# Effect of foliar application of different hydrogen peroxide concentrations on the biochemical traits of several cotton cultivars

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**Abstract:**

A field trial implemented during the 2025 growing season at the first research station of the College of Agriculture, University of Anbar. to evaluate the effect of foliar application of hydrogen peroxide at different concentrations on selected biochemical traits of several cotton cultivars. The trial followed a randomized complete block design (RCBD) with split-plot arrangement. Three concentrations of hydrogen peroxide: 0, 50, and 75 mg/L allocated to the main plot. The sub-plots contained five cotton genotypes: W888, Coker 310, Montana, Lashata, and Spiro. The results showed that plants of the Lashata variety showed the highest catalase enzyme activity. They also had the highest leaf MDA content, SOD enzyme activity, proline acid concentration, and relative water content. These values reached 39.56 absorbance units/min/g fresh weight, 19.10 µmol/g fresh weight, 34.59 absorbance units/g fresh weight, 21.93 µg/g fresh weight, and 89.48%, respectively. Hydrogen peroxide concentrations had a significant effect. The dose of 75 mg/L gave a significant superiority in proline acid concentration, catalase enzyme activity, and leaf MDA content. These values reached 27.80 µg/g fresh weight, 39.48-absorbance units/min/g fresh weight, and 24.78 µmol/g fresh weight, respectively. Meanwhile, the control treatment showed superiority in relative water content of leaves, reaching 89.60%. The concentration of 50 mg/L recorded superiority in SOD enzyme activity, reaching 34.63-absorbance units/g fresh weight. Interactions between study factors were observed. Control treatment combined with the Lashata variety gave a significant superiority in relative water content of leaves, reaching 94.60%. However, the concentration of 75 mg/L combined with the Lashata genotype recorded significant superiority in the rest of the studied traits

**Keywords: Cotton cultivars; Hydrogen peroxide; Catalase; Superoxide dismutase; Proline.**

## Introduction

Cotton [*Gossypium hirsutum*] is considered the second largest oil-producing crop after soybean, representing 35% of global fiber production. The continuing global water crisis confirms the urgent need for drought-resistant cotton cultivars [25]. Cotton is also considered highly sensitive to drought-induced stress, which negatively affects root structure, physiological functions, and fiber productivity [19]. Cotton plants show moderate drought tolerance during the vegetative growth stage and severe sensitivity to water deficit during the flowering stage [24].

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Stress leads to weakness in the photosynthesis process by inhibiting chlorophyll synthesis, damaging essential enzymes, increasing lipid peroxidation, and producing reactive oxygen species [1]. In response to these challenges, plants produce enzymatic antioxidants, such as peroxidase, catalase, and ascorbate peroxidase, and non-enzymatic antioxidants, including proline and phenolic compounds, to reduce oxidative stress and damage [13]. Studies have shown that, in water-deficit environments, genotypes that maintain higher levels of proline and relative water content are considered drought-resistant genotypes and can be used to develop drought-resistant cultivars in crops [15]. Drought causes flower shedding and boll shedding, as ethylene synthesis leads to boll shedding and a decrease in total productivity. In addition, drought weakens electron transport and reduces photosystem efficiency, which further hinders growth and boll development [20].

Hydrogen peroxide is formed during several biological reactions. At high concentrations, it can be toxic to cells because it induces oxidative stress. However, when added in small quantities, it plays a basic role in activating plant defense mechanisms [16]. It is one of the reactive oxygen species [ROS] and is the most stable at the cellular level. It plays a vital role in plants by sending chemical signals that lead to plant resistance to stress [23]. At low concentrations, it acts as a molecular signal that causes plant tolerance against biotic and abiotic stresses and regulates plant biological processes, such as photosynthesis and plant growth and development [18]. However, its high concentrations cause disturbance in cellular and physiological functions and consequently lead to the release of factors inducing programmed cell death [21].

The study aims to determine the extent of the impact of hydrogen peroxide concentrations on the defense system of enzymatic and non-enzymatic antioxidants in cotton plants.

**Materials and methods:**

A field trial implemented during the 2025 growing season at the First Research Station located in the Al-Buaytha area, associated with the College of Agriculture, University of Anbar. The study aims to determine the extent of the impact of hydrogen peroxide concentrations on the defense system of enzymatic and non-enzymatic antioxidants in cotton plants. Random samples were collected from the field soil at a depth of 30 cm prior to planting to investigate certain physical and chemical properties of the soil. The samples were subsequently analyzed at the Central Laboratory of the College of Agriculture, University of Anbar, as presented in Table (1).

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**Table (1).** Some chemical and physical properties of the experimental soil before planting for the summer season of 2025.

| Trait | Value | Unit of measurement |
|---|---|---|
| Electrical conductivity (EC) | 1.4 | dS m-1 |
| Total dissolved salts (TDS) | 512 | mg L-1 |
| pH | 7.89 | ----- |
| Soil separates | | |
| Clay | 205 | g kg-1 |
| Silt | 470 | g kg-1 |
| Sand | 325 | g kg-1 |
| Texture | Loam | |
| Nitrogen | 13.6 | mg kg-1 |
| Phosphorus | 7.85 | mg kg-1 |
| Potassium | 234 | mg kg-1 |
| Calcium | 4.15 | mmol L-1 |
| Magnesium | 3.25 | mmol L-1 |
| Sodium | 1.23 | mmol L-1 |
| Bicarbonate | 1.11 | mmol L-1 |
| Carbonate | Nil | |
| Gypsum | 0.48 | g kg-1 |
| Lime | 278 | g kg-1 |
| Cation exchange capacity (CEC) | 21 | cmolc kg-1 soil charge |

The trial followed a randomized complete block design (RCBD) with split-plot arrangement. Three concentrations of hydrogen peroxide: 0, 50, and 75 mg/L(Hydrogen peroxide was obtained from the laboratories of the College of Agriculture at Anbar University) allocated to the main plot according to what was mentioned by Al-Mansouri and Ali [8]. The sub-plots contained five cotton genotypes: W888, Coker 310, Montana, Lashata, and Spiro.( Table 2) The field was partitioned into 45 experimental units. Each measuring of 7.5 $m^2$. DAP fertilization applied once before plant at a rate of 100 kg $ha^{-1}$, alongside with nitrogen fertilizer at a rate of 80 kg /ha was added in the form of urea (46% N) in two equal splits: half of the quantity 30 days after planting and the second at the beginning of flowering (5). Drip irrigation pipes were extended, and then the seeds were planted on 20/4/2025. Planting was carried out on lines with a distance of 75 cm between them and 25 cm between one hill and another, with a plant density estimated at 53333 plants $ha^{-1}$. Three to four seeds were placed in each hill at a depth of 2–3 cm followed by light soil covering. The experimental field was irrigated immediately after planting. Crop management practices, including irrigation, weeding, hoeing, and weed control, were conducted whenever required.

Hydrogen peroxide was sprayed in the evening using a 10-L backpack sprayer. Only distilled water was sprayed for the control treatment, A wetting agent was added to reduce water surface tension, ensure sufficient wetting of the leaves, and enhance the ability of the spray solution to penetrate the outer leaf surface. Hydrogen peroxide was sprayed in three doses: the first dose after the appearance of the sixth true leaf, the second dose at flowering, and the third dose two weeks after the second. The first picking was carried out on 3/9/2022, and the second picking was carried

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out on 5/11/2025 after the opening of the remaining bolls.

Table 2. Origin and source of the genotypes.

| Genotypes | Origin | sourse |
|---|---|---|
| W888 | U.S.A | University of Anbar - College of Agriculture |
| Coker 310 | U.S.A | University of Anbar - College of Agriculture |
| Montana | U.S.A | University of Anbar - College of Agriculture |
| Lachata | Uzbekistan | University of Anbar - College of Agriculture |
| Spiro | Greece | University of Anbar - College of Agriculture |

**Studied traits**

**Estimation of catalase enzyme activity (CAT) in leaves**

It was estimated according to the method of Aebi [2] "Fresh tissues were collected and ground with ice-cooled phosphate buffer. They were placed in a centrifuge at 12000 rpm for 10 minutes at 4°C, and then the supernatant was collected. The spectrophotometer was prepared at 240 nm and calibrated with a mixture of 2 mL of cooled phosphate buffer and 1 mL of hydrogen peroxide at a concentration of 30 mM in a 3-mL quartz cuvette as a blank sample. In an experimental cuvette, 1 mL of PB was added with 1 mL of the diluted sample. To start the analysis, 1 mL of hydrogen peroxide at a concentration of 30 mM was added to the cuvette, and then it was quickly placed in the spectrophotometer to observe the absorbance. Note: the initial absorbance should be about 0.500–0.520. If necessary, PB was added to reduce the absorbance, or H2O2 was added to increase the absorbance. It was mixed with a flat-ended glass rod, and then the decrease in absorbance was followed directly with the recorder for 3 minutes every 30 seconds. Catalase solution was used as a control sample. A volume of 1.9 mL of PB was placed in the cuvette, and 1 mL of 30 mM H2O2 and 0.1 mL of catalase solution (~10 units) were added. Catalase activity was calculated according to the following formula:

$$U/mg = (A0 - A180) \times Vt / E240 \times d \times Vs \times Ct \times 0.001$$

where (A0 − A180) is the difference between the initial absorbance and the final absorbance, Vt is the total reaction volume (3 mL), E240 is the molar absorption coefficient at a wavelength of 240 nm, d is the optical path length of the cuvette, which is 1 cm, Vs represents the sample volume, 1 mL, and Ct is the total protein concentration in the sample."

**Estimation of Malondialdehyde (MDA) concentration in leaves, µmol g-1 fresh weight**

It was estimated according to the method of Heath and Packer [14] "TCA (trichloroacetic acid) at a concentration of 0.1%, and TBA (thiobarbituric acid), dissolved in TCA at 0.5%, a spectrophotometer, a porcelain mortar, and steam or a water bath at 95°C. A weight of 0.5 g of fresh plant leaves was taken and ground with 5 mL of TCA (0.1%) until it became homogeneous. It was filtered using a centrifuge at 10000 rpm for 10 minutes. Then, 1 mL of the

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filtrate + 4 mL of 0.5% TBA solution dissolved in 10% TCA were taken. The tubes were heated in a water bath at 95°C for 30 minutes, and then they were cooled directly in an ice bath to stop the reaction and placed again in the centrifuge to remove impurities. The absorbance was measured at wavelengths of 532 and 600 nm, and the following equation was applied:

$MDA = ((A532 - A600) / 155000) \times 10^6$

The value 155000 is the molar absorption coefficient."

**Estimation of superoxide dismutase enzyme activity (SOD) (absorbance unit $mL^{-1}$)**

It was estimated according to the method of Beyer and Fridovich [11]" Solutions used**:**

Solution (1): K2HPO4 (82.4 mmol) and EDTA-2Na (165 µmol). It was prepared by dissolving 3.5880 g of K2HPO4 and 0.0154 g of EDTA-2Na in a small quantity of deionized distilled water, and the volume was completed to 250 mL with distilled water.

Solution (2): KH2PO4 (82.4 mmol) and EDTA-2Na (165 µmol). It was prepared by dissolving 2.8034 g of K2HPO4 and 0.0154 g of EDTA-2Na in a small quantity of distilled water, and then the volume was completed to 250 mL with distilled water.

Potassium phosphate buffer was prepared by gradually adding Solution (2) to 250 mL of Solution (1) until the pH value became equal to 7.8.

Solution (B): the amino acid L-methionine (14 mmol). It was prepared by dissolving 150 mg of the amino acid L-methionine in 5 mL of distilled water.

Solution (C): Triton X-100 (1% v/v). It was prepared by dissolving 0.1 mL of Triton X-100 in 10 mL of distilled water.

Solution (D): Nitro blue tetrazolium (NBT). It was prepared by dissolving 14.4 mg of NBT in 10 mL of distilled water and kept inside an opaque bottle in the refrigerator.

Solution (E): working mixture. It consisted of 18.35 mL of Solution A, 1.50 mL of Solution B, 0.75 mL of Solution C, and 1.00 mL of Solution D, until the total volume became 21.60 mL.

Solution (F): riboflavin (47.7 µM). It was prepared by dissolving 0.0018 g of riboflavin in a small quantity of distilled water, and then the volume was completed to 100 mL with distilled water.

**Procedure:**

1.5 mL of the working mixture was placed in test tubes, and 500 µL of distilled water was added to it. A volume of 40 µL of the sample was added to the tubes. The blank treatment was then prepared in the same way, but it differed only in containing distilled water instead of the sample. A volume of 40 µL of Solution F was added to each tube. The contents were then mixed well, and the absorbance was read at a wavelength of 560 nm. After that, the tubes were exposed to light for 10 minutes using a lighting box containing a fluorescent lamp with a power of 18 watts, and then the absorbance was read directly at a wavelength of 569 nm. The standard inhibition curve of the enzyme was prepared as follows:

Different volumes of the sample were taken (10, 20, 40, 60, 80, 100, 120, and 140) units, and the standard curve showing the inhibition percentages and the sample volume was drawn."

**Calculations:**

After extracting the highest inhibition percentage from the standard inhibition curve, SOD activity was calculated according to the following equation:

$SOD\ inhibition\ \% = ((A1S - A2S) - (A1B - A2B)) / ((A1B - A2B))$

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where:
A1B = absorbance value of the blank before illumination
A2B = absorbance value of the blank after illumination
A1S = absorbance value of the sample before illumination
A2S = absorbance value of the sample after illumination
Then, SOD activity was estimated as follows:
SOD activity (unit mL-1) = (inhibition percentage of the sample / highest inhibition percentage) × (dilution factor / sample volume)
Dilution factor: 2000 µL
Sample volume: 40 µL

**Estimation of the concentration of the amino acid proline (µg $g^{-1}$ fresh weight)**

The concentration of proline in fresh plant leaves was estimated according to the method of Bates et al. [10] " A weight of 0.5 g of leaves was taken and placed in a porcelain mortar after adding 10 mL of 3% sulfosalicylic acid. The plant samples were crushed and then placed in a centrifuge at 2000 rpm for 10 minutes. A volume of 2 mL of the filtrate was withdrawn, and 2 mL of glacial acetic acid and 2 mL of ninhydrin solution were added. The ninhydrin solution was prepared by mixing 1.25 g of ninhydrin with 30 mL of glacial acetic acid and 20 mL of phosphoric acid. The samples were then placed in tubes in a water bath at 100°C for one hour, and the red color began to appear at varying degrees according to the proline concentration in the sample. The tubes were then placed in an ice bath to cool. After that, the red layer was separated by adding 4 mL of toluene, and the mixture was mixed for one minute, so the color began to rise into the toluene layer. Then, 3 mL of this layer was withdrawn, and the color intensity was measured using a spectrophotometer at a wavelength of 520 nm. These readings were compared with the standard curve of pure proline."

**Standard curve of proline:**

The standard curve was constructed using varying concentrations of pure proline (10, 30, 50, 70, 90, 110, and 130 µg $mL^{-1}$). Subsequently, 2 mL of each dilution was combined with 2 mL of glacial acetic acid and ninhydrin solution. The mixture was thoroughly homogenized and incubated in a water bath at 100°C for one hour. Following incubation, the solutions were cooled, and 4 mL of toluene was added. The mixtures were then agitated for one minute prior to measuring absorbance at 520 nm using a spectrophotometer. The proline standard curve was plotted, and sample readings were compared to determine the proline content in the leaves.

**Estimation of relative water content in leaves**

Determination of fresh weight was performed at the end of stress period and tender leaves were placed in 1,000 mL distilled water for turgid weight measurement after 24 h. Subsequently, dry weights were obtained by drying samples at 65oC for 72 hours. Relative water content (%) was calculated as follows: (FW-DW)/(TuW-DW)×100, where FW is fresh weight, DW dry weight and TuW turgid weight.

$RWC = (FW - DW) / (TuW - DW) \times 100$ (Barrs and Weatherley, 1962).

**Statistical Analysis:**

The data for the studied traits were statistically analyzed using the Genstat statistical software. The Least Significant Difference (LSD) test was used to differentiate between the arithmetic means at a probability level of 0.05.

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## Results and Discussion

### CAT enzyme activity (absorbance units/min/g fresh weight)

Results in Table (3) show significant differences among genotypes. Differences also exist among hydrogen peroxide spray concentrations. The interaction between them was also significant for catalase enzyme activity. Plants of the Lashata genotype were superior. They gave the highest average catalase activity. It reached 39.56 AU $min^{-1}$ $g^{-1}$ FW . The least value was obtained in Spiro (28.46 AU $min^{-1}$ $g^{-1}$ FW). This variation may be attributable to genetic diversity among cultivars and/or differences in resource-use efficiency. The production of enzymes takes energy, and requires amino acids. This is in agreement with the findings by Al-Juhayshi and Al-Laila [6], and Khalid and Mueen [17].

For hydrogen peroxide spraying, the dosage of 75 mg/L was better. It showed maximum value of catalase enzyme activity best. It has peaked at 39.48 absorbance units/min/g fresh weight The control treatment had the lowest mean for this trait. It reached 21.82 AU $min^{-1}$ $g^{-1}$ FW. The reason may be that hydrogen peroxide acts as a signaling molecule. It stimulates genes responsible for building antioxidant enzymes. This agrees with findings by Bhardwaj et al. [12].

The results revealed a presence of interaction between genotypes and hydrogen peroxide spray concentrations. The Lashata genotype interacted with the 75 mg/L concentration. It recorded the highest average of 52.20 AU $min^{-1}$ $g^{-1}$ FW. Meanwhile, the W888 genotype interacted with the control treatment, recorded the least mean of 20.33 AU $min^{-1}$ $g^{-1}$ FW.

**Table (3):** Effect of genotypes, spraying with hydrogen peroxide, and their interaction on the mean activity of catalase enzyme (absorbance unit $min^{-1}$ $g^{-1}$ fresh weight) in cotton crop, 2025.

| Hydrogen peroxide | Genotypes | | | | | Mean |
|---|---|---|---|---|---|---|
| | **W888** | Coker | Montana | Lachata | Spiro | |
| **0** | 20.33 | 22.56 | 21.40 | 24.18 | 20.62 | **21.82** |
| **50** | 36.49 | 38.40 | 38.40 | 42.29 | 36.40 | **38.40** |
| **75** | 40.31 | 49.98 | 26.51 | 52.20 | 28.37 | **39.48** |
| Mean | **32.38** | **36.98** | **28.77** | **39.56** | **28.46** | |
| **L.S.D 0.05** | Genotypes | | Concentrations | | Interaction | |
| | 0.34 | | 0.41 | | 0.61 | |

### Leaf content of malondialdehyde (MDA) (µmol g-1 fresh weight)

Table (4) shows significant differences among genotypes. Differences also exist among hydrogen peroxide spray concentrations. The interaction between them was also significant for leaf MDA. The plants of the genotype Lachata were superior in leaf content of MDA, as they gave the highest mean, reaching 19.10 µmol $g^{-1}$ fresh weight, whereas the genotype Spiro recorded the least mean for the trait, reaching 13.45 µmol g-1 fresh weight. Such variation may be attributed to genetic differences among cultivars and their ability to exploit available growth resources. This agrees with what was reached by Al-Juhaishi and Al-Layla [6], and Khalid and Mueen [17].

The 75 mg L-1 hydrogen peroxide treatment resulted in the highest mean MDA content, reaching 24.78 µmol g-1 fresh weight, whereas the control treatment gave the lowest mean, 6.82 µmol g-1 fresh weight. The increase in

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MDA may be associated with membrane disturbance caused by hydrogen peroxide-induced oxidative stress, which leads to the formation of secondary toxic compounds such as MDA. This agrees with Al-Ghazzi [4] and Shani et al. [24].

This trait was influenced significantly by the interaction between these two experimental factors. Lachata sprayed with 75 mg $L^{-1}$ obtained the highest mean MDA content (30.13 µmol $g^{-1}$ fresh weight) while Spiro under control treatment recorded the lowest value, 5.43 µmol $g^{-1}$ fresh weight.

**Table (4):** Effect of genotypes, spraying with hydrogen peroxide, and their interaction on leaf content of Malondialdehyde (MDA) (µmol $g^{-1}$ fresh weight) in cotton crop, 2025.

| Hydrogen peroxide | Genotypes | | | | | Mean |
|---|---|---|---|---|---|---|
| | **W888** | Coker | Montana | Lachata | Spiro | |
| **0** | 5.83 | 7.97 | 6.20 | 8.67 | 5.43 | **6.82** |
| **50** | 12.70 | 18.01 | 14.13 | 18.50 | 13.64 | **15.40** |
| **75** | 22.15 | 29.87 | 20.47 | 30.13 | 21.28 | **24.78** |
| Mean | **13.56** | **18.61** | **13.60** | **19.10** | **13.45** | |
| **L.S.D** | Genotypes | | Concentrations | | Interaction | |
| **0.05** | 0.36 | | 0.28 | | 0.59 | |

**SOD enzyme activity (absorbance units/g fresh weight).**

Table (5) shows significant differences among genotypes. Differences also exist among hydrogen peroxide spray concentrations. The interaction between them was also significant for SOD enzyme activity. Results indicate that plants of the Lashata genotype were superior. They gave the highest value for SOD enzyme activity, reaching 34.59 AU $min^{-1}$ $g^{-1}$ FW. Meanwhile, plants of the W888 genotype recorded the lowest average of 26.04 AU $min^{-1}$ $g^{-1}$ FW. This difference may result from genetic variation among the cultivars. It may also be due to their different response to available growth resources. This agrees with what was mentioned by Al-Bazi [3] and Al-Khazraji [7].

Meanwhile, the 75 mg L-1 concentration had a significant ($P \leq 0.05$) effect on SOD level with an average of 32.00 AU $min^{-1}$ $g^{-1}$ FW that is the highest compared to control treatment which only yields as high as 21.84 AU $min^{-1}$ $g^{-1}$ FW. The much larger increase may be as simple as hydrogen peroxide signaling the activation of genes which produce antioxidant enzymes, consistent with Bhardwaj et al. [12] and Shani et al. [24].

The interaction effect revealed that with SOD activity of 42.55 AU $min^{-1}$ $g^{-1}$ FW. , Lachata sprayed with the higher concentration (75 mg $L^{-1}$) outperformed all other treatments. On the other hand, Montana in control treatment produced lowest value (18.78 AU $min^{-1}$ $g^{-1}$ FW. ).

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**Table (5):** Effect of genotypes, spraying with hydrogen peroxide, and their interaction on the mean activity of SOD enzyme (absorbance unit g-1 fresh weight) in cotton crop, 2025.

| Hydrogen peroxide | Genotypes | | | | | Mean |
|---|---|---|---|---|---|---|
| | **W888** | Coker | Montana | Lachata | Spiro | |
| **0** | 20.38 | 22.39 | 18.78 | 24.39 | 23.25 | **21.84** |
| **50** | 33.56 | 39.01 | 32.48 | 36.81 | 31.28 | **34.63** |
| **75** | 24.19 | 42.26 | 24.90 | 42.55 | 26.09 | **32.00** |
| Mean | **26.04** | **34.55** | **25.39** | **34.59** | **26.87** | |
| **L.S.D** | Genotypes | | Concentrations | | Interaction | |
| **0.05** | 0.66 | | 0.39 | | 1.06 | |

**Leaf content of proline acid (µg g-1 fresh weight)**

The results revealed significant differences among the genotypes, concentrations and their interaction of spraying with hydrogen peroxide (Table 6). The plants of the genotype Lachata were superior in leaf content of proline acid, as they gave the highest mean, reaching 21.93 µg $g^{-1}$ fresh weight, whereas the genotype Montana recorded the least mean for the trait at 7.59 µg $g^{-1}$ fresh weight. This difference could be attributed to the genetic nature of the cultivars and their efficiency in using available growth resources. This agrees with what was reached by Al-Bazi (3) and Al-Khazraji [7].

The highest mean proline content among treatments (27.80 µg g-1 fresh weight), was recorded in plants treated with 75 mg L-1 hydrogen peroxide and the least means of 4,07µg g-1 fresh weight were observed for control treatment. This significant increase is attributed to the defensive response of the plant to adapt to oxidative stress, as proline acts as an osmotic regulator that maintains cell turgor pressure and membrane integrity from degradation, in conjunction with the noticeable increase in catalase level (Table 16). Despite the increase in MDA levels as evidence of the occurrence of oxidative damage, the accumulation of proline and the increase in catalase activity helped prevent this damage from reaching lethal toxic levels. This agrees with Rana et al. [22] and Almansouri and Ali [8].

Regarding the interaction between genotypes and hydrogen peroxide concentrations, we note that plants of the Lashata genotype interacting with the 75 mg/L concentration were superior. They gave the highest average for proline content, reaching 41.15 µg/g fresh weight. Meanwhile, plants of the W888 genotype interacting with the control treatment recorded the least mean for this trait at 3.25 µg/g fresh weight.

**Table (6):** Effect of genotypes, spraying with hydrogen peroxide, and their interaction on the mean concentration of proline acid (µg $g^{-1}$ fresh weight) in cotton crop, 2025.

| Hydrogen peroxide | Genotypes | | | | | Mean |
|---|---|---|---|---|---|---|
| | **W888** | Coker | Montana | Lachata | Spiro | |
| **0** | 3.25 | 5.11 | 3.57 | 4.85 | 3.56 | 4.07 |
| **50** | 17.42 | 20.16 | 7.71 | 19.79 | 8.80 | 14.78 |
| **75** | 32.40 | 37.21 | 11.47 | 41.15 | 16.77 | 27.80 |
| Mean | 17.69 | 20.83 | 7.59 | 21.93 | 9.71 | |
| **L.S.D** | Genotypes | | Concentrations | | Interaction | |
| **0.05** | 0.50 | | 0.50 | | 0.85 | |

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**Estimation of relative water content in leaves (%)**

Table (7) illustrates the existence of significant differences among the genotypes., spraying with hydrogen peroxide, and the interaction between them in relative water content in leaves. The plants of the genotype Lachata were superior in relative water content in leaves, as they gave the highest mean, reaching 89.48%, whereas the genotype Spiro recorded the least average for the trait, reaching 74.03%. This difference could be attributed to the genetic nature of the cultivars and their efficiency in using available growth resources. This agrees with what was reached by Shani et al. [24], who indicated that genotypes differ among themselves in relative water content in leaves.

The control treatment showed the highest mean in relative water content, 89.60%, and the least was with a dose of 75 mg $L^{-1}$ which provided means relative to this variable of up to 72.87%. This decrease may be attributed to cellular damage in leaves resulting from oxidative stress. This agrees with findings by Shani et al. [24].

There was a significant two-way interaction between them. The highest value (94.60%) of relative water content was observed in Lachata under control treatment and the least was recorded for Montana sprayed with 75 mg $L^{-1}$ (62.53%).

**Table (7):** Effect of genotypes, spraying with hydrogen peroxide, and their interaction on relative water content in leaves (%) in cotton crop, 2025.

| Hydrogen peroxide | Genotypes | | | | | Mean |
|---|---|---|---|---|---|---|
| | **W888** | Coker | Montana | Lachata | Spiro | |
| **0** | 83.67 | 93.31 | 90.15 | 94.60 | 86.27 | 89.60 |
| **50** | 79.40 | 87.27 | 74.53 | 89.40 | 72.21 | 80.56 |
| **75** | 72.16 | 81.60 | 62.53 | 84.43 | 63.60 | 72.87 |
| Mean | 78.41 | 87.39 | 75.74 | 89.48 | 74.03 | |
| **L.S.D** | Genotypes | | Concentrations | | Interaction | |
| **0.05** | 0.52 | | 1.05 | | 1.16 | |

## CONCLUSIONS

We conclude from the study that the cotton genotypes showed variation in the biochemical traits, with the genotype Lachata being distinguished in all traits, as it showed significant superiority in the activity of CAT and SOD enzymes, which confirms that it possesses a defense system for decomposing free radicals. The study also concluded that spraying with hydrogen peroxide stimulated the defense mechanisms in the plant. The study also showed that the cultivar Lachata exhibited tolerance to oxidative stress with increasing peroxide concentrations

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