliness by washing with 0.3% $HgCl_2$ for 3 minutes, followed by autoclaving with distilled water. The seeds were then sown in plastic pots (8 × 12 cm^2) filled with a 1:1 mixture of clay and sand, and placed in a greenhouse at the Department of Botany, University of Science and Technology, Bannu.

Treatment of Arsenic and Ascorbic acid

The stock solution was prepared by dissolution of 0.5 g ascorbic acid in 500 ml distilled water. The stock solution of 100ppm ascorbic acid was prepared and then kept in the lab for further use. The seeds were dipped in solution of ascorbic acid for 120 minutes. Two different solutions (15ppm& 25ppm) used. The treatment of arsenic & ascorbic acid was done to the plants and the pots put in the containers. Three replicas were used for each treatment. Holes were made in each pot for absorption of solution from the container (Table 1)

Table 1: Lay out of the treatments.

S. No	Treatments	Concentrations
1	Control	Untreated water
2	Ascorbic acid	100ppm
3	Arsenic	15ppm
4	Arsenic	25ppm
5	Arsenic+ Ascorbic acid	15ppm+100pm
6	Arsenic+ Ascorbic acid	25ppm+100ppm

Parameters

After arsenic and ascorbic acid treatment, the plants were allowed to grow. After 25 days of sowing, the samples were harvested and checked for the following morphological qualities such as dry and fresh weight, germination percentage, germination index, germination rate index and physiological attributes such as analysis of leaf photosynthetic compounds, Total Phenolics Contents & Anti-oxidant Potential.

Statistical Analysis

One way (ANOVA) was used for statistical analysis of the research data and minimum significant differences tests, all the treatments were compared (Steel and Torrie, 1984). Coefficient of correlation was measured by using Statistics (version 8,1 USA).

Results

Root Length

Ascorbic acid shows positive effect on root length of *Cucumis sativus L*. as compared to other treatments. Ascorbic acid along with arsenic significantly enhanced the length of roots. Arsenic concentration suppressed the length of root.

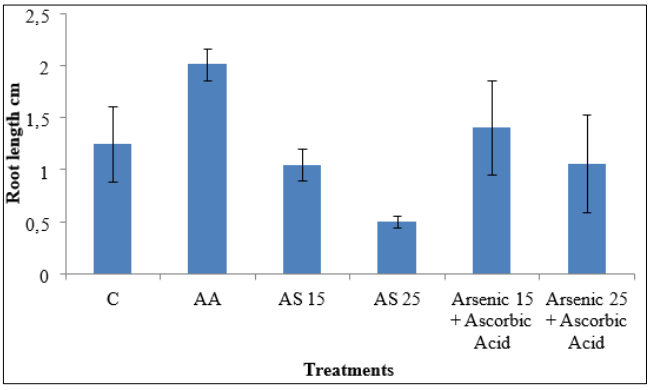

Fig 1: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 0.5354).

Root Fresh Weight

Arsenic concentrations reduced the fresh weight of *Cucumis sativus L*. Ascorbic acid increased the fresh weight of root. The mixture of ascorbic acid and arsenic also shows positive

effect on root fresh weight, as arsenic boosts the activity of ascorbic acid.

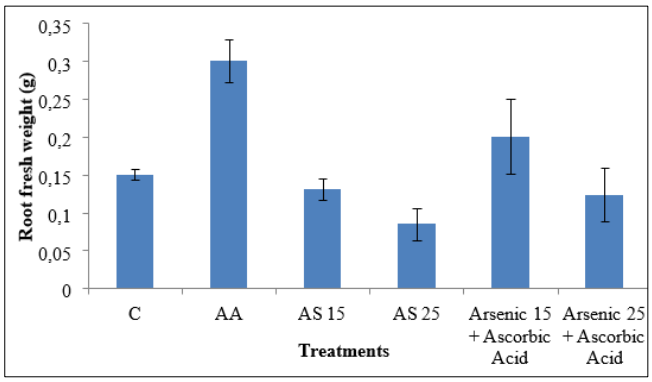

Fig 2: Role of ascorbic acid on *Cucumis sativus* L. (LSD. 0.0353).

Root dry weight

The root dry weight of *Cucumis sativus L* was badly affected by high amount of arsenic concentration. The mixed solution of ascorbic acid and arsenic significantly improved the dry weight of root. Ascorbic acid showed a defensive role against the arsenic stress and increased the dry weight of root.

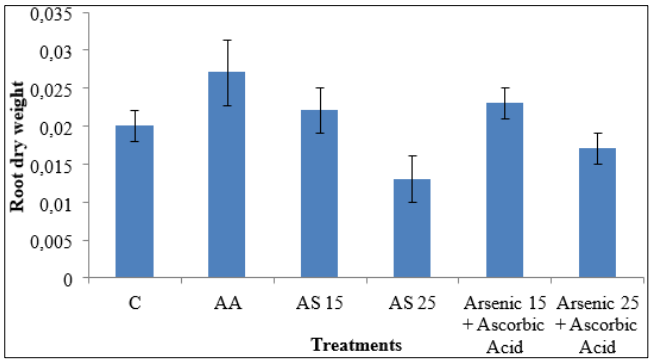

Fig 3: Role of ascorbic acid on *Cucumis sativus* L. (LSD. 3.727).

Root Antioxidant

Ascorbic acid stimulated the defensive mechanism of *Cucumis sativus L* against the arsenic stress and increased the quantity of root antioxidant. The mixture of arsenic and ascorbic acid also results in promotion of root antioxidant contents. The high amount of arsenic also induced the root antioxidant quantity.

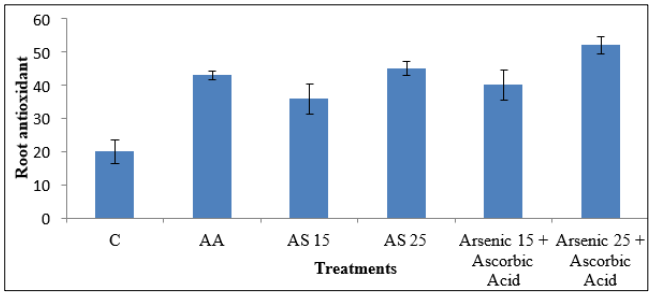

Fig 4: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 3.1257)

Root Phenolics

Ascorbic acid shows a positive influence on then root phenolics of *Cucumis sativus L*. The graph of root phenolics also increased by the treatment of mixed solution of arsenic and ascorbic acid. The moderate amount of arsenic promotes root phenolics contents up to some range.

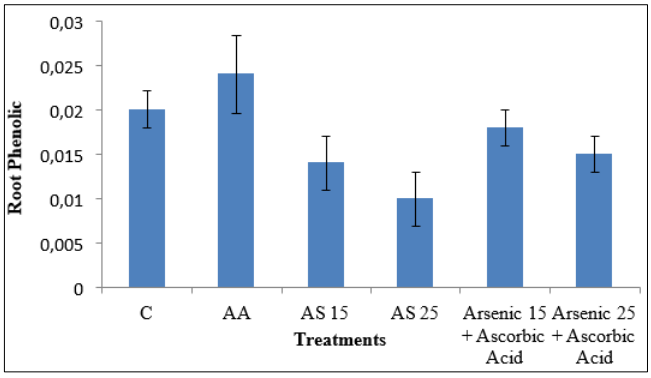

Fig 5: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 07.727)

Shoot Length

The externally applied ascorbic acid played a chief role in elongation of shoot in *Cucumis sativus* L. Addition of arsenic to ascorbic acid solution also enhanced the shoot length interestingly. High concentration of arsenic reduced the shoot length due to its toxic effect.

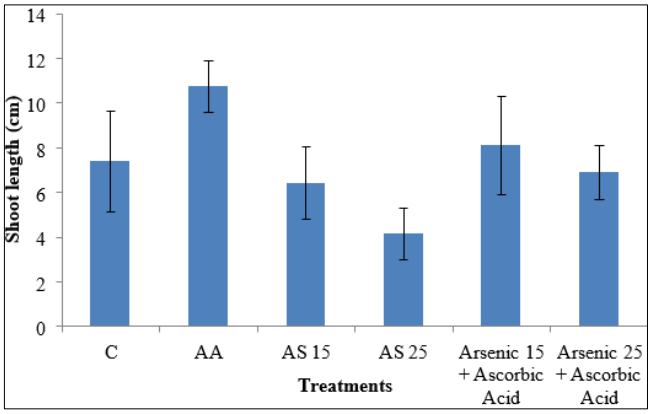

Fig 6: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 1.656).

Shoot Fresh Weight

Ascorbic acid enhanced the fresh weight of shoot of *Cucumis sativus* L. The untreated and other samples show less fresh weight than ascorbic acid treated samples. High amount of arsenic reduced the fresh weight. The mix solution of arsenic and ascorbic acid promote the fresh weight of shoot as it detoxifies the effect of arsenic.

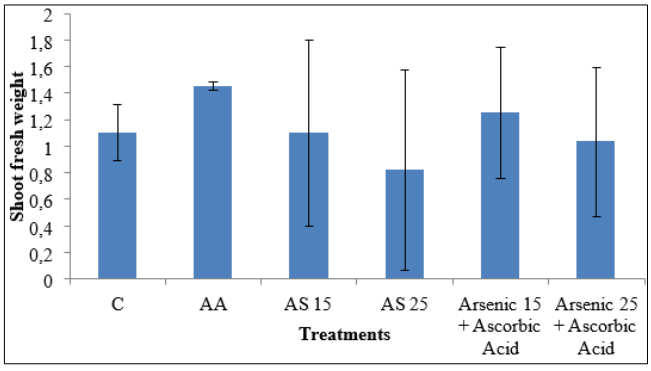

Fig 7: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 0.2036).

Shoot Dry weight

Ascorbic acid promotes shoot dry weight of *Cucumis sativus*

L. It also improves the dry weight of shoots when applied along with arsenic. The mix form of ascorbic acid and arsenic improved the dry weight of shoot as it suppresses the toxicity of arsenic.

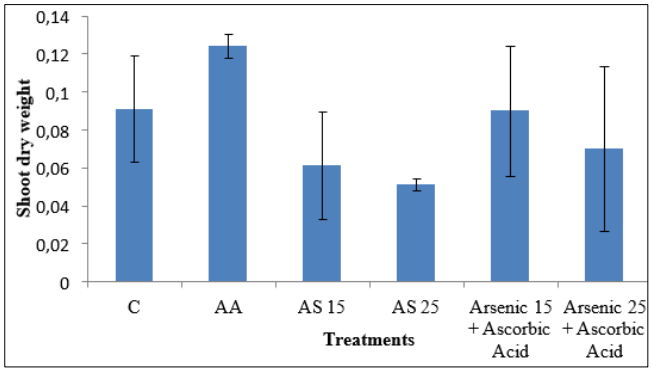

Fig 8: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 0.0257)

Shoot Antioxidant

Using DPPH solution, the antioxidant of shoots contents in *Cucumis sativus* L was enhanced as compared to other samples. The shoot antioxidant content is promoted by applying exogenously mixed solution of ascorbic acid and arsenic. The high amount of arsenic also increases the antioxidant quantity up to some extent.

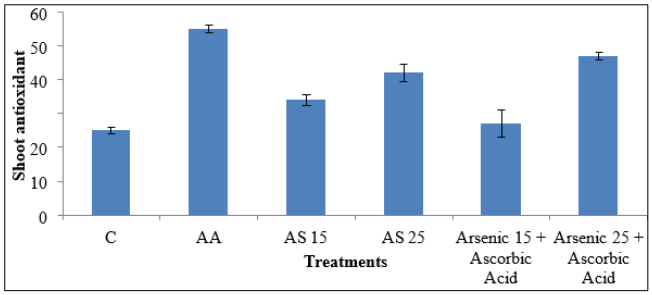

Fig 9: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 3.0379)

Shoot Phenolics

The shoot phenolic compound concentration of *Cucumis sativus* L is increased by the exposure to ascorbic acid. The shoots phenolics also show positive change in mixed solution of ascorbic acid and arsenic. The high amount of arsenic reduces the shoot phenolics contents, showing its toxicity upon the plant.

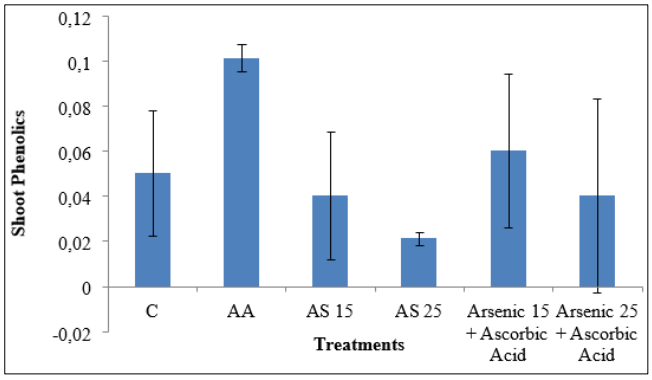

Fig 10: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 09.789)

Seed Germination Percentage (%)

The ascorbic acid has positive influence on seed germination percentage of *Cucumis sativus* L. The percentage of seed germination is increased by the combined solution of ascorbic acid and arsenic. The seed germination percentage is reduced with increase of arsenic concentration. High concentration of arsenic has badly affected the seed germination percentage.

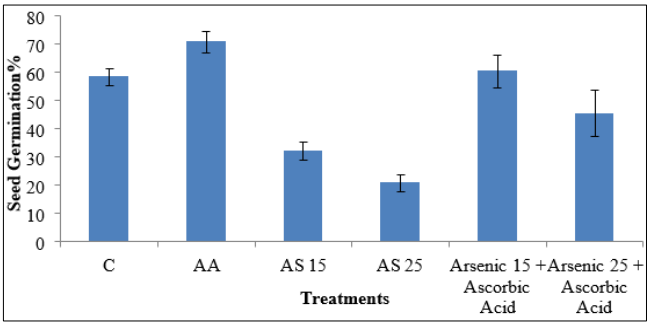

Fig 11: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 7.786)

Seed Germination Index

The ascorbic acid has significant role in seed germination index of *Cucumis sativus L*. The seed germination index is improved by the combined solution of ascorbic acid and arsenic. The seed germination index is decreased with increase of arsenic. High quantity of arsenic shows negative affected on seed germination index.

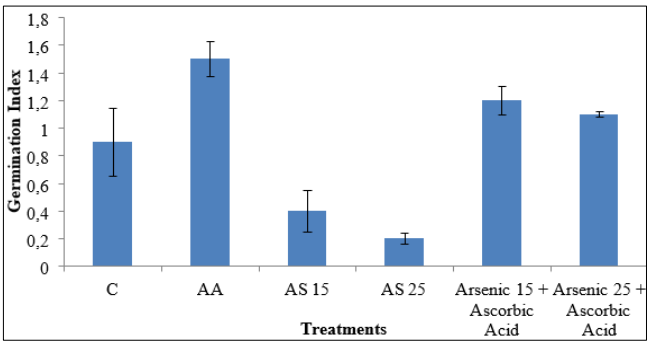

Fig 12: Role of ascorbic acid on seed germination index of *Cucumis sativus* L under arsenic. (LSD: 0.1476)

Photosynthetic Pigment (Chlorophyll "a" content)

The exogenously applied ascorbic acid improved the chlorophyll a content in *Cucumis sativus* L. The combination of arsenic and ascorbic acid also maximized the quantity of chlorophyll a. The high amount of arsenic shows negative effect & hence decreases the chl a.

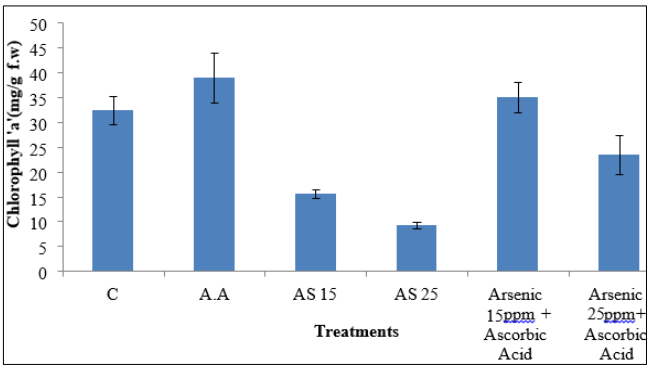

Fig 13: Role of ascorbic acid on *Cucumis sativus* L under arsenic. (LSD: 1.2576)

Photosynthetic Pigment (Chl "b")

The role of ascorbic acid increased the chlorophyll b content in *Cucumis sativus L*. The mixed solution of arsenic and ascorbic acid also enhanced the quantity of chlorophyll b. The high amount of arsenic causes toxicity and hence lowered the quantity of chlorophyll b.

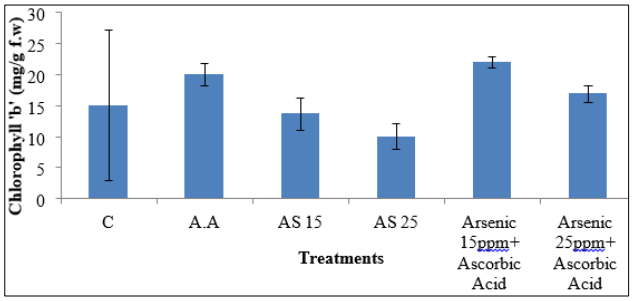

Fig 14: Role of ascorbic acid on *Cucumis sativus* L. (LSD: 5.1459)

Photosynthetic Pigment (Leaf carotenoid content)

The Leaf carotenoid content of *Cucumis sativus* L was increased by the application of ascorbic acid. The control and other samples show less leaf carotenoid than ascorbic acid treated samples. High concentration of arsenic reduced the leaf carotenoid. The mix solution of arsenic and ascorbic acid increases the quantity of leaf carotenoid as it detoxifies the effect of arsenic.

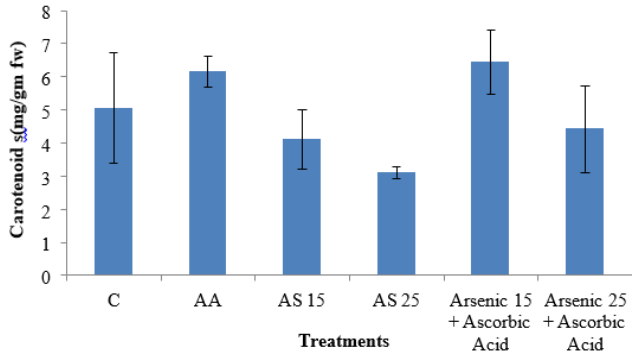

Fig 15: Role of ascorbic acid on carotenoid content of *Cucumis sativus* L. (LSD: 2.5787)

Discussion

Heavy metal pollution is a well-known environmental problem, which is dangerous for biotic and abiotic components. Due to overpopulation the need for food is increasing day by day which leads towards release of many pollutants in excess amount that consequently disturb the food chain. Heavy metals have adverse effect on plants growing in heavy metals polluted soil. Feeding on such affected plants leads towards serious health issues. It also disturbs the texture and profile of fertile soil that negatively affect the uptake of primary minerals. The present study notice that ascorbic acid play an important role as an antioxidant that protect the *Cucumis sativus* from the toxic effect of arsenic and hence it increases the rate of photosynthesis, antioxidant, phenolics contents and defence mechanism of *Cucumis sativus*. Ascorbic acid along with other phytohormones like gibberellins and ABA regulates the rate of seed germination. The role of plant growth regulators in seed germination were observed by some researchers which stated that "Low level of ABA in seed increases the concentration of ROS which leads towards stimulation of Ascorbic Acid and it enhances the production of gibberellins which shows significant increase in germination process, as gibberellins stimulates amylase to convert the starch into sugar, resulting promotion of seed germination" [9].

The seed soaking activity in the solution of ascorbic acid gives very positive results. The mixed solution of ascorbic acid and arsenic enhanced various parameters like root and shoot length, antioxidant and phenolics and seed germination percentage. The antioxidant ability of ascorbic acid shows great stimulation of defence mechanism and fights against the toxicity caused by arsenic and neutralized the effect of reactive oxygen species (ROS) [10].

The present study examined that the toxicity of arsenic badly affected the content leaf pigments like chlorophyll "a", chlorophyll "b" and carotenoids, that's why it reduced the rate of photosynthesis. The research study showed that toxicity of heavy metals like arsenic minimized the chlorophyll "a" content in *Cucumis sativus* [11]. It is also observed that the structure of chlorophyll is degraded by arsenic that replaces the Magnesium atom [12].

The research findings showed that the seed germination index is badly affected by toxic effect of arsenic. Due to lack of defence mechanism in seed of *Cucumis sativus* the percentage of seed germination is also decreased by the arsenic. This toxic effect is neutralized with the help of ascorbic acid. The application of Ascorbic acid increased the expansion of seeds cell wall that significantly improved the seed germination in *Cucumis sativus* [13].

The research believes that low amount of arsenic can stimulate the growth of plants. When plants are exposed to low amount of arsenic concentration then it produces reactive oxygen species (ROS) which can leads towards the production of many enzymes and antioxidant metabolites that plays a major role in antioxidant defence mechanism. When plants uptake high amount of arsenic then it can affect the plants both directly and indirectly. The direct effect is caused by the deposition of arsenic in plants which cannot be broken down easily, due to this reason they block the active sites of enzymes and disturb the cellular activities. The indirect effect of the arsenic toxicity disturbs the absorption of primary nutrients as the arsenic replaces the uptake of nutrients [14].

The research study concluded that the high quantity of arsenic inhibits the growth of root and shoot and hence it minimized the weight of root and shoot. The low amount of arsenic has some positive impacts on various phytochemicals as phenolics, antioxidant and carotenoids contents are increased by the action of arsenic. The solution of arsenic and ascorbic acid in combined form enhanced the physiological activities of *Cucumis sativus* [15].

Conclusion

On the basis of our research findings, it was concluded that the ascorbic acid has strong influence on the *Cucumis sativus* grown in various concentration of arsenic. Ascorbic acid showed positive impact on physiology and phytochemical enhancement of *Cucumis sativus* as compared to untreated samples. Amongst all the treatment, application of 100 ppm Ascorbic acid gives very fruitful results in the growth of root and shoot, dry and fresh weight of shoot and root, antioxidant and phenolic contents, leaf pigments and seed germination. Based on the current study, we found that high amount of arsenic has very toxic influence on the growth and physiology of *Cucumis sativus* while low quantity of arsenic can stimulate the growth of plants. In low amount arsenic

produces reactive oxygen species (ROS) which stimulates the production of antioxidant and metabolites that play a key role in antioxidant defence mechanism. The mixture of ascorbic acid and arsenic shows positive effect on physiology of Cucumis sativus, as low amount of arsenic boosts the antioxidants content up to some level and induces the activity of ascorbic acid. Ascorbic acid increases the stress tolerance potency of plants by coping with the reactive oxygen species (ROS) hence neutralizes the toxic effect of arsenic. In short, the research study would recommend the use of ascorbic acid in arsenic polluted soil. *Cucumis sativus* exhibited strong activities against a wide range of parameter which means that it contains compounds which have broad spectrum of activity.

References

1. Nwaichi EO, Dhankher OP. Heavy metals contaminated environments and the road map with phytoremediation. J Environ Prot. 2016;7(1):41.
2. Fatoki JO, Badmus JA. Arsenic as an environmental and human health antagonist: a review of its toxicity and disease initiation. J Hazard Mater Adv. 2022;5:100052. doi:10.1016/j.hazadv.2022.100052.
3. Smedley PL, Kinniburgh DG. A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem. 2002;17(5):517-68.
4. Mittler R. ROS are good. Trends Plant Sci. 2017;22(1):11-9.
5. Rao MJ, Duan M, Zhou C, *et al*. Antioxidant defense system in plants: reactive oxygen species production, signaling, and scavenging during abiotic stress-induced oxidative damage. Horticulturae. 2025;11(5):477. doi:10.3390/horticulturae11050477.
6. Mishra S, Sharma A, Srivastava AK. Ascorbic acid: a metabolite switch for designing stress-smart crops. Crit Rev Biotechnol. 2024;44(7):1350-66. doi:10.1080/07388551.2023.2286428.
7. Wu P, Li B, Liu Y, *et al*. Multiple physiological and biochemical functions of ascorbic acid in plant growth, development, and abiotic stress response. Int J Mol Sci. 2024;25(3):1832. doi:10.3390/ijms25031832.
8. Nadi R, Mateo-Bonmatí E, Juan-Vicente L, Micol JL. The 2OGD superfamily: emerging functions in plant epigenetics and hormone metabolism. Mol Plant. 2018;11(10):1222-4.
9. Ye N, Zhang J. Antagonism between abscisic acid and gibberellins is partially mediated by ascorbic acid during seed germination in rice. Plant Signal Behav. 2012;7(5):563-5.
10. Batth R, Singh K, Kumari S, Mustafiz A. Transcript profiling reveals the presence of abiotic stress and developmental stage specific ascorbate oxidase genes in plants. Front Plant Sci. 2017;8:198.
11. Balal RM, Shahid MA, Javaid MM, *et al*. Chitosan alleviates phytotoxicity caused by boron through augmented polyamine metabolism and antioxidant activities and reduced boron concentration in Cucumis sativus L. Acta Physiol Plant. 2017;39(1):1-15.
12. Sharma L, Priya M, Bindumadhava H, Nair RM, Nayyar H. Influence of high temperature stress on growth, phenology and yield performance of mungbean [Vigna radiata (L.) Wilczek] under managed growth conditions. Sci Hortic. 2016;213:379-91.
13. Ullah MZ, Hasan MJ, Chowdhury AZMKA, Saki AI, Rahman AHMA. Genetic variability and correlation in exotic cucumber (Cucumis sativus L.) varieties. Bangladesh J Plant Breed Genet. 2012;25(1):17-23.
14. Taiz L, Zeiger E. Plant physiology. 3rd ed. Sunderland: Sinauer Associates; 2002.
15. Joshi S, Shah S, Kalantar-Zadeh K. Adequacy of plant-based proteins in chronic kidney disease. J Ren Nutr. 2019;29(2):112-7.