Effect of Hydrogen Peroxide and Zinc Spraying on the Growth, Yield and Antioxidant Enzyme of Wheat *Triticum aestivum* L.

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Abstract. Wheat (*Triticum aestivum* L.) is one of the most important crops in the world. Thus, the importance of this study stems from its attempt to investigate the effect of foliar spraying with hydrogen peroxide and foliar application of zinc on some growth, yield and antioxidant enzymes of the wheat crop. A field experiment was organized in a randomized complete block design (RCBD) with 3 replications with Split plot arrangement. The first factor was three concentrations of hydrogen peroxide (*H*$_2$*O*$_2$) (25, 50, and 75) ppm as well as the control treatment that was sprayed with distilled water only. The second factor was three concentrations of zinc (*Zn*) (50, 100, and 150) mg L$^{-1}$ as well as the control treatment that was sprayed with distilled water only. The result of this study showed that the higher concentrations of *H*$_2$*O*$_2$ gave low value of plant height, number of spikes per m$^2$ and grain yield, while they increased the activity of antioxidant enzymes SOD, CAT and also increased the proline content. The higher concentration of *Zn* application significantly increased the plant height, SOD, CAT, proline content, number of spikes per m$^2$ and grain yield.

1.Introduction

Wheat *Triticum aestivum* L. is considered as one of the most significant crops in the world. Additionally, it is a predominant cereal and more than half of the world population regarded it as a staple food [1]. Despite the technical progress, agricultural production, specially cereals, is the focus of attention in all countries of the world. This state of affairs is attributed to the problem of food shortage and the increasing demand on these food stuffs as a result of the rapid increase in the population. In order to meet the growing food need, it is necessary to think of increasing the productivity of this crop, which is still very low in Iraq. A long time ago, focus began to be directed towards increasing the yield through scientific methods that establish an integrated system by the addition of foliar nutrients that help increase productivity and improve quality [2]. Zinc is one of the important elements for plant growth and development. It plays an important role in various metabolic processes, such as respiration, photosynthesis, and assimilation of other main nutrients. It is involved in the activation of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [3]. Hydrogen peroxide is defined as a reactive oxygen species (ROS) created from molecular oxygen (O$_2$) with relatively high stability in the cell. H$_2$O$_2$ is a type of reactive oxygen species produced in plants under both biotic and abiotic conditions and plays a major role against O$_2$ resulting cytotoxicity [4]; [5]. These authors state that H$_2$O$_2$ works as a messenger molecule involved in adaptive signaling, resulting in tolerance against many abiotic stresses at low concentrations, but at high concentrations, it regulates programmed cell death. Moreover, [6] indicated that a lower level of treatment with H$_2$O$_2$ can have a significant positive result on plant growth, growth regulators, antioxidant enzyme activity, grain yield and quality. It has also been stated in a study by [7] that exogenous application of H$_2$O$_2$ increased the plant growth, physiological activities and biochemical properties of plant. Based on the
previous survey, the study aimed to find out the effect of foliar spraying with hydrogen peroxide and foliar application of zinc on some growth, yield and antioxidant enzymes of the wheat crop.

2. Materials and methods

2.1. Experiments Site and Design

An experiment was carried out during the winter season of 2020 - 2021 in a private farm in Fallujah District, Anbar Province in order to study the effect of foliar spraying with hydrogen peroxide and zinc on some growth, yield and antioxidant characteristics of wheat plants. A field experiment was organized in a randomized complete block design (RCBD) with 3 replications with Split plot arrangement. The treatment design was the combination of two factors. The first factor was three concentrations of hydrogen peroxide (25, 50, and 75) ppm as well as the control treatment that was sprayed with distilled water only. The second factor was three concentrations of zinc (50, 100, and 150) mg. L\(^{-1}\) as well as the control treatment that was sprayed with distilled water only. Wheat seeds were planted in 20/11/2020 and the crop service operations of irrigation, fertilization and weeding control were carried out according to the needs of the plant during the growing season. Hydrogen peroxide (H\(_2\)O\(_2\)) was sprayed twice, first in the tillering and stem extension stage (after the wheat plant finishes forming tillers, it starts elogation of its internodes), and the second in the boot stage (created when the head starts to form inside the flag leaf). Zinc (Zn) was sprayed twice; first in the heading stage (where the head will fully emerge from the stem) and the second time in the flowering stage.

2.2. Data Collection.

2.3. Plant height (cm).

Ten plants were selected randomly from the center of each experimental unit and measured from the level of soil surface to the highest peak of the plant.

2.4. Superoxide dismutase (SOD) was examined according to the procedure of [8].

2.5. Catalase (CAT) was determined by the method defined by [9].

2.6. Proline contents were examined according to the procedure developed by [10].

2.7. Number of spikes per m\(^2\). was measured as a rate to the number of spikes harvested in (m\(^2\)) for each experiment unit.

2.8. Grain yield (t. ha\(^{-1}\)). was measured as grain weight per m\(^2\) after being harvested and the weight of each experiment unit was taken then converted to (t. ha\(^{-1}\)).

2.9. Statistical analyses.

All data were analyzed using GenStat software window. One-way analysis of variance (ANOVA) at a confident level p=0.05 was used to determine the difference between Hydrogen peroxide and foliar application of Zinc and followed by Least Significant Difference (LSD) at a confident level p=0.05

3. Results and Discussion

3.1. Plant height (cm)

Results demonstrate that there was a significant effect (ANOVA, P<0.05) of Hydrogen peroxide and foliar application of Zinc on Plant height as shown in Table (1). Maximum Plant height of wheat was recorded when the concentration 25 ppm of H\(_2\)O\(_2\) was 104.01 cm. However, minimum Plant height of wheat was recorded when the concentration 75 ppm of H\(_2\)O\(_2\) was 96.99 cm. The increase in hydrogen peroxide concentration stimulated the production of free radicals ROS, which act to disrupt cell division as well as its elongation. A similar result was arrived at by [11], who found a decrease in plant height of barley with the increase of the concentration of H\(_2\)O\(_2\). The results in the table (1) show that increasing the zinc concentration to 150 mg. L\(^{-1}\) gave the highest value of 105 cm and an increase of 7.11% compared with the control treatment that gave the lowest value of 98.59 cm. The direct role of zinc is to form Tryptophan acid form hormone (IAA) which is necessary in cell division and growth increase as well as creating chlorophyll, amino acid and carbohydrates and also increasing the effectiveness and activity of pollen grains, which led to an increase in growth characteristics by
increasing the concentrations of zinc. This result was also indicated by [3]. The interaction between the concentrations of hydrogen peroxide and zinc was significant giving the highest value of the interaction at a concentration of 50 ppm H$_2$O$_2$ and a concentration of 150 mg. L$^{-1}$ 107.31 cm, whereas the lowest interaction value at 75 ppm concentration of hydrogen peroxide and concentration of 50 mg. L$^{-1}$ of zinc was 92.1 cm.

Table (1) Effect of Hydrogen peroxide (H$_2$O$_2$) and Foliar application of Zinc (Zn) and their interaction on Plant height (cm) of Wheat

| Zn concentration mg.L$^{-1}$ | H$_2$O$_2$ Ppm | 0 | 50 | 100 | 150 | means |
|-----------------------------|----------------|----|----|------|------|-------|
| 0                           | 100.59         | 98.02 | 104.74 | 109.3 | 103.16 |
| 25                          | 100.95         | 106.59 | 104.53 | 103.99 | 104.01 |
| 50                          | 98.51          | 100.69 | 103.75 | 107.31 | 102.56 |
| 75                          | 94.3           | 92.1 | 99.73 | 101.82 | 96.99 |
| Means                       | 98.59          | 99.35 | 103.19 | 105.6 |       |
| LSD5%                       | P Zn P*Zn      | 1.11 | 1.318 | 2.635 |       |

3.2. Superoxide dismutase (SOD).

Results show that there was a significant effect (ANOVA, P<0.05) of Hydrogen peroxide and foliar application of Zinc on Superoxide dismutase as presented in Table (2). Maximum SOD activity was shown when the concentration 50 ppm of H$_2$O$_2$ was 44.19 units. g$^{-1}$ fresh weight, with an increase rate of 61.1%, while the control treatment gave the lowest value of SOD which was 27.43 units. g$^{-1}$ fresh weight. Perhaps the reason for the increase in the overall activity of the enzyme SOD is that the addition of hydrogen peroxide led to the accumulation of hydrogen peroxide inside the cell [12]. This result is in agreement with [13]. The results in table (2) indicate that increasing the zinc concentration to 150 mg. L$^{-1}$ gave the highest value of 46.88 units. g$^{-1}$ fresh weight, with an increase of 71.41% compared with the control treatment that gave the lowest value of 26.37 units. g$^{-1}$ fresh weight. This may be due to the fact that the soil suffers from a deficiency of zinc, especially the surface soil, under biotic and abiotic stress conditions. Therefore, the movement of zinc is very slow. Therefore, adding this element as a spraying on the leaves is necessary. SOD is usually used as an indicator of zinc deficiency, so it can be concluded that zinc deficiency in soil has a significant effect on the activity of this enzyme [14]. This result agrees with [15]. Result in table (2) showed that the highest value of the interaction was at a concentration of 75 ppm H$_2$O$_2$ and a concentration of zinc 150 mg. L$^{-1}$ which reached 53.96 units. gm$^{-1}$ fresh weight, while the control treatment gave the lowest value which was 22.44 gm$^{-1}$ fresh weight.

Table (2) Effect of Hydrogen peroxide (H$_2$O$_2$) and Foliar application of Zinc (Zn) and their interaction on Superoxide dismutase (gm$^{-1}$ fresh weight) of Wheat

| Zn concentration mg.L$^{-1}$ | H$_2$O$_2$ Ppm | 0 | 50 | 100 | 150 | means |
|-----------------------------|----------------|----|----|------|------|-------|
| 0                           | 22.44          | 29.13 | 25.35 | 32.81 | 27.43 |
| 25                          | 24.57          | 32.15 | 40.32 | 50.33 | 36.84 |
| 50                          | 34.58          | 43.44 | 48.34 | 50.41 | 44.19 |
| 75                          | 23.88          | 34.39 | 52.75 | 53.96 | 41.25 |
3.3. Catalas Activity (CAT).

Results indicate that there was a significant effect (ANOVA, P<0.05) of Hydrogen peroxide and foliar application of Zinc on Superoxide dismutase as presented in Table (3). Maximum CAT activity was shown when the concentration 75 ppm of H\textsubscript{2}O\textsubscript{2} was 31.84 units g\textsuperscript{-1} fresh weight, while the control treatment gave the lowest value of SOD which was 14.92 units g\textsuperscript{-1} fresh weight. The results in table (3) show that increasing the zinc concentration to 150 mg L\textsuperscript{-1} indicated the highest value of 26.37 units g\textsuperscript{-1} fresh weight, with an increase of 78.65% compared with the control treatment which gave the lowest value of 14.76 units g\textsuperscript{-1} fresh weight. It is shown that Zn foliar application under stress rises the activities of CAT in cotton and rice, which proposes that the application of Zn significantly improves the action of the antioxidant system, therefore contributing to the improvement of oxidative stress [16] ; [17].

Results in table (3) show that the highest value of the interaction was at a concentration of 75 ppm H\textsubscript{2}O\textsubscript{2} and a concentration of zinc 100 mg L\textsuperscript{-1}, which reached 37.7 units gm\textsuperscript{-1} fresh weight, while the control treatment showed the lowest value which was 11.75 gm\textsuperscript{-1} fresh weight.

Table (3) Effect of Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and Foliar application of Zinc (Zn) and their interaction on Catlase (CAT) (gm-1 fresh weight) of Wheat

| Zn concentration mg.L^{-1} | H\textsubscript{2}O\textsubscript{2} Ppm | 0 | 50 | 100 | 150 | means |
|----------------------------|--------------------------------------|---|----|-----|-----|-------|
| 0                          | 11.75                                | 15.38 | 16.49 | 16.07 | 14.92 |
| 25                         | 13.23                                | 19.16 | 20.93 | 24.2  | 19.38 |
| 50                         | 16.23                                | 26.73 | 27.05 | 28.85 | 24.72 |
| 75                         | 17.81                                | 35.46 | 37.7  | 36.37 | 31.84 |
| Means                      | 14.76                                | 24.18 | 25.54 | 26.37 |       |
| LSD5%                      | P                                    | Zn  | P*Zn |      |       |
| 0.655                      | 0.873                                | 1.746 |     |      |       |

3.4. Proline content.

Results show that there was a significant effect (ANOVA, P<0.05) of Hydrogen peroxide and foliar application of Zinc on Proline as presented in Table (4). The results shown in table (4) indicate that Proline increased with an increase in H\textsubscript{2}O\textsubscript{2} concentrations, whereby the concentration of 75 ppm gave the highest rate of the proline which was 34.98 µmol.gm\textsuperscript{-1} fresh weight compared to the control treatment that gave the lowest value of proline which was 9.73 µmol.gm\textsuperscript{-1} fresh weight. Maybe proline has a role in protecting plants from free radicals (ROS) and working in improving the ability of plants to adapt against oxidative stress. Proline plays an important role in plant tolerance of abiotic stresses through many functions proline performs, since proline acts as a molecular guardian to protect the protein and prevent its destruction and acts as an osmotic regulator and resistant to inappropriate conditions [11].

The results exposed in table (4) indicate that proline increased with an increase in Zn concentrations, whereby the concentration of 150 mg L\textsuperscript{-1} gave the highest rate of the proline which was 31.47 µmol.gm\textsuperscript{-1} fresh weight, with an increase of 92.83% compared to the control treatment which gave the lowest value of proline which was 16.32 µmol.gm\textsuperscript{-1} fresh weight. The production of organic solvents, including the amino acid proline, is one of the most important mechanisms in stress-resistant plants (Tarighaleslami et al., 2012). These results are in agreement with [15]. Results in table (4) show that the highest value of the interaction was at a concentration of 75 ppm H\textsubscript{2}O\textsubscript{2} and a
concentration of zinc 150 mg. l\(^{-1}\), which reached 46.09 µmol. gm\(^{-1}\) fresh weight, while the control treatment showed the lowest value which was 8.61 µmol.gm\(^{-1}\) fresh weight.

Table (4) Effect of Hydrogen peroxide (H\(_2\)O\(_2\)) and Foliar application of Zinc (Zn) and their interaction on Proline content (µmol.gm\(^{-1}\) fresh weight) of Wheat

| H\(_2\)O\(_2\) ppm | Zn concentration mg.L\(^{-1}\) | means   |
|------------------|-----------------------------|---------|
|                  | 0                           | 50      | 100     | 150     |
| 0                | 8.61                        | 9.3     | 10.3    | 10.73   | 9.73    |
| 25               | 15.37                       | 24.7    | 32.07   | 32.74   | 26.22   |
| 50               | 18.84                       | 27.84   | 37.34   | 36.34   | 30.09   |
| 75               | 22.48                       | 30.65   | 40.73   | 46.09   | 34.98   |
| Means            | 16.32                       | 23.12   | 30.11   | 31.47   |
| LSD\(\%5\)       | 1.132                       | 1.682   | 3.363   |

3.5. Number of spikes per m\(^2\).

Results presented show that there was a significant effect (ANOVA, P<0.05) of Hydrogen peroxide and foliar application of Zinc on the number of spikes per m\(^2\). as Table (5) indicates. The results show that the concentration of 25 ppm was superior and gave the highest value of this trait which amounted to 499.7 spikes.m\(^2\) with an increase rate of 23.96%, while the concentration of 75 ppm recorded the lowest value of this trait amounting to 403.1 spikes.m\(^2\). This is due to the negative effect of hydrogen peroxide in causing a significant decrease in plant height, as in table (1), and then the effect on the vegetative growth of the plant, as well as the division and elongation of cells, which negatively affected the characteristics of the components of the wheat plant. Hydrogen peroxide concentrations negatively affected the plant in terms of low absorption of nutrients from the soil, mainly nitrogen, phosphorous and potassium due to the weakness of the root system, which affected the photosynthesis process. This leads to a decrease in the amount of manufactured and stored materials, which affected the filling of the grains, as well as the decrease in the amount of water absorbed during the filling of the grains. This mainly affected their small size and low weight, meaning that the stressful factor hydrogen peroxide affected the process of transferring photosynthesis products from the source to the sink [18] ; [19].

The results shown in Table (5) indicate that the concentration 150 mg. L\(^{-1}\) was superior and recorded the highest value of the trait which was 485.6 spikes.m\(^2\) and an increase of 9.67%, while the concentration of 50 mg. L\(^{-1}\) recorded the lowest value of 442.3 spikes.m\(^2\). This is considered the most important component of the yield because this trait is associated with the nature of the genetic structure [20]. It may be due to the fact that the number of spikes is determined in the early stages of the plant’s life, during the period of production of ribs and formation of spikes [21]. These results agree with those of [22]. Results in table (5) show that the highest value of the interaction was at a concentration of 25 ppm H\(_2\)O\(_2\) and a concentration of zinc 150 mg. l\(^{-1}\), which reached 527.3 spikes.m\(^2\), while the concentration of 75 ppm H\(_2\)O\(_2\) and a concentration of zinc 50 mg. l\(^{-1}\) show the lowest value which was 371 spikes.m\(^2\).
Table (5) Effect of Hydrogen peroxide (H$_2$O$_2$) and Foliar application of Zinc (Zn) and their interaction on Number of spikes per m$^2$ of Wheat

| H$_2$O$_2$ ppm | Zn concentration mg.L$^{-1}$ | means |
|----------------|-----------------------------|-------|
|                | 0                           | 50    | 100   | 150   |
| 0              | 416                         | 460.7 | 468.3 | 504.7 | 462.4 |
| 25             | 517.7                       | 465   | 488.7 | 527.3 | 499.7 |
| 50             | 433.3                       | 472.7 | 504.7 | 501.3 | 478   |
| 75             | 403.7                       | 371   | 428.7 | 409   | 403.1 |
| Means          | 442.7                       | 442.3 | 472.6 | 485.6 |
| LSD5%          | zn                          | p     | zn x p|
|                | 15.75                       | 37.37 | 31.5  |

3.6. Grain yield (t. ha$^{-1}$).

Results presented show that there was a significant effect (ANOVA, P<0.05) of Hydrogen peroxide and foliar application of Zinc on grain yield (t. ha$^{-1}$) as presented in Table (6). The results exposed in the table (6) indicate that the concentration of 25 ppm was superior and gave the highest value of this trait amounting to 4.13 t. ha$^{-1}$ and an increase rate of 21.46 %, while the concentration of 75 ppm recorded the lowest value of this trait amounting to 3.35 t. ha$^{-1}$. This is due to the negative effect of hydrogen peroxide in causing a significant decrease in plant height, as in table (1), and then its effect on the vegetative growth of the plant, as well as the division and elongation of cells, which negatively affected the characteristics of the components, table (5), of the wheat plant. Hydrogen peroxide concentrations negatively affected the plant in terms of low absorption of nutrients from the soil, mainly nitrogen, phosphorous and potassium due to the weakness of the root system, which affected the photosynthesis process. This leads to a decrease in the amount of manufactured and stored materials, which affected the filling of the grains, as well as the decrease in the amount of water absorbed during the filling of the grains. This mainly affected their small size and low weight, meaning that the stressful factor hydrogen peroxide affected the process of transferring photosynthesis products from the source to the sink [21] ; [22]. The results indicated in table (6) show that the concentration 150 mg. L$^{-1}$ was superior and recorded the highest value of the trait which was 4.33 t. ha$^{-1}$ and an increase of 27.12 %, compared to the control treatment which gave the lowest value of grain yield which was 3.41 t. ha$^{-1}$. Perhaps this is due to the positive role of zinc in increasing the vitality of pollen and pollination. In the event of a deficiency of this element, the pollen grains formed are small and in few numbers, and most of them have little cytoplasm content, shrink and become weak and may fail in germination and that zinc has an effect on metabolic and chemical processes inside the cell. This leads to producing carbohydrates and proteins and then contributing to the production of reproductive parts in the plant, on the other hand. This explains the increase in the fertility rate by increasing zinc concentrations in the cell. These results agree with those of [23] ; [22].

Results in table (6) revealed that the highest value of the interaction was in the control treatment without the addition of H$_2$O$_2$ and a concentration of zinc 150 mg. L$^{-1}$, which reached 4.76 t. ha$^{-1}$, while the concentration of 75 ppm H$_2$O$_2$ and control treatment without the addition of zinc gave the lowest value which was 3.00 t. ha$^{-1}$. 
Table (6) Effect of Hydrogen peroxide (H$_2$O$_2$) and Foliar application on Zinc (Zn) and their interaction with Grain yield (t. ha$^{-1}$) of Wheat

| $H_2O_2$ ppm | Zn concentration mg.L$^{-1}$ | means |
|--------------|-----------------------------|-------|
|              | 0   | 50  | 100 | 150 |
| 0            | 3.609 | 3.447 | 4.23 | 4.756 | 4.01 |
| 25           | 3.397 | 3.949 | 4.426 | 4.748 | 4.13 |
| 50           | 3.616 | 3.875 | 4.087 | 4.696 | 4.068 |
| 75           | 3.004 | 3.685 | 3.581 | 3.124 | 3.349 |
| Means        | 3.407 | 3.739 | 4.081 | 4.331 |
| LSD5%        | zn  | p   | zn x p |
|              | 0.2261 | 0.2974 | 0.4523 |

4. Conclusion
To conclude, spraying wheat plants with high concentrations of hydrogen peroxide led to a significant decrease in plant height, yield and its components. However, it led to a significant increase in the activity of antioxidant enzymes (SOD and CAT) and the content of proline. Moreover, spraying wheat plants with high concentrations of zinc led to a significant increase in all studied parameters.

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