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Original Research

Fulvic Acid Observations Associated to Physiology and Biochemistry on Arsenic Treated Wheat (*Triticum Aestivum* L.)

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Abstract

The recent study aimed to determine whether the use of fulvic acid can enhance the inhibitory effects of arsenic stress on the growth and physiology of wheat, thereby inducing arsenic tolerance in wheat. One specific variety of wheat, known as Lasani wheat (*Triticum aestivum L*), was obtained from the Ayyub Agricultural Research Institute in Faisalabad, Pakistan. Seeds of this variety were planted in jars filled with sandy loamy soil (dimensions: 25 cm in height, circumference of 20 cm at the top, and 16 cm at the bottom). The research was conducted using a completely randomized design with three replicates. At the vegetative stage, wheat plants were subjected to one level of arsenic (3 mM) along with a control group. After 15 days of arsenic treatment, a foliar spray of fulvic acid (1.5 mM) was applied to the plants. Some plants received no spray, while others were sprayed with water. Tissue samples were collected after 21 days of treatment to assess growth, physiological, and biochemical characteristics. The results clearly showed that arsenic stress significantly affected the growth and physiochemical traits of wheat. Foliar application of fulvic acid increased various growth factors, such as root and shoot length, fresh and dry weight, and leaf area in wheat. Furthermore, fulvic acid enhanced the activities of antioxidant enzymes, including POD, SOD, APX, and CAT. It also increased the levels of total soluble protein, chlorophyll, proline, and total amino acids under arsenic stress. Malondialdehyde and H_2O_2 levels increased under arsenic stress, but a foliar spray of fulvic acid mitigated the oxidative stress.

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All experimental data were analyzed using the software COSTAT through analysis of variance (ANOVA) procedures.

Keywords: fulvic acid (FA), plants, arsenic, growth, enzyme, soluble protein, metallic

Introduction

The most popular food source around the world is wheat, which provides almost 30% of global food energy. Its high starch content, about 60-70% of animal grain, and its high protein content make it an important source of protein. Wheat production is increasing in Europe and Pakistan, with wheat production reaching over 20% million tons. Its nutritional value is significant, especially in less populated countries, where it is used in bread, noodles, and various products. Wheat genotypes have a strong defense mechanism [1]. Abiotic stress, such as soil salt, drought, heat, cold, flooding, and ion stress, significantly influences crop growth and output around the world. Metal deposition in plants' edible parts poses a significant threat to humans, animals, and plants. Some essential micronutrients, such as copper, zinc, and iron, are essential for plant and animal movement. However, the outcomes of metallic accumulation often result in deadly compounds like copper, mercury, zinc, chromium, nickel, lead, cadmium, and arsenic. These lethal compounds can destroy soil and biodiversity, disturb food webs, and pose severe health risks [2]. Arsenic is used in plants in two ways: through the roots and the 26-like protein nodule. Arsenic stress in plants reduces biomass collection and growth, upsets photosynthesis, and induces reactive oxygen species (ROS), injuring cellular macromolecules [3]. Artificial arsenic is extra dangerous due to its biological system and extraordinary toxicity. It interacts with sulfhydryl accumulation, limiting cell activity and possibly leading to cell death [4]. Research advises that naturally, arsenic may be more dangerous than toxic metals. Arsenic interaction influences antioxidant defense mechanisms, such as the making of phytochelatins in roots and decreased plant evaporation force [5]. Plants require various strategies to filter harmful substances, mainly containing metallic compounds. Hereditary variations in plant life reactions can improve agriculture varieties and make them more fit to increase arsenic in soils. Advance species may either lessen arsenic transport to seeds or bound arsenic transport to seeds, leading to lessened food contact with arsenic. Certain plant classes, particularly those in areas with increasing metal concentrations, have the exceptional capability to adjust and change to significant elements. Plant resistance to metals is significantly influenced by plant classes and genetic makeup [6]. Hyper accumulators mature on unprocessed loams and can bloom on contaminated soils and produce high amounts of minor components in biofuel. Multiple defense mechanisms have been suggested for enhanced plants' physiological response to harmful metals, including complex particles, reduced metal flow, and better enzyme creation. The occurrence of heavy metals in undergrowth may distinguish the ability of plants to persist in soils poisonous to different plants [7].

One of the naturally occurring organic compounds is Fulvic acid (FA), which is found in plants and soil and consists of high-atomic weight substances that are made through secondary amalgamation responses [8]. These compounds are formed of ore, protein, nucleic acid, carbohydrates, vitamins, phytoconstituents, and plant DNA. FA is more physiologically responsive due to the presence of numerous OH and COOH groups. It is found in fruits and vegetables and takes part in cellular respiration due to its lesser mass [8]. FA is created by microorganisms in soil and originates in fruits and vegetables. It has a variety of beneficial uses in diabetes, diuretic, hypoglycemic effects, antihypertensive effects, anti-oxidant, antimicrobial, anti-inflammatory, immunomodulation, gut health, and Alzheimer's infections. It also has a powerful free radical hunter and lessens oxidative pressure markers. FA is vital for plant growth and health, as it is an important component of the plant's metabolic system [9]. The durable antioxidant action of FA may present its beneficial effect on human health. Likewise, used in the biomedical field, FA has additional industrial applications in bioremediation, photo catalytic reactant, and lactase-based catalysis. FA is frequently used in limited fields such as veterinary medicine, agribusiness, ecological safety, human medication, and life science. FA has discovered anti-inflammatory and immune-modulation effects across recommendations of dissimilar genes intricate in lipid digestion and inflammation. It is validated that FA treatment for respiratory tract infection, cancer, brain syndrome, tiredness, allergy, and asthma reduces heavy metal toxicity [10]. The order of arsenic is as follows: roots>leaves>shoot>achenes. The comparatively minor arsenic accumulation in achenes recommended that there was a greater number of arsenic deposited in the root and sap of the leaves. This discovered that roots and leaves prevailed over achene. Moreover, it has been stated that with stress tolerance in plants, the uphill transport of arsenic components was reduced, resulting in a higher collection of arsenic in the roots of the plants [11].

This study involves the exogenous application of fulvic acid to improve arsenic stress tolerance for cost-effective crop production. It also analyzes the physiological processes controlling the growth and yield of wheat.

Materials and Methods

Seed and Chemicals Collection

All the chemicals used in the experiment were purchased from Biorad, Sigma, Merck, BDH, Fluka, and Aldrich, as well as ACS or AR grades. The Department of Botany at Government College University, Faisalabad, has performed an experiment in a pot to examine how wheat's growth, physiological makeup, and biochemical properties vary over time (*Triticum aestivum* L). The Ayyub Agricultural Research Institute (AARI) in Faisalabad, Pakistan, has provided one variety of wheat (Lasani-2008). Every pot has a hole drilled in the base for leaking throughout the solution changeover. Some seeds of one variety were spread in a plastic container (dimension 25 cm in height, boundary of 20 cm in the upper and 16 cm at the end). The experiment was conducted in plastic pots filled with sandy, loamy soil.

Experimental Design

Five uniform seedlings were maintained in each pot. At the vegetative stage, arsenic stress (3 mM) was applied to plants. Various foliar treatments, including

- control (no spray),
- water spray,
- and fulvic acid (1.5 mg L^{-1})

FA was applied to plants after 15 days of arsenic application. Shoot sampling was done 21 days after the application of the FA to record physiochemical characteristics. A control group without arsenic application was maintained for comparison. Two weeks after germination, plants were sprayed with a concentration of FA (1.5 mg L^{-1}) solution for each wheat seedling. An equivalent amount of urea was added to the controlled plants to compensate for the differences resulting from the FA application. The controlled plants were sprayed with distilled water in the same amount. After two weeks of arsenic treatment, the plants were removed, and the fresh leaves were frozen at -20ºC to record various growth and biochemical characteristics. The experiment was carried out in CRD by four repetitions. A statistical analysis of the data was performed with the help of the appropriate software.

Growth Attributes

The plants were carefully uprooted from the container soil to prevent any harm to the entire intact plant. Before phenotypic analysis could begin, the intact uprooted plants were thoroughly cleaned with water to get rid of any remaining dust and soil particles. The metric scale was utilized to calculate the lengths of the roots and shoots because the tissues of the two organs are entirely separated. After being rooted up, their fresh weight was determined immediately. Some plant samples were then wrapped in paper bags, labeled, and oven dried for 72 h at 70ºC to determine the dry weight of the plants.

Physiological Characteristics

Several physiological characteristics are analyzed such as:

Chlorophyll Contents / Photosynthetic Pigments

The chlorophyll *a*, *b*, and carotenoids were measured using 0.5 g leaves, crushed in methanol (80%) with the help of mortar and pestle. Then, they were centrifuged at 15000 rpm for 15 min with the volume of material retained up to 5 mL. In a UV-VIS (Hitachi U–2910) spectrophotometer, the optical density (OD) of supernatants was noted at 663 nm, 645 nm, and 480 nm. Carotenoids were discovered utilizing DAVIS (1975) methodology. The following formula was used for chlorophyll *a* and *b* and total carotenoid contents.

$$\text{Chl.}a \text{ (mg/g of leaf fresh weight)} = [12.7(OD_{663})\text{-}2.69(OD_{669})]\times V/1000\times W$$

$$\text{Chl.}b \text{ (mg/g of leaf fresh weight)} = [22.9(OD_{645})\text{-}4.68(OD_{663})]\times V/1000\times W$$

$$\text{Total chl. (mg/g of leaf fresh weight)} = [20.2(OD_{645})\text{+}8.02\ (OD_{663})]\times V/1000\times W$$

$$\text{Carotenes (mL/g of fresh leaf)} = \{[OD480)\text{+}0.114(OD663)]/2500\}$$

Where V = extract volume of acetone in, and W = weight of newly picked leaf tissue in milligrams

Biochemical Characteristics

Hydrogen Peroxide (H_2O_2) Contents

The H_2O_2 content was calculated using 0.25 g plant leaves, ground in 2 mL TCA (0.1 percent (w/v)), and centrifuged at 12,000 *g* for 15 min. The KH_2PO_4 (0.5 mL) with pH 7.0 and 10 mM, in addition to 1 mL of PI (1M), was then added to the 0.5 mL extract. The materials were kept for a long period. After that, absorbance was measured by UV-VIS spectrophotometer (Hitachi U–2910) at 390 nm, and blank values were taken with TCA (0.1 % (w/v)). A stranded curve was utilized to calculate the H_2O_2 concentration using 35% H_2O_2.

Malondialdhyde (MDA) Contents

For MDA concentration measurement, 1 mL of leaf matter (0.25 g) was ground (5% TCA) at 12,000 *g* and centrifuged for 15 min. One milliliter of the extract was combined with one milliliter of TBA 0.5%

and 1 mL of TCA 20% (w/v). The sample was then kept in water bathed for 50 min at 95°C before being cooled to room temperature. Using a UV-VIS (Hitachi U–2910) spectrophotometer, absorbance measurements were performed at 600 nm and 532 nm. However, TCA (5%) was employed as a blank.

$$MDA\ (\mu mol\ mL^{-1}) = [(A532\text{-}A600)/155000]10^{6}$$

Ascorbic Acid Concentration

A 0.5 g leaf material was ground in a 5 mL solution of trichloroacetic acid (TCA) at a concentration of 6% TCA. Then 2 mL dinitrophenyl hydrazine (2%) in solution (4 mL) was added, followed by one drop of thiourea (70% ethanol). Place the reading combo inside the steam bath for 15 min at hundred degrees Celsius. Allow the mixture to cool to room temperature before adding 0.7 mL H_2SO_4. Then, using a spectrophotometer UV-VIS (Hitachi U–2910), check the absorbance at 530 nm.

Total Soluble Protein Contents

Bradford technique (1976) [12] was used to calculate total soluble protein. In 2 mL of potassium buffer (pH = 7), 0.25 g of leaf tissue was ground. The produced sample was placed in an Eppendorf tube and centrifuged for 15 min at 12000 rpm. In the test tube, 0.1 g of supernatant was taken, and up to 2 mL of Bradford reagent was added. Before using the test tube, it was kept at room temperature for 35 min. At 595 nm, a reading was taken by using a UV-VIS spectrophotometer (Hitachi U-2910, Tokyo, Japan), and bovine serum albumin (BSA) was used as standard. As a blank, 0.1 mL buffer and 2 mL Bradford reagent were employed.

Total Soluble Sugars

Riazi et al. (1985) [13] method was used to assess the TSS concentration. Plant leaves 0.5 g were immersed in 5 mL methanol for 60 min at 60ºC in an incubator. The liquid was then transferred to a container and diluted with alcohol to reach a level of 25 mL. 1 mL material, 1 mL phenol (5%), and analytical grade H_2SO_4, 5 mL were transferred to test tubes and mixed before being chilled at ambient temperature. The OD at 480 nm was measured by a spectrophotometer (Hitachi U–2910).

Reducing Sugar Content

Riazi et al. (1985) method [13] was used to determine reducing sugar content. In 0.1 g of plant leaves, 1 mL of 80% methanol was poured and grinding. 0.5 mL DNS reagent follows. In a 100 mL supernatant, 1 mL NaOH, 30 g KNO_3, 100 mL dH_2O, 3, 5-dinitro salicylic acid, and one milliliter of sanitized water were added. The reaction mixture cooled in the water bath. The optical fate was assessed using a UV-VIS spectrophotometer (Hitachi U-2910) at 540 nm absorbance measurement**.**

Free Amino Acid Content

The free amino acid content was detected using the Hamilton and Van–Slyke (1943) method. For this reason, 0.25 g of the leaf was immersed in a potassium buffer solution for the whole night. 1 mL material was transferred to a 25 mL test tube, 0.5 mL ninhydrin solution (10%). Then, they injected 0.5 mL of pyridine solution (2%). The material was then held in a water bath for 20 min at 100ºC and transferred distilled water to all test tubes up to 25 mL to keep the volume. Using a spectrophotometer, OD was evaluated at 570 nm.

$$\text{Total amino acid (mg/g fresh weight)} = \text{Graph reading of sample} \times \text{volume of sample dilute /Weight of the tissue} \times 1000$$

Weight of the tissue × 1000

Phenolic Determination

To determine the total phenolic content, a 0.5 g leaf grind in 5 mL methanol (80%), then centrifuged for 10 min, 0.1 mL sample, 2 mL distilled water, 1 mL Folin–Ciocalteus phenol chemical, and 2 mL $NaCO_3$ were added to the test tube for readings. After shaking the sample for 5 min, a reading at 750 nm was recorded.

Flavonoids Content Determination

The flavonoids were determined using 1 mL plant sample, 2 mL NaOH and 0.3 mL sodium nitrite, 0.3 mL aluminum chloride to make up the reaction mixture. Readings were taken at 510 nm after shaking the test tube.

Anthocyanin Content

Hodges & Nozzolillo (1996) method [14] was used to evaluate the anthocyanin content. Another 100 mM potassium phosphate solution (PH = 7.8) was prepared, and 0.5 g of plant leaves were crushed in it. The solution was centrifuged at 14000 rpm over 5 min at 4ºC, and a reading at 600 nm was recorded using a spectrophotometer Hitachi (U-2910).

Glycine Betaine Content

Taken a 0.5 g sample and crushed it in 20 ml of suitably deionized water. At 25ºC, the leaf mixture was continuously shaken for 2 hours. The sample material was filtered to use a filter. The mixture was then combined in a ratio of 1:1 with $2NH_2SO_4$. In a

cleaned test tube, 0.5 ml of precipitate was poured, and the solution was chilled in a water bath for one hour. The material was maintained at 40ºC for 16 hours, such as the tubes. The supernatant was then collected and chilled in a test tube. The crystal was then dissolved in 9 ml dichloromethane by whisking the mixture. The sample was placed at 25°C for 2-2.5 hours and the nm was recorded with a spectrophotometer set to 350 nm.

Activities of Antioxidant Enzymes

0.5 g of leaf material was crushed in 10 mL in phosphate buffer 50 mM at pH 7.8 to evaluate the activities of antioxidants. The enzyme activity of POD, CAT, and SOD samples was examined by centrifuging for fifteen minutes at 12000 rpm at 4ºC and placing in a freezer being utilized to assay ascorbate peroxidase (APX) activities, peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD). Ascorbate peroxidase (APX) activity was investigated by using the Nakano and Asada technique (1987). PBS (50 mM; pH 7.5), Hydrogen peroxidase (0.1 mM), 0.5 mM ascorbic acid, and enzyme excision make up the mixture (200 µL). The spectrophotometer (Hitachi U–1800) is used to determine the absorbance of the sample every 20 s at 290 nm after every 20 s for 2 min and EU mg^{-1} protein states the APX activity. One unit of APX is the amount of protein used to analyze 1.0 µmol of substrate per min at 25°C. Likewise, POD activity must be determined by the method of Comba et al. (1998) [15]. 0.25 g of fresh leaf ground in K_2HPO_4 (pH = 7). 1.5 mL Guaiacol (5 mM), 1.5 mL Hydrogen peroxidase (15 mM) added to the cuvette, along with 0.1 mL plant extract. Every 20 min, the cuvette was placed in the spectrophotometer (Hitachi U–1800) and the sample absorbance was measured at 470 nm.1mL phosphate buffer, 1.5 mL Guaiacol, and 1.5 mL H_2O_2 were used as blanks. Accordingly, Cakmak et al. (1993) [16] determined the catalase activity. 0.2 g of wheat leaves were ground in 50 Mm $NaH_2PO_4.H_2O$ solutions (pH = 7.2), and 3 mL of phosphate buffer (pH = 7.2) was placed in a quartz cuvette. Then, in a cuvette, 0.1mL of 5.9 mM H_2O_2 and 0.1mL of enzyme material (leaf sample) were added. As soon as the enzyme source was applied, the response began. Then, every 20 seconds, readings were obtained on a spectrophotometer at 240 nm. Superoxide dismutase (SOD) activity of fresh leaves of wheat was estimated with Gong et al. (2005) protocol [17] to assay the activity of SOD. Then 0.5 g fresh sample was chopped in 100Mm (pH 7.8) cooling potassium phosphate buffer before being centrifuged at 4ºC for 10 min at 14000 rpm. 0.5 mL Methionine, 0.1 mL Na_2Co_3, 0.5 mL potassium phosphate buffer, 0.5 mL enzyme extract, 0.5 mL riboflavin, and 0.1 mL NBT were included in the sample mixture. The process was maintained at 78 $\mu molm^{-2}s^{-1}$ beneath light (15-W Fluorescent bulb). The absorbance of SOD at 390 nm was measured using a spectrophotometer (Hitachi U–1800) for 15 min.

Statistical Analysis

All data analysis was made in triplicate from this completely randomized experiment. The presence or absence of significant differences in different factors was determined with analysis of variance (ANOVA). Computer software COSTAT (CoHort software, 2003, Monterey, California) was used for all statistical analysis and MS-Excel was used to graphically present the data.

Results

Shoot Length

For the shoot length of the plants, analysis of data indicated that there was a significant difference (P≤0.001) in arsenic stress, fulvic acid spray (P≤0.01), and non-significant (P≥0.05) interaction between these factors (Table 1). Shoot length was decreased (18%) under arsenic stress (3 mM) compared to the control plants. Foliar-applied FA (1.5 mg/L) increased the shoot length in control (10%) and stressed plants (27%), respectively. Water spray enhanced the shoot length in the control (0.63%) as well as in stressed plants (4%).

Table 1. Analysis of variance (ANOVA) of data showing the change in shoot length of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	388.81	388.815	32.17	0.0000***
FA spray (B)	2	449.12	224.558	18.58	0.0000***
A x B	2	54.93	27.466	2.27	0.1318ns
Error	18	217.57	12.087		
Total	23	1110.44			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

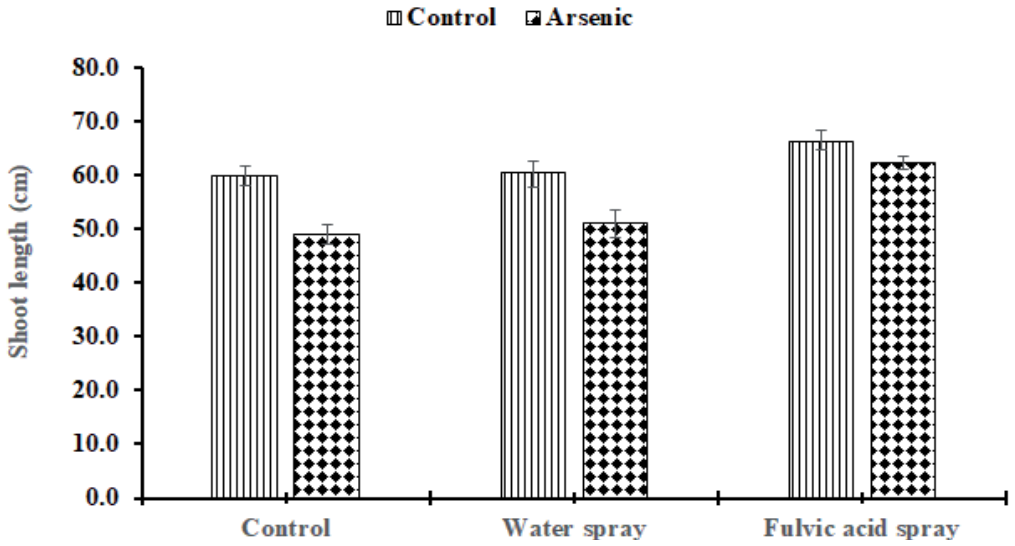

Fig. 1. The change in shoot length of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

The maximum increase in shoot length was recorded in both control and stressed plants by foliar application of FA (Fig. 1).

Root Length

For the root length of the plants, the data analysis found that there was a significant difference (P≤0.001) in arsenic stress, and foliar spray of FA (P≤0.05) with non-significant (P≥0.05) interaction between these factors (Table 2). Root length was reduced (44%) under arsenic stress (3 mM). Foliar application of FA (1.5 mg/L) enhanced (5%) the root length in control plants. FA spray eradicated the harmful effect of arsenic stress and improved the root length (15%). Whereas water spray increased the root length in both controls (0.71%) and stressed plants (3%). A greater increase in root length was noted in both controls as well as arsenic stress by FA spray (Fig. 2).

Shoot Fresh Weight

For the fresh weight of the shoot of the plants, analysis of the data presented that there was a significant difference (P≤0.001) in As stress, FA foliar spray (P≤0.01), and non-significant (P≥0.05) contact between these factors (Table 3). Shoot fresh weight was repressed (50%) under arsenic stress (3mM), respectively, as compared to control plants. Foliar application of FA (1.5 mg/L) the shoot fresh weight enhanced under control conditions (54%). The foliar application FA distant the negative effect of As stress and raises the shoot fresh weight (74%) under (3 mM) of arsenic stress. Shoot fresh weight was increased (14%) in the water spray of control prominent as stress (10%). The maximum increase in shoot fresh weight is noted under arsenic stress by foliar application of FA (Fig. 3).

Root Fresh Weight

For the fresh weight of the roots of the plants, data analysis revealed that there was a significant difference

Table 2. Analysis of variance (ANOVA) of data showing the change in root length of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	326.639	326.639	262.36	0.0000***
FA spray (B)	2	6.619	3.310	2.66	0.0974*
A x B	2	0.438	0.219	0.18	0.8402ns
Error	18	22.410	1.245		
Total	23	356.105			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

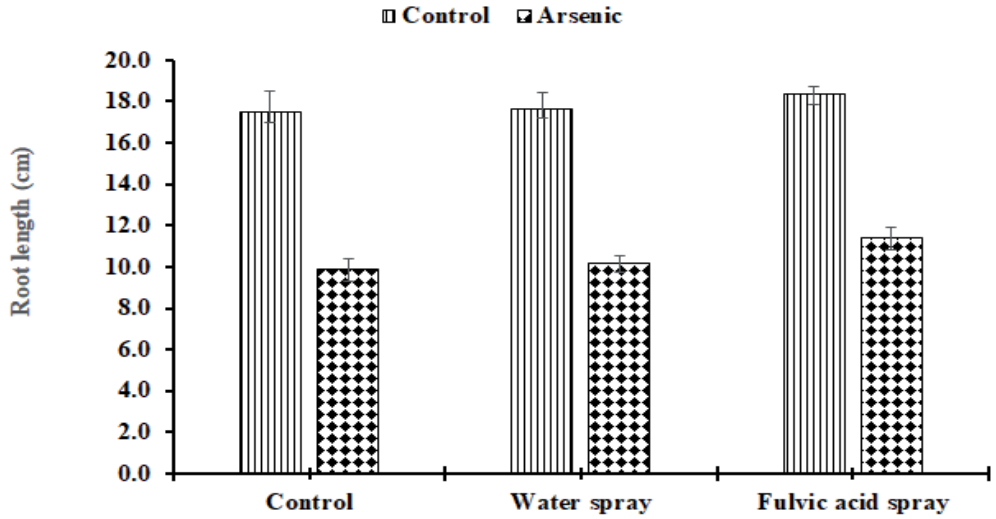

Fig. 2. The change in root length of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

(P≤0.001) in arsenic stress (P≤0.01) and non-significant (P≥0.05) interaction between these factors (Table 4). Root fresh weight was reduced (59%) under arsenic stress (3 mM), as compared to control plants. Foliar application of FA (1.5 mg/L) and combination uplifted the root fresh weight under control conditions (45%). The foliar application of FA minimized the disastrous effects of arsenic stress and increased the root fresh weight (80%) under both arsenic stresses. When the record of water spray increased the root fresh weight of control (3%), arsenic stress plant (16%). The maximal increase in root fresh weight was recorded under arsenic stress (3 mM) by foliar application of Fulvic acid, separately (Fig. 4).

Shoot Dry Weight

For the dry weight of shoots of the plants, data analysis indicated that there was a significant difference (P≤0.001) in arsenic stress, FA foliar spray (P≤0.01), and non-significant (P≤0.05) interaction between these factors (Table 5). Shoot dry weight was reduced (49%) under Arsenic stress (3 mM), respectively, as compared to control plants. Foliar application of FA (1.5 mg/L) improved the shoot dry weight under control conditions (22%). The foliar application of FA eliminated the suppressing effects of arsenic stress and enhanced the shoot dry weight (71%) under arsenic stress, respectively. The water spray enhances the shoot dry weight in the control (1%) and stressed plants (6%). The maximum increase in shoot dry weight was noted under arsenic stress by foliar application of FA (Fig. 5).

Root Dry Weight

For the dry weight of roots of the plants, analysis of the data showed that there was a significant difference (P≤0.001) in As stress, FA foliar spray (P≤0.05) with non-significant (P≥0.05) interaction between these factors (Table.6). Root dry weight was minimized (60%) of stress (3 mM), correspondingly as associated to control plants. FA-injected foliar (1.5 mg/L) reduced the root dry weight under control conditions (16%). The FA spray foliar eradicated the effects of arsenic

Table 3. Analysis of variance (ANOVA) of data showing the change in shoot fresh weight of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	221.738	221.738	234.15	0.0000***
FA spray (B)	2	101.238	50.619	53.45	0.0000***
A x B	2	3.127	1.563	1.65	0.2196ns
Error	18	17.046	0.947		
Total	23	343.148			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

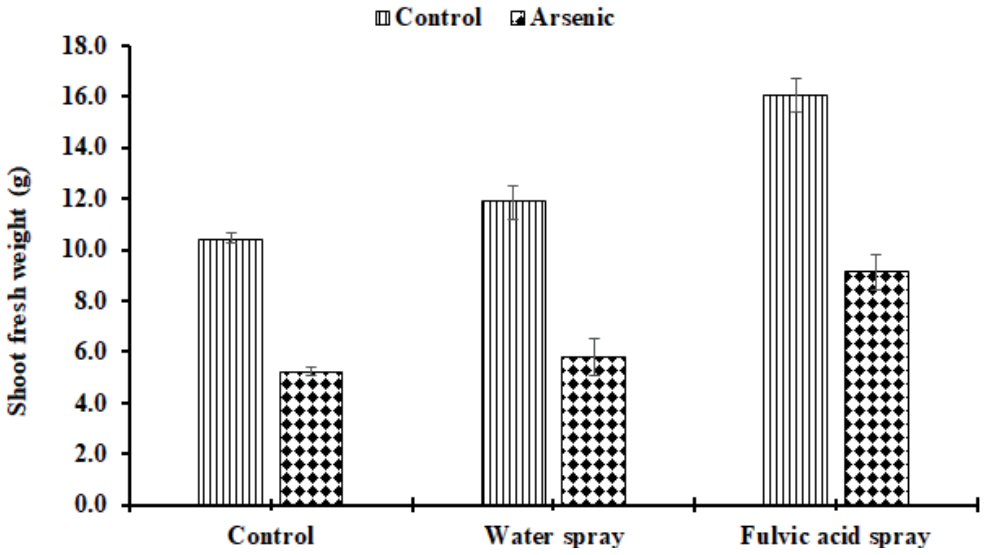

Fig. 3. The change in shoot fresh weight of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

Table 4. Analysis of variance (ANOVA) of data showing the change in root fresh weight of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	12.2408	12.2408	224.54	0.0000***
FA spray (B)	2	3.9751	1.9875	36.46	0.0000***
A x B	2	0.1622	0.0811	1.49	0.2523ns
Error	18	0.9813	0.0545		
Total	23	17.3594			
*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.					

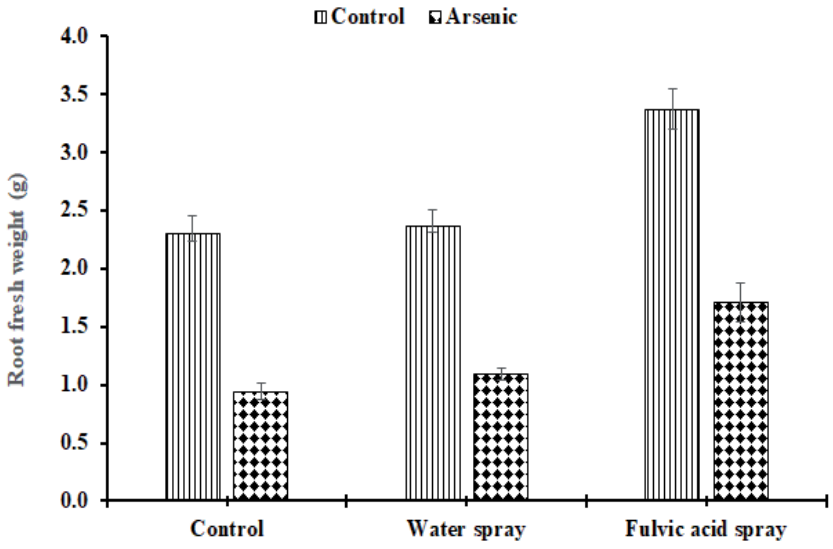

Fig. 4. The change in root fresh weight of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

Table 5. Analysis of variance (ANOVA) of data showing the change in shoot dry weight of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	10.4280	10.4280	98.15	0.0000***
FA spray (B)	2	3.7292	1.8646	17.55	0.0001***
A x B	2	0.1927	0.0963	0.91	0.4215ns
Error	18	1.9124	0.1062		
Total	23	16.2623			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

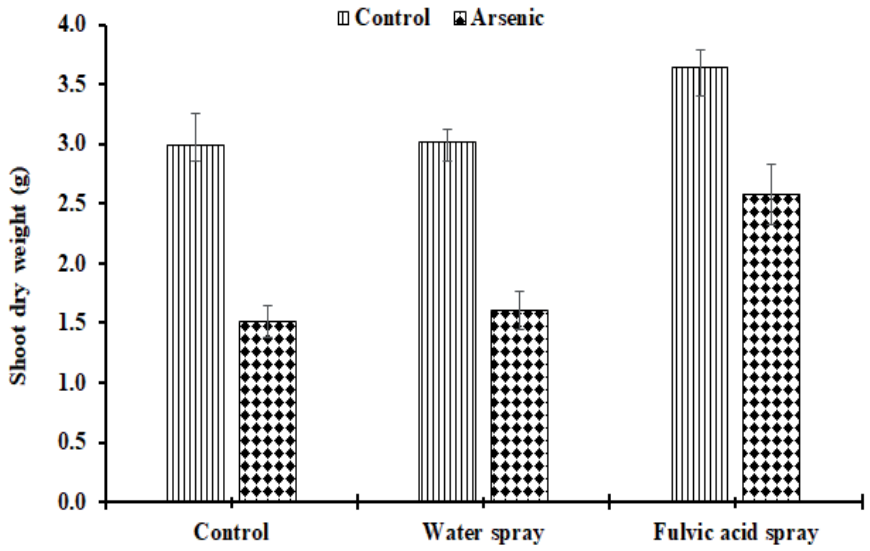

Fig. 5. The change in shoot dry weight of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

stress and improved the root dry weight (44%) under (3mM) level of arsenic stress. Because water sprays root dry weight in control (8%), As affected plant (5.2%). While Ars stress, FA application showed a greater rise in root dry weight (Fig. 6).

Leaf Area

For the leaf area of the plants, analysis of the data examines that there was a significant change (P≤0.001) in arsenic stress, FA application, and significant (P≥0.001) with (P≤0.01) interface between these factors (Table 7). Leaf area was lowered (24%) undervalues of cobalt stress, in that order as related to control plants. When Foliar sprayed FA (1.5 mg/L), the leaf area decreased (12%) as compared to control conditions. Foliar application FA did not remove the impact of arsenic stress and decreased the leaf area (34%) under concentrations (3 mM) of arsenic stress, correspondingly. Water spray increases the Leaf area in control (0.7%) while in stress plants (8%). A greater rise in leaf area is represented in both controls other than arsenic stress by FA spray (Fig. 7).

Number of Leaves

For the number of leaves of the plant, data analysis informed that there was a significant difference (P≤0.001) in arsenic stress, foliar spray of FA significant (P≤0.01) with non-significant relations between these factors (Table 8). The number of leaves was diminishing (59%) below arsenic stress (3 mM), as associated with control plants. Foliar FA spray (1.5 mg/L) improved the number of leaves under control conditions (45%). Where the FA spray narrows arsenic stress and higher (81%) in arsenic stress. With the water spray, the number of leaves was greater in arsenic control (15%) while in FA (2%). An extreme rise in the number of leaves was detected due to arsenic stress by foliar spray of fulvic acid (Fig. 8).

Table 6. Analysis of variance (ANOVA) of data showing the change in root dry weight of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	0.27094	0.27094	105.05	0.0000***
FA spray (B)	2	0.01390	0.00695	2.69	0.0947*
A x B	2	0.00070	0.00035	0.14	0.8740ns
Error	18	0.04642	0.00258		
Total	23	0.33196			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

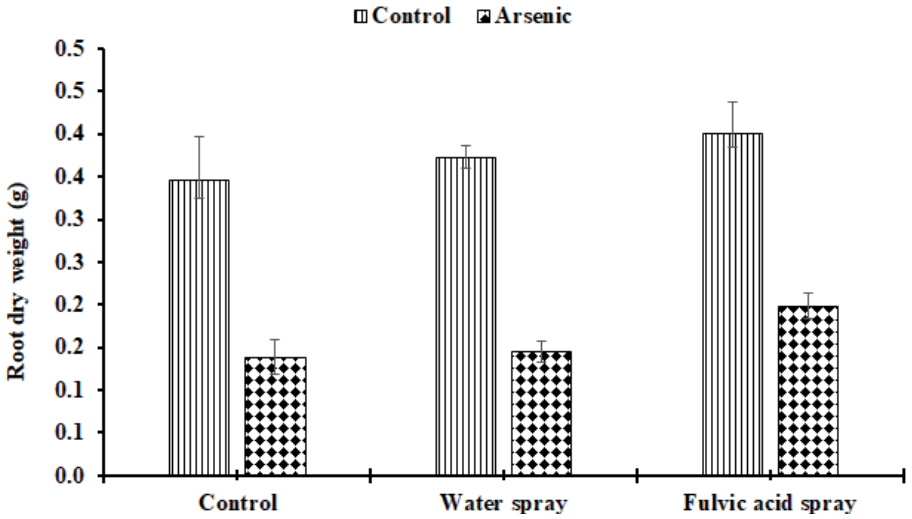

Fig. 6. The change in root dry weight of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

Chlorophyll *a* Contents

For the chlorophyll *a* content of the plant, statistical analysis of data represents that there is a significant difference (P≤0.001) in As stress, foliar FA spray (P≤0.01) with a non-significant difference (P≥0.05) reaction between these features (Table 9). Chlorophyll content was minimized (49%) level of arsenic stress (3 mM), as linked to control plants. Foliar FA treatment was applied (1.5 mg/L) to the chlorophyll a content in the control condition (12%). The FA foliar use excluded the effects of as stress and increased (27%) with arsenic stress (3 mM). While the water spray increased the chlorophyll a in control (0.20%), control of arsenic (3.34%). The utmost (27%) in chlorophyll a content was detailed As stress (3 mM) by foliar FA applied (Fig. 9).

Chlorophyll *b* Contents

For the chlorophyll *b* content of the plant, analysis of the data observed that there was a significant difference (P≤0.001) in Arsenic stress, Fa foliar spray (P≤0.001), non-significant difference (P≥0.05) in the interaction between these factors (Table 10). Chlorophyll b content was reduced (26%) by arsenic stress (3 mM), and matched to control plants. Foliar FA rate (1.5 mg/L), the chlorophyll b content increased (16%) under control conditions. With FA spray reduced the effects of arsenic stress and enhanced the chlorophyll b content (36%) in (3 mM) of Ars stress. With water spray, chlorophyll b content is greater in control (16%) and also in stress plants. The top growth (6%) in chlorophyll b content was famous for arsenic stress (3 mM) by FA spray (Fig. 10).

Total Chlorophyll Contents

For the total chlorophyll content of the plant, statistical analysis of data showed a significant difference (P≤0.001) in arsenic stress with FA foliar (P≤0.001) and non-significant difference (P≥0.05) relations between these factors (Table 11). Total chlorophyll content was decreased (38%) with arsenic stress (3 mM) as matched to control plants. When Foliar FA (1.5 mg/L)

Table 7. Analysis of variance (ANOVA) of data showing the change in leaf area of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	25.4616	25.461	83.33	0.0000***
FA spray (B)	2	22.5721	11.2860	36.94	0.0000***
A x B	2	2.8415	1.4207	4.65	0.0236**
Error	18	5.4996	0.3055		
Total	23	56.3748			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

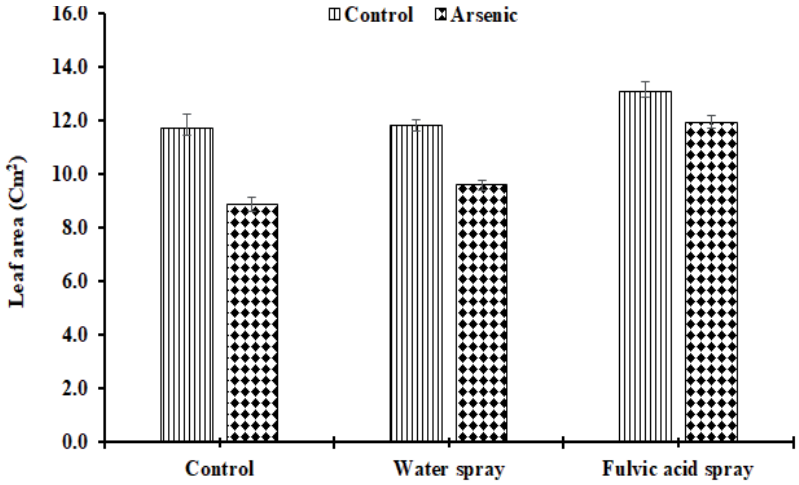

Fig. 7. The change in leaf area of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

Table 8. Analysis of variance (ANOVA) of data showing the change in the number of leaves per plant of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	96.000	96.0000	80.37	0.0000***
FA spray (B)	2	11.583	5.7917	4.85	0.0207**
A x B	2	0.250	0.1250	0.10	0.9012ns
Error	18	21.500	1.1944		
Total	23	129.333			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

total chlorophyll content greater than control conditions (14%). Foliar FA spray lessened the effects of As stress and improved (32%) the level of As stress (3mM), whereas the water sprayed the total chlorophyll in control (1.96%), stress plant (5%). The best growth (32%) in total chlorophyll content was detected under arsenic stress (3 mM) with FA spray (Fig. 11).

Total Carotenoid Contents

For the carotenoid content of the plant, statistical analysis of the data illustrated that there was a significant difference (P≤0.001) in Ars stress, foliar FA spray (P≤0.01) with a non-significant difference (P≥0.05) interaction between these factors (Table 12). The

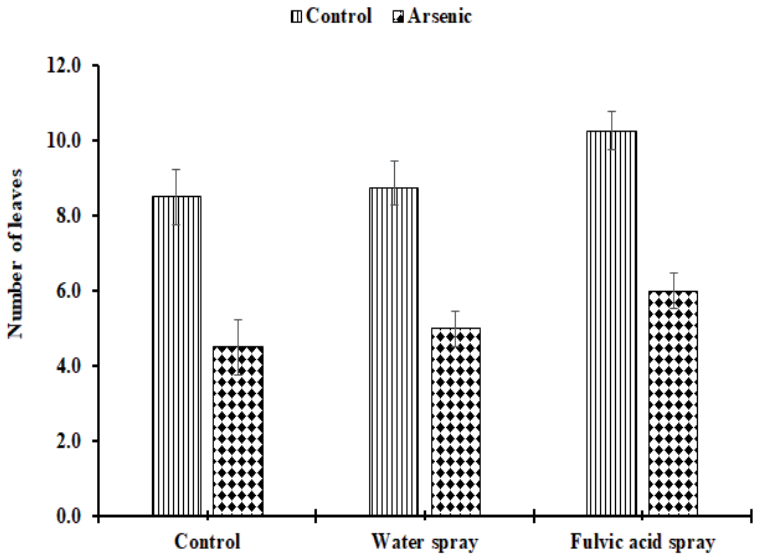

Fig. 8. The change in the number of leaves per plant of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

Table 9. Analysis of variance (ANOVA) of data showing the change in chlorophyll *a* contents of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	22.2284	22.2284	134.48	0.0000***
FA spray (B)	2	1.3567	0.6784	4.10	0.0340**
A x B	2	0.0052	0.0026	0.02	0.9843ns
Error	18	2.9751	0.1653		
Total	23	26.5655			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

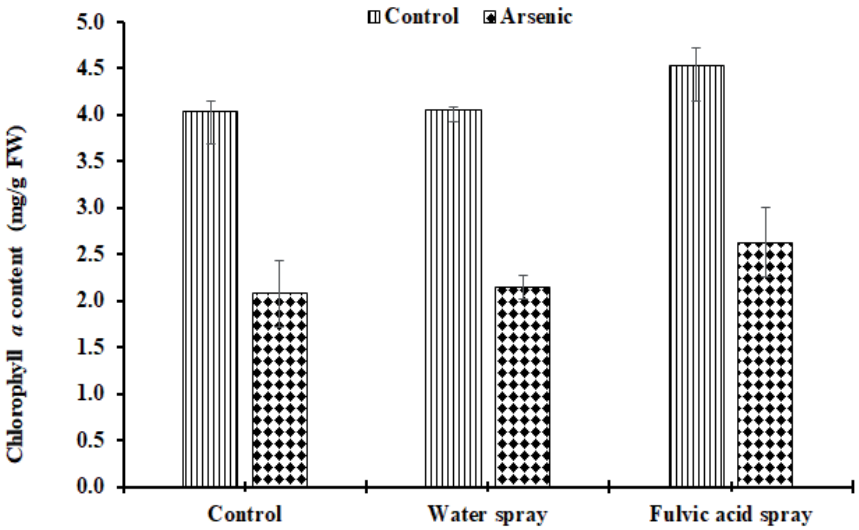

Fig. 9. The change in chlorophyll *a* contents of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

Table 10. Analysis of variance (ANOVA) of data showing the change in chlorophyll *b* contents of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	3.32165	3.32165	30.51	0.0000***
FA spray (B)	2	2.39588	1.19794	11.00	0.0008***
A x B	2	0.16446	0.08223	0.76	0.4842ns
Error	18	1.95970	0.10887		
Total	23	7.84168			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

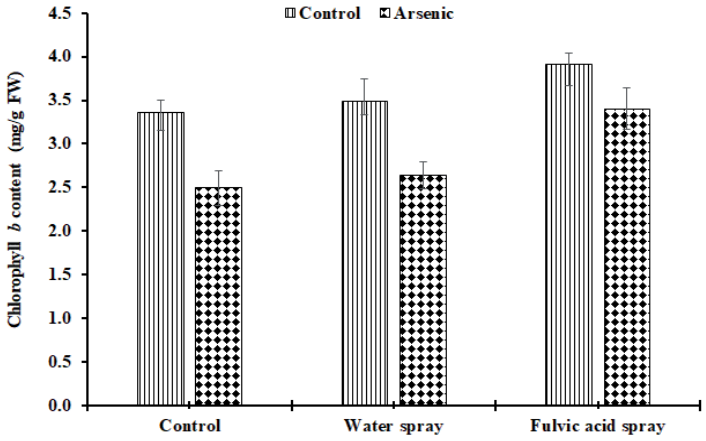

Fig. 10. The change in chlorophyll *b* contents of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

Table 11. Analysis of variance (ANOVA) of data showing the change in total chlorophyll contents of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	42.7200	42.7200	193.69	0.0000***
FA spray (B)	2	7.3467	3.6733	16.65	0.0001***
A x B	2	0.2009	0.1005	0.46	0.6413ns
Error	18	3.9700	0.2206		
Total	23	54.2376			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

carotenoid content was dropped (48%) in arsenic stress (3 mM), as harmonized to control plants. Foliar spray FA (1.5 mg/L) and better the carotenoid content under control conditions (17 %). The foliar Fulvic acid reduced the arsenic stress and enhanced the carotenoid content (39%) under (3 mM) of arsenic stress. Water spray increased the total carotenoids in control (9.1%) while in arsenic stress (12%). The maximum increase (38.5%) in carotenoid content was experiential under Ars stress (3 mM) by applying FA spray (Fig. 12).

Malondialdehyde (MDA) Contents

For the malondialdehyde content of the plant, analysis of the data exposed that there was a significant difference (P≤0.001) in arsenic stress, FA spray (P≤0.001),

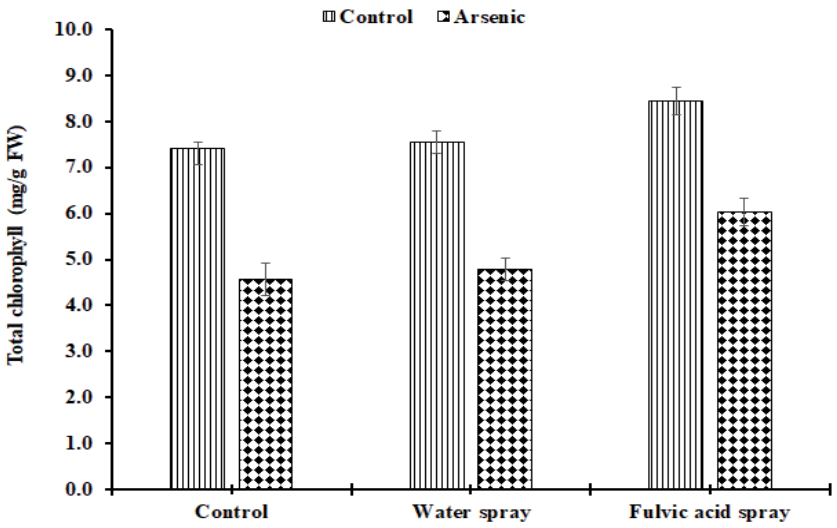

Fig. 11. The change in total chlorophyll contents of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

Table 12. Analysis of variance (ANOVA) of data showing the change in total carotenoid contents of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress.

SOV	df	SS	MS	F	P
Main Effects					
Arsenic stress (A)	1	0.42805	0.42805	107.39	0.0000***
FA spray (B)	2	0.04068	0.02034	5.10	0.0176**
A x B	2	0.00111	0.00056	0.14	0.8706ns
Error	18	0.07175	0.00399		
Total	23	0.54160			

*, **, *** = significant at P<0.05, P<0.01and P<0.001, respectively. ns = no significant at P>0.05.

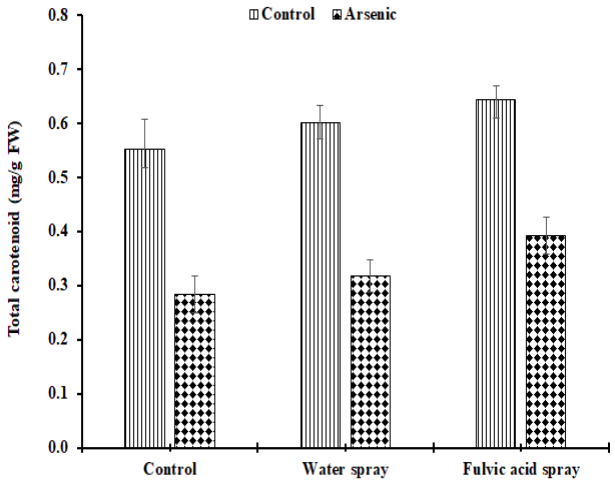

Fig. 12. The change in total carotenoid contents of wheat by exogenous application of fulvic acid at the vegetative stage under arsenic stress (n = 3; Mean±S.E).

non-significant (P≥0.05) contact between these factors (Table S1). The MDA content was increased (64%) with arsenic stress (3 mM) as linked to control plants. Foliar fulvic acid (1.5 mg/L) declined the MDA content in control conditions (52%). Whether FA foliar sprays could not alleviate the effects of arsenic stress and drop in arsenic stress (30%). The water spray reduced the MDA content in control (10%) As stress (3%). The determined turn down (51%) in MDA contents content was recorded in FA spray under As stress (3mM), respectively (Fig. S1).

Hydrogen Peroxide (H_2O_2) Contents

For the hydrogen peroxide content of the plant, analysis of the data determined that there was a significant difference (P≤0.001) in As stress, foliar FA spray (P≤0.001), non-significant (P≥0.05) dealings between these factors (Table S2). The H_2O_2 content was elevated (54%), As stress (3 mM), in comparison

to control plants. Foliar FA spray decreased the H_2O_2 content control conditions (24%). The foliar FA lessened the effects of As stress and dropped the H_2O_2 (32%) under arsenic (3mM). Water spray decreased the H_2O_2 content in the control (2%) as well as in the stress plant (6%). The concentrated diminish (32%) in H_2O_2 content was seen under As stress by foliar application FA (Fig. S2).

Total Ascorbic Acid Content

For the ascorbic acid content of the plant, statistical analysis of the data stated that there was a significant difference ($P \leq 0.001$) in As stress, foliar FA spray ($P \leq 0.001$) with the non-significant difference ($P \geq 0.05$) dealings between these factors (Table S3). The ascorbic acid content decreased (29%) the level of arsenic stress (3 mM) in comparison to that of control plants. Foliar FA sprays elevated the ascorbic acid content under control conditions (28%). Fulvic acid treatment mitigated the effects of arsenic stress and increased the ascorbic acid content (53%) under arsenic stress. Meanwhile, the water spray increased the ascorbic acid content in both control (3%) and stressed plants (4%). The extreme rise (52.9%) in ascorbic acid content was prominent under arsenic stress by FA spray (Fig. S3).

Total Soluble Protein Contents

For the total soluble protein content of the plant, data analysis discovered a non-significant difference ($P \geq 0.05$) in As stress, foliar FA spray was also non-significant ($P \geq 0.05$), a significant difference ($P \leq 0.01$) interface between these factors (Table. S4). The total soluble protein content was increased (6.4%) in arsenic stress as linked to control plants. Foliar FA application (1.5 mg/L) enhanced the total soluble protein content under control conditions (8.2%). The foliar FA spray eradicated the effects of arsenic stress and enlarged the total soluble protein content (15%) under arsenic stress, correspondingly. While water spray increased the total soluble protein in the control (0.53%) and the stressed plant (1.57%). The maximum rise (15%) in total soluble protein content was perceived under arsenic stress by FA spray (Fig. S4).

Total Soluble Sugar Content

For the total soluble sugar content of the plant, statistical analysis of data indicated a significant difference ($P \leq 0.001$) in arsenic stress, foliar FA spray ($P \geq 0.05$) with a non-significant difference ($P \geq 0.05$) interface between these factors (Table S5). Total soluble sugar content was increased (80%) with arsenic stress (3 mM) as compared to control plants. Foliar FA spray (1.5 mg/L) elevated the total soluble sugar content (16%) under control conditions. Foliar FA spray detached the effects of arsenic stress and boosted (20%) in applications of arsenic stress. The water spray increased the TSS in control (5.8%) as well as in arsenic plant (6.3%). The highest (19.84%) in total soluble sugar content was verified under arsenic stress by FA spray (Fig. S5).

Reducing Sugar Content

For the reducing sugar content of the plant, analysis of the data revealed a significant difference ($P \leq 0.001$) in arsenic stress, foliar FA spray ($P \leq 0.001$), and a significant difference ($P \leq 0.001$) dealings between these factors (Table S6). The reduced sugar content was decreased (90%) in arsenic stress (3 mM), as associated with control plants. Foliar FA (1.5 mg/L) greater reducing sugar content below control conditions (25%). The foliar FA spray reduced the effects of arsenic stress and increased the sugar content (36%) beneath (3 mM) of arsenic stress. The water spray increased the sugar content in the stressed plant (3%) and control (2.21%). The maximum improvement (36%) in reducing sugar content was illustrious under arsenic stress by the application of FA (Fig. S6).

Total Free Amino Acids Content

For the free amino acid content of the plant, analysis of data presented a significant difference ($P \leq 0.001$) in arsenic stress, foliar FA spray ($P \leq 0.001$), and non-significant difference ($P \geq 0.05$) relations between these factors (Table S7). The free amino acid content was decreased (31%) with arsenic stress (3 mM) as equaled to control plants. Foliar FA (1.5 mg/L) enhanced the free amino acid content with control conditions (23%). The FA spray lessened the effects of arsenic stress (25%). Meanwhile, the water spray increased the free amino acid in both the control (7%) and stressed pant (5.2%). The main rise (25.07%) in free amino acid content was detected under arsenic stress by FA (Fig. S7).

Total Phenolic Content

For the phenolic content of the plant, statistical analysis of the data suggested that there was a significant difference ($P \leq 0.001$) in arsenic stress, foliar FA spray ($P \leq 0.001$), non-significant difference ($P \geq 0.05$) contact between these factors (Table S8). The total phenolic content was depressed (32%) with arsenic stress (3 mM) as well as control plants. Foliar FA spray (1.5 mg/L) enhanced the phenolic content under control conditions (19%). The foliar FA spray eradicated the effects of As stress enhanced (66%) in 3 mM of arsenic stress. The phenolic content in water spray of control (0.7%) as in stress plant (0.51%). The maximum increase (66%) in phenolic content was detailed under As stress (3 mM) by foliar FA application (Fig. S8).

Flavonoid Content

For the flavonoid content of the plant, analysis of the data exhibited a significant difference (P≤0.001) in arsenic stress, foliar FA spray (P≤0.001) in non-significant difference (P≥0.05) in dealings between these factors (Table S9). The flavonoid content was dropped (32%) under arsenic stress (3 mM), as likened to control plants. Foliar FA (1.5 mg/L) higher the flavonoid content in control conditions (29%). The foliar FA removed the effects of arsenic stress and improved (36%) with (3 mM) of arsenic stress. The water spray increased the flavonoid content in the stress plants (0.03%) as well as in the control (8%). The higher increase (35.7%) in flavonoid content was practical under arsenic stress by foliar FA spray (Fig. S9).

Anthocyanin Content

For the anthocyanin content of the plant, analysis of data displayed a significant difference (P≤0.001) in arsenic stress, foliar FA spray (P≤0.001) with the non-significant difference (P≥0.05) contact between these factors (Table S10). Anthocyanin content was condensed (22%) in arsenic stress (3 mM) related to control plants. Foliar fulvic acid (1.5mg/L) further to the anthocyanin content with control conditions (32%). The foliar fulvic acid removed the effects of As stress and made the anthocyanin content (48%) with (3 mM) of arsenic stress. The water spray increased the anthocyanin content in control (1.1%) as in the arsenic stress plant (2.3%). The greater anthocyanin content was detected under arsenic stress (3 mM) by foliar FA treatment (Fig. S10).

Proline Content

For the proline content of the plant, analysis of data showed a significant difference (P≤0.001) in arsenic stress, foliar FA spray (P≤0.001), and non-significant difference (P≥0.05) interface between these factors (Table S11). The proline content was increased (25%) in arsenic stress as linked to control plants. Foliar FA (1.5 mg/L) produced the proline content with control conditions (11%). The foliar FA application excluded the effects of stressed plants (12%). The proline content increased a little bit with water spray in control (1.5%) as well as in stressed plants (0.4%). Greater growth (12%) in proline content was recorded under arsenic stress by foliar FA spray (Fig. S11).

Glycine Betaine Content

For the glycine betaine content of the plant, analysis of the data supposed that a significant difference (P≤0.001) in arsenic stress, foliar fulvic acid spray (P≤0.001) with the non-significant difference (P≥0.05) contacts between these factors (Table S12). The GB content was increased (65%) in the concentration of arsenic stress (3 mM) compared to control plants. When FA spray (1.5 mg/L) was upraised, the GB content in control conditions (133%). The foliar FA spray diminishes the effects of arsenic stress and is higher (79) than arsenic stress (3 mM). Meanwhile, the glycine betaine content in water spray is in control (99%) and in arsenic stress (54%). Fulvic acid spray increased the glycine betaine content (133.5%) under arsenic stress (Fig. S12).

Superoxide Content (SOD)

For the superoxide dismutase content of the plant, statistical analysis of the data showed a significant difference (P≤0.001) in arsenic stress, foliar fulvic acid spray (P≤0.001) with the non-significant difference (P≥0.05) dealings between these factors (Table S13). The SOD content was amplified (225%) under level arsenic stress (3 mM), paralleled to control plants. Foliar FA spray (1.5 mg/L) raised the SOD content in control conditions (28%). FA spray removed the effects of arsenic stress and increased the SOD contents (18%) under arsenic stress. while the SOD content increased by water spray in control (6%) and arsenic stress (5%). Foliar FA spray enhanced the SOD content (18.05%) under arsenic stress (Fig. S13).

Peroxidase (POD) Contents

For the peroxidase content of the plant, analysis of data displayed that there was a significant difference (P≤0.001) in arsenic stress, foliar FA spray (P≤0.001) with the non-significant difference (P≥0.05) in contact between these factors (Table S14). The POD content was improved (31%) under As stress (3 mM), matched to control plants. Foliar FA treatment (1.5 mg/L) greater the POD content under control conditions (37%). The foliar fulvic acid eradicated the effects of arsenic stress and raised the POD (30%) under arsenic stress. The POD content was high when water was sprayed in the stressed (5.4%) as well as in control (3%) plants. Foliar FA increased the POD content (34.6%) under arsenic stress (Fig. S14).

Catalase (CAT) Contents

For the catalase content of the plant, statistical analysis of data designated a significant difference (P≤0.001) in arsenic stress, foliar FA spray (P≤0.01), and non-significant difference (P>0.05) relations between these factors (Table S15). The CAT content was improved (43%) with arsenic stress, separately as matched to control plants. Foliar FA (1.5 mg/L) increased the CAT content under control conditions (29%). The foliar FA application removed the effects of arsenic stress and developed the CAT content (35%) with the concentration of arsenic stress. The CAT activity increased when water spray on stressed (8.5%) as well as control plants

(7.4%). The FA spray revealed an increase (35.2%) under arsenic stress (Fig. S15).

Ascorbic Acid Peroxidase (APX) Contents

For the ascorbic acid peroxidase content of the plant, analysis of data labeled that significant difference ($P \geq 0.05$) in arsenic stress, foliar FA spray ($P \leq 0.001$), non-significant difference $P \geq 0.05$) dealings between these factors (Table S16). The APX content was enhanced (11%) under arsenic stress (3 mM). Foliar FA (1.5 mg/L) decreased the APX content in control plants (14%). The FA spray mitigated the effects of arsenic stress and lowered the APX content (33%) under arsenic stress. APX activity increased by water spray in stress (9%) as well as in control (13%) plants (Fig. S16).

Discussion

Nearby environments can impact plant crop yield [18]. When arsenic stress was imparted to wheat-grown plants in pots with sandy loamy soil, plant height and yield declined [19]. In our present research, the growth parameters length of the wheat plants was also reduced (Fig. 1 and Fig. 2). Fulvic acid (FA) serves the purpose of refining the crop's level of tolerance. FA applied as a foliar application, on the other hand, increases the germination rate by raising the root and shoot fresh and dry weight and improving the plant size. Since heavy metals reached the leaves and collected excessively in various zones, they merged with protein or substituted for manganese, iron, zinc, etc. injuring the chloroplast's form and structure. According to our findings, low levels of arsenic might increase the root and shoot growth of wheat plants. A plant's overall growth was limited, and its biomass eventually reduced, as nutrient absorption in the roots was obstructed [20]. Several examinations have found that high levels of heavy metals in wheat caused in raised levels of an oxygen radical-stimulating species [21].

H_2O_2 contents are the proposed result of decreased capabilities of POD, APX due to Arsenic stress. In our research, activity of SOD, POD and CAT was amplified under stress situations and reduced by the foliar application of FA (supplementary Fig. S13 and Fig. S14). It is showed that anti-oxidative activity increases with plants treated to small level of arsenic, and when greater arsenic concentration the enzyme activity drop [22]. Metal stress resistance has been increased by secondary metabolites such as phenolic, flavonoids, and anthocyanin. According to previous studies, arsenic-stressed showed considerably random activity. It was found that their levels increased when exposed to a low concentration of arsenic, but that they typically reduced when exposed to high levels of arsenic. In our investigation, we also detected similar effects. The use of FA and arsenic stress caused a significant enhancement (supplementary Fig. S8 and Fig. S10). Our discoveries showed that, when arsenic content rises, the level of active oxygen species is elevated in wheat seedling leaf cells, due to the increase in O_2. Content that outpaces plants' capacity to eliminate oxidative stress. Due to the disturbed equilibrium between production and abolition of the oxygen-free radical in stressed cells, the wheat organism's cells were polluted with toxic materials [23]. Arsenic contact in plants causes' oxidative stress, that is known as a toxicity indication and starts the indicating process and also the action of plants' defensive systems [24]. In certain plants, oxidative stress has mainly been used as a marker for arsenic toxicity and the metabolic consequences it produces. During electron transport, excessive ROS generation in chloroplasts and mitochondria can damage photosynthetic mechanisms such as breathing, as well as the stability of membranes, homeostasis. Reduced growth and efficiency may also be caused by changes in respiratory metabolism when exposed to arsenic toxicity [25].

Though arsenic poisoning causes ROS generation, the protein content of plants was decreased. While arsenic poisoning causes ROS generation, the protein content of plants was reduced. Wheat plants seeded in pots revealed levels (3 mM) of arsenic stress reduced in content, (supplementary Fig. S4). Arsenic stress reduced food absorption, which in turn reduced chlorophyll a and b levels. Metal poisoning decreases plant chlorophyll levels, making it difficult for the plants to absorb essential nutrients [25]. We found the same thing in our most current study (Fig. 9 and Fig. 11). Excessive levels of arsenic in plants cause a wide range of physiological ailments. ROS production, hydroxyl radical generation, hydrogen peroxide production, elevated MDA, proline concentration, and changes in antioxidant enzyme activity are the most common. This is what we revealed during the course of our research (Fig. S1). At an arsenic concentration of 3 mM, MDA concentrations increase. MDA, a lipid peroxidation byproduct, was also shown to increase in wheat when exposed to arsenic. The high amount of arsenic usage assisted in retaining extra water in cells and avoiding drying, which enhanced macromolecule carbohydrate ruin but reduced cell synthesis [26]. This was valuable for gathering water in cells. It also immediately converted into molecular mass and soluble sugar, glycogen, glucose, leading to rise in soluble sugar content. Arsenic slow down the plant growth and development [27]. The polypeptide that was not required by organisms was subsequently collected, leading to a rise for protein. Stronger stress such as arsenic exposure could exceed these defensive systems and cause cellular harm due to rising production of reactive oxygen species (ROS) [28]. Apoptosis can happen because of this damage. It was shown that arsenic treatment impacted wheat germination rate, plant length, productivity, nutrient absorption, chlorophyll, photo synthetically active radiation, stomata closure, and also lipid peroxidation [29]. Plants have a defensive mechanism system to defend from the influence of

oxidative stress. Enzymes such as catalase, peroxidase, superoxide, include the APX [30]. However, other investigations have found a marked fall in concentration under different stress conditions, showing that plants are susceptible to stress. In conclusion, arsenic toxicity decreased the physiological features of wheat plants while other variables specified resistance to arsenic stress, as mentioned earlier. When FA was applied, the physiological and biochemical growth characteristics were enhanced.

Conclusions

The study was conducted to improve the arsenic tolerance in wheat (*Triticum aestivum* L.) genotype by foliar application of FA on agronomic, physiological, biochemical. The results revealed that arsenic stress reduced chlorophyll content and carotenoids, leading to a decrease in ascorbic acid content. However, FA spray (1.5 mg/L) increased chlorophyll and carotenoid content, whereas decreasing ascorbic acid content. Proline and glycine betaine increased under arsenic acid, but FA spray managed to increase these levels. Hydrogen peroxide and malondialdehyde content were maximum during arsenic stress, but decreased with FA spray. Arsenic stress also enhanced the total phenolic and flavonoids content, but pretreated plants managed to manage cell functioning. Sugar content decreased during arsenic stress, but was normal during FA spray. Catalase, super oxidase dismutase, and peroxidase activities were also increased under arsenic stress. All things considered, foliar application of Fulvic acid reduces arsenic stress in wheat by influencing growth, physiological, biochemical, and antioxidant activities. Based on the results of this study, it can be concluded that FA sprayed to the wheat plants improved the resistance in wheat to arsenic stress. The destructive effects of arsenic could be reduced by the application of FA.

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Conflict of Interest

The authors declare no conflict of interest.

Author's Contribution Statement

Conceptualization, Ayesha kanwal Siddique; Formal analysis, Ayesha kanwal Siddique; Investigation, Ayesha kanwal Siddique; Methodology, Ayesha kanwal Siddique; Project administration, Rizwan Munir; Software, Hafiza Aqsa Tariq, Hafiz M, Saleem Akhtar and, Tawaf Ali Shah; Supervision, Rizwan Munir; Funding acquisition, streamlining the idea of research and editing Kotb A. Attia, Yaser M Hafez, Arif Ahmed Mohammed, Sajid Fiaz and Tawaf Ali Shah; Writing – original draft, Ayesha kanwal Siddique; Writing – review & editing, Tawaf Ali Shah and Sajid Fiaz.

Availability of Data and Materials

All the data has been included in the manuscript.

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Supplementary Material

Link to supplementary material https://www.pjoes.com/SuppFile/194238/1/