Raed Mohammed Sarhan AL-Janabi et al /J. Pharm. Sci. & Res. Vol. 11(6), 2019, 2341-2346

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Treatment the adverse effect of the Oxidative stress of hydrogen peroxide with Humic Fulvic acid, Zinc in barley plant (*Hordeum vulgare L.*)

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Abstract

An experiment was carried out in the field of the botanical garden of the Department of Biology at the Faculty of Education for Pure Sciences / Ibn al-Haytham / University of Baghdad for the winter 2017 season to study spraying of humic fulvic acid (0, 25,50) $mg.L^{-1}$ and Zinc element (0,50,100,150) $mg.L^{-1}$ for revealing the oxidative stress caused by hydrogen peroxide (0,2,4) % and the interaction of the three factors on barley plant (*Hordeum vulgare* L.) in estimating the effectiveness of antimicrobial agents, Oxidation enzyme (superoxide dismutase, Peroxidase, Catalase Enzyme), the experiment was designed according to Randomized Complete Block Design (RCBD) as a Worker experience (3×3×4×3). The results showed that the 2% concentration of hydrogen peroxide increased the activities of all antioxidants enzymatic, while at the 4% concentration of hydrogen peroxide the activities on the antioxidant enzyme decreased compared to the concentration of 2% In addition, the spraying of humic fulvic acid and the zinc element resulted in an increase in all studied antioxidants enzymes.

Key words: Hydrogen Peroxide. Zinc Oxide. Antioxidants. humic fulvic acid.

INTRODUCTION

The barley plant (*Hordeum vulgare* L.) family Poaceae, is one of the most all around important winter grain crops grown in large areas all around the world. It is ranked fourth cultivated crop in the world after wheat, rice and maize [1]. Barley is a tolerant plant for unsuitable growth conditions in dry and semi-arid regions in terms of coldness, drought, salinity, basal and barley, It is more mature than wheat [2]. is also has a dual-use crop where as it is in the food industry, also is used in pharmacological manufacture [3]. is a source of starch and it is need as well as, production vinegar and as fat of in as animal feeding [4]. As into the medical uses, which are important and many, also it reduce cholesterol in the blood, promotes the healing of gastric ulcer , has effective properties against some types of cancers and has the effectiveness against infections and allergies, more over green barley is considered as an anti-oxidant potent [5]. Barleys niche active substances as Maltine and Hordenine, which strengthens the nerves, tonic to the liver, stabilize blood pressure and blood glucose level also is used in the treatment of chest diseases, infections of the urinary system and the treatment of gout and milk management in infants [6].

Hydrogen peroxide which cause oxidative stress on plant also called oxygen water, a chemical compound with H_2O_2 formula is a weak acid, and plays many of the basic roles in the process of food metabolism of the plant and has a wide range of interactions, organizing the closure and open the gaps and participate in the processes of metabolism and natural growth of the plant [7]. hydrogen peroxide in low concentrations gives a partial indication of the organization of biological and physiological processes as photosynthesis, cell cycle, plant growth and development, plant response, gene expression, and stress for biotic and a-biotic stress [8].

Micronutrients are essential for crop production and their deficiency reduce growth, metabolism and reproductive phase of crop plants, animals and human beings. Among the micronutrients, zinc deficiency in plants and soils has been reported all over the world [9]. Zinc co-directs the process of photosynthesis during the reproductive stage by participating in electron transport [10]. Micronutrients also help to increase the efficiency of macronutrients[11]. zinc deficiency may inhibit the activities of a number of antioxidant enzymes causing damage to membrane lipids, proteins and nucleic acids [12].

Fulvic acid and hardly soluble in water [13]. Fulvic acids are a mixture of weak aliphatic and aromatic organic acids [14]. Humic acid the fraction are not soluble in water under pH < 2 conditions, but are soluble hardly athigher pH [13,15]. Humic acids are termed 14 polydisperse because of their variable chemical features [16]. Humic acids are brown-black polymeric acids of plant origin that are ubiquitous at the Earth's surface [17]. Due to the lack of studies of these factors, the experiment aims to determine the effect of the flavic acid and the zinc element in improving the growth properties of barley plant under the influence of chemical stress caused by hydrogen peroxide.

MATERIALS AND METHODS

Site and design experience

The field experiment was conducted during the winter growth season 2018-2017 in the Botanical Garden of the Department of Biology within the College of Education for Pure Sciences - Ibn Al-Haytham/ Baghdad University for the purpose of studying the treatment of the harmful effect of hydrogen peroxide with the humic fulvic acid and the zinc element and their overlap in the growth and yield of barley plant (*Hordeum vulgare* L.). The experiment was designed according to Randomized Complete Block

Design (RCBD) as a Worker experiment (3×3×4×3). The experiment was divided into three replicates each containing 36 experimental units. The experimental unit area with dimensions (100 cm × 60 cm) Unit and another distance of 30 cm. The cultivation of barley seeds was done on 1/12/2017 and the plants were harvested on 2/5/2018.

The experiment included three factors:

1. Hydrogen peroxide concentrations (2%, 4% plus control treatment)
2 - Concentrations of humic fulvic acid (25 $mg.L^{-1}$, 50 $mg.L^{-1}$ In addition to the treatment of control)
3. Zinc sulphate concentrations (50 $mg.L^{-1}$, 100 $mg.L^{-1}$, 150 $mg.L^{-1}$ plus control treatment)

Determination of the efficacy of enzyme antioxidants for barley plant.

Determination of the efficacy of the enzyme superoxide dismutase (absorption unit.mL^{-1}) Estimated by [18].

Used Solutions:

A-Nitro Blue Tetrazolioum

B- Ribovlavin: The rahiboflavin solution was 47.7 micromol at a weight of 0.0018 gm and soluble in a small amount of distilled water and supplemented to 100 ml of distilled water.

Preparation of volume of solutions:

The solution	1	2	3	4	The total solution
Ingredients	Potassium phosphate is 82.4 mmol	Lymethionine ammonia is 14 mM	Ttitron-X 1%	14.4 mg + 10 ml distilled water	……..
Size (ml)	18.35	1.5	0.75	1	21.60

Procedures : 1 gram of soft plant tissue was crushed at 78 days with 10 mL potassium phosphate (0.1 M) and kept in the refrigerator under 3 ° C for a whole day. The centrifuge was separated by a speed of 1,000 cycles / minute for 15 minutes , Placing 1.5 mL in test tubes of the total volume of solutions in the above table, adding 40 microliters of sample leachate, adding 40 µl of ribovlavin solution, and then reading absorption at 560 nm with Spectrophotometer, I took the sample of the plank to compare in the same way above, as it differed only by not containing the leachate and replacing it with 40 microliters of distilled water. The samples were then transferred to a box containing two 20 watt lamps for 10 minutes, then the absorption was read at the same wavelength, Drawing the standard curve The percentage of inhibition was calculated from the following equation:

The ratio

whereas :

AB_1 = Blank Absorption value before lighting

AB_2 = Blank Absorption value after lighting

AS_1= The absorption value of the sample before lighting

AS_2 = Sample value after lighting

The following equation was then applied to estimate the efficacy of the enzyme (absorption unit.

(D.f.) / (Sample size) × (sample inhibition ratio) / (higher inhibition ratio) = enzyme efficacy

That is

D.f. = Dilution factor

Sample size = 40 µL.

Determination of the efficacy of peroxidase enzyme (absorption unit.mL^{-1}):

As estimated by the method described by [19].

Used Solutions:

A - Solution Base: Guaiacoal solution Prepare to mix 1.36 ml in 250 ml of distilled water

B - hydrogen peroxide solution H_2O_2 0.1% concentration Prepare to take 0.4 ml of H2O2 and complete the volume to 120 mL distilled water.

Procedures: 1 mL of Guaiacoal solution was mixed with 1 mL of H_2O_2 solution. Absorption was obtained at 420 nm wavelength using optical spectrometer. The enzyme efficacy was estimated by adding 2 mL of the Spectrophotometer reaction mixture and 0.1 mL of sample leachate was added. Difference in absorption of light every 30 seconds and for 3 minutes and the same wavelength, and then calculated the effectiveness of the enzyme POD as follows:

(Device change reading) / ((time change) / (0.1 × 0.01) = (absorption unit mL^{-1}) peroxidase enzyme activity

whereas:

0.1 = sample size

0.01 = the amount of enzyme that causes an increase in light absorption of 0.01 ppm at the same wavelength.

Determination of catalase enzyme efficacy (absorption unit mL^{-1})

Estimated according to the method described by [20].

Used Solutions:

A-DRI Phosphate Solution: Prepare a 50 mL buffer phosphate solution at pH 7

B - hydrogen peroxide solution 30 mmol (the record of 0.34 ml of hydrogen peroxide) and completed the size to 100 mL of phosphate.

Procedures: Mix 0.1 mL of the sample sample leachate with SOD with 1.9 mmol of phosphate drip solution and then apply 1 mL of hydrogen peroxide solution and mix with the man. Then read the sample absorption by UV Spectrophotometer at a wavelength of 470 nm , Followed by a change in absorbance for 3 minutes at 30 seconds. Attended the Blank treatment in the same way above without leaching and 0.1 mL of distilled water.

(Change in the device) / (time in change) / (0.1 × 0.01)) = (1-ml. Absorption unit) catalase enzyme efficiency

whereas:

0.1 = sample size

0.01 = the amount of enzyme that causes an increase in light absorption of 0.01 ppm at the same wavelength.

RESULTS

Effect of the enzyme superoxide dismutase (absorption unit. mL^{-1}).

The results of Table (1) showed a significant ($P<0.05$) increase in the effectiveness of the superoxide dismutase

when the concentration of hydrogen peroxide increased from zero to 4%. The average enzyme activity increased by 201.33%, while the concentration of 2% exceeded 4% concentration giving the highest percentage increase of 280.30% when compared to 0%. group The results of the same table showed a significant effect of spraying of humic fulvic acid in increasing the effectiveness of the enzyme, which increased by an increase of 18.53% when lifting the concentration to 50 mg.L-^{1} compared to zero concentration. The results of the table confirm the morphological role of zinc supplementation in increasing the average efficacy of the enzyme, which exceeds the concentration of 150 mg.L-^{1} on the other concentrations, giving an increase rate of 15.73% compared with zero concentration of zinc. The results of the table showed a significant overlap between hydrogen peroxide concentrations and humic fulvic acid. There were significant differences in the increase in the efficiency of the superoxide dismutase. When the concentration of hydrogen peroxide was 4% and the acid spray was 50 mg.L-^{1} the increase was 26.98% compared to the zero concentration of humic fulvic acid Same focus above. The results of the effect of the interaction between hydrogen peroxide concentrations and zinc element concentrations were similar to the above interference and the highest average efficiency of the enzyme at the concentration of 2% hydrogen peroxide and 150 mg.L-^{1}with an average of 215.29 absorption units. mL^{-1} compared with an average of 190.62 absorption units. mL^{-1} when not sprayed with zinc and under the concentration of hydrogen peroxide itself or the lowest value of the average efficacy of the enzyme 47.41 absorption units. mL^{-1} at zero concentration for both workers.The results of the table showed a significant increase in the mean effect of the enzyme, with 42.35% at 50 and 150 mg.L-^{1} concentration for both the acid and zinc in the sequence compared to the non-spray, confirming the role of acid and zinc as an antagonist. Strong oxidation. The results showed that there were significant differences in this effect due to the triple interference between the three factors above in the value of the enzyme's effectiveness, with the highest value of this characteristic being 226.63 absorption units. ml^{-1} at 50 mg.L-^{1}-acid concentration and 150 mg.L-^{1} zinc and 2% hydrogen peroxide-37.22 absorption units. mL^{-1} for this characteristic was at zero concentration of the inhibitory agent and not sprayed the catalysts. The results of the effect of the tripartite interference of acid and zinc in reducing the harmful effect of hydrogen peroxide and increasing concentrations of acid and zinc at the concentration of 4% hydrogen peroxide and the spraying of the workers 50 and 150 mg.L-^{1} gave an increase in the effectiveness of the enzyme 56.31 % Compared to non-sprayed workers and under the influence of the same concentration of hydrogen peroxide.

Table (1) Effect of concentration of Humic Fulvic acid and zinc in the activity of the superoxide oxide dismutase (absorption units. mL^{-1}) of barley plant exposed to hydrogen peroxide stress.

P (%)	H and F (mg.L^{-1})	Concentrations of Z (mg.L^{-1})				Average of P× H and F
		0	50	100	150	
0	0	37.22	42.83	47.18	52.12	44.84
	25	49.36	52.87	53.41	58.28	53.48
	50	55.64	59.89	61.26	69.42	61.55
2	0	182.72	193.86	196.92	205.98	194.87
	25	191.06	195.06	210.78	213.27	202.54
	50	198.07	201.91	215.62	226.63	210.56
4	0	121.87	141.01	148.82	155.79	141.87
	25	152.99	157.50	160.47	167.89	159.72
	50	168.87	177.75	183.49	190.49	180.15
Average of Z		128.64	135.85	141.99	148.88	
L.S.D (0.05)		Effect of Z 0.304				0.456
		Effect of triple interference 0.913				

Effect of P×Z

P (%)	Concentrations of Z (mg.L^{-1})				Average of P
	0	50	100	150	
0	47.41	51.86	53.95	59.94	53.29
2	190.62	196.94	207.77	215.29	202.66
4	147.91	158.75	164.26	171.39	160.58
L.S.D (0.05)	0.527				0.264

Effect of H and F×Z

H and F (mg.L^{-1})	Concentrations of Z (mg.L^{-1})				Average of H andF
	0	50	100	150	
0	113.93	125.90	130.97	137.97	127.19
25	131.14	135.14	141.55	146.48	138.58
50	140.86	146.52	153.46	162.18	150.76
L.S.D (0.05)	0.527				0.264

P= hydrogen peroxide ** H and F= humic fulvic acid ** Z= zinc

Table (2) Effect of concentration of Humic Fulvic acid and zinc in the effectiveness of peroxidase enzyme (absorption units. mL^{-1}) of barley plant exposed to hydrogen peroxide stress.

P (%)	H and F ($mg.L^{-1}$)	Concentrations of Z ($mg.L^{-1}$)				Average of P× H and F
		0	50	100	150	
0	0	5.11	8.07	9.46	10.10	8.19
	25	8.20	10.23	9.55	11.69	9.91
	50	9.69	11.21	13.30	12.70	11.73
2	0	10.19	14.30	16.12	17.21	14.46
	25	14.86	15.31	17.09	18.24	16.37
	50	17.37	16.15	18.22	20.08	17.96
4	0	6.95	8.14	9.65	9.76	8.63
	25	9.15	9.98	9.97	11.25	10.09
	50	10.52	10.30	10.65	12.11	10.90
Average of Z		10.23	11.52	12.67	13.68	
L.S.D (0.05)		Effect of Z 0.039				0.059
		Effect of triple interference 0.118				

Effect of P×Z

P (%)	Concentrations of Z ($mg.L^{-1}$)				Average of P
	0	50	100	150	
0	7.67	9.84	10.77	11.50	9.94
2	14.14	15.25	17.14	18.51	16.26
4	8.87	9.47	10.09	11.04	9.87
L.S.D (0.05)	0.068				0.034

Effect of H and F×Z

H and F ($mg.L^{-1}$)	Concentrations of Z ($mg.L^{-1}$)				Average of H and F
	0	50	100	150	
0	7.42	10.17	11.74	12.36	10.42
25	10.74	11.84	12.20	13.73	12.13
50	12.53	12.55	14.05	14.96	13.53
L.S.D (0.05)	0.068				0.034

P= hydrogen peroxide ** H and F= humic fulvic acid ** Z= zinc

Table (3) Effect of concentration of Humic Fulvic acid and zinc in the activity of the catalase enzyme (absorption units. mL^{-1}) of barley plant exposed to hydrogen peroxide stress.

P (%)	H and F ($mg.L^{-1}$)	Concentrations of Z ($mg.L^{-1}$)				Average of P× H and F
		0	50	100	150	
0	0	1.18	1.96	2.27	2.41	1.96
	25	1.86	2.18	2.27	2.26	2.14
	50	2.12	2.17	2.40	2.49	2.29
2	0	1.88	3.10	3.65	4.22	3.21
	25	3.18	4.05	4.79	4.44	4.12
	50	3.85	4.42	5.05	4.39	4.43
4	0	1.51	1.67	1.72	1.75	1.66
	25	1.82	1.75	1.89	1.85	1.83
	50	1.87	1.90	2.14	2.11	2.01
Average of Z		2.15	2.58	2.91	2.88	
L.S.D (0.05)		Effect of Z 0.016				0.024
		Effect of triple interference 0.049				

Effect of P×Z

P (%)	Concentrations of Z ($mg.L^{-1}$)				Average of P
	0	50	100	150	
0	1.72	2.10	2.31	2.39	2.13
2	2.97	3.86	4.50	4.35	3.92
4	1.73	1.77	1.92	1.90	1.83
L.S.D (0.05)	0.028				0.014

Effect of H and F×Z

H and F ($mg.L^{-1}$)	Concentrations of Z ($mg.L^{-1}$)				Average of H and F
	0	50	100	150	
0	1.52	2.24	2.55	2.79	2.28
25	2.29	2.66	2.99	2.85	2.70
50	2.61	2.83	3.20	2.99	2.91
L.S.D (0.05)	0.028				0.014

P= hydrogen peroxide ** H and F= humic fulvic acid ** Z= zinc

Effectiveness of the enzyme peroxidase (absorption units. mL^{-1}).

The results of Table (2) showed the significant ($P<0.05$) effect of increasing the concentration of hydrogen peroxide in increasing the effectiveness of the enzyme peroxidase and the highest average efficiency of the enzyme 16.26 absorption units. mL^{-1} concentration at 2% concentration of hydrogen peroxide concentration and the lowest average concentration of 4% hydrogen peroxide with 9.87 absorption units. mL^{-1} while at zero concentration of hydrogen peroxide the average was 9.94 absorption units. mL^{-1}. In the same table, there was a significant effect of humic fulvic acid in increasing the effectiveness of peroxidase when the concentration of acid was increased from 0 to 50 $mg.L^{-1}$an increase of 29.85%. The effect of the zinc element was also significant in the effectiveness of the enzyme at a concentration of 150 $mg.L^{-1}$ gave 13.68 absorption units. mL^{-1} with an increase of 33.72% compared with control treatment, which gave 10.23 absorption units. mL^{-1}. The results of the binary interaction between the concentration of hydrogen peroxide and the concentration of the humic fulvic acid showed significant differences in the mean effectiveness of the enzyme. When the concentration of hydrogen peroxide was 2% and the acid spray at 50 $mg.L^{-1}$ concentration was 24.20% Of hydrogen peroxide, while the effect of the overlap between the concentration of hydrogen peroxide and zinc concentration between the zinc spray at different concentrations has a significant effect compared to the non-spray, where the increase rate of 24.46% at the concentration of 150 $mg.L^{-1}$ zinc under the influence of 4% concentration of hydrogen peroxide. The results showed that there was a significant effect on the increase in the average efficiency of the enzyme by 101.62% when spraying with the highest concentration of acid and zinc element compared to the concentration of zero for the workers. The results of the effect of the triple interference of acid and zinc in reducing the effect Hydrogen peroxide and increased concentrations of acid and zinc when the concentration of 4% of hydrogen peroxide and the lack of spraying of workers amounted to the effectiveness of the enzyme 6.95 absorption units. mL^{-1}. When sprayed with concentrations 50 and 150 $mg.L^{-1}$ the value of 12.11 absorption units was given. ML with an increase of 74.24% and the highest interference value of 20.08 absorption units. mL^{-1} at 2% of the concentration of hydrogen peroxide and spray at the highest concentration of workers while the lowest value was 5.11 absorption units. mL^{-1} at zero concentration of hydrogen peroxide and non-spraying of acid and element.

Effect of catalase enzyme (absorption units. mL^{-1})

The results of Table (3) showed that the highest average efficiency of catalase was at 2% concentration of hydrogen peroxide, giving 3.92 absorption units. mL^{-1} while the lowest average concentration was 4% hydrogen peroxide with 1.83 absorption units. Ml, while at zero concentration of hydrogen peroxide the average efficiency was 2.13 absorption units. mL^{-1}. The effect of spraying of the humic fulvic acid was significant at zero concentration of acid. Ml and the average of this characteristic increased with acid concentration with a concentration increase to 50 $mg.L^{-1}$. The average efficiency was 2.91 absorption units. mL^{-1} with an increase of 27.63%. When the zinc was sprayed, the results showed that there was a significant effect on the mean effectiveness of the enzyme. The average concentration increased by 100 $mg.L^{-1}$ and by 35.34%. The results of the double interference between the concentrations of hydrogen peroxide and the concentrations of the humic fulvic acid were significant in the mean of this characteristic. At the concentration of 2% hydrogen peroxide and spray with a concentration of 50 $mg.L^{-1}$ the increase was 38.00% compared to the non-spraying of acid and the same concentration above. The results of the effect of the interaction between hydrogen peroxide concentrations and zinc element concentrations were similar to the above interference, increasing the concentration of zinc to 150 $mg.L^{-1}$ At 2% concentration of hydrogen peroxide, the average was increased by 46.46% compared to non-zinc spraying. The results showed a significant effect on the average activity of the catalase enzyme. The highest mean was 50 and 100 $mg.L^{-1}$of humic fulvic acid and zinc with 3.20 absorption units. mL^{-1} and an increase of 110.53% compared to the non-spraying of acid and zinc, which amounted to 1.52 absorption units. Ml-1. The results of the triple interference between the factors studied above showed a significant effect on the effectiveness of the enzyme when spraying with acid and zinc at concentrations of 50 and 150 $mg.L^{-1}$ In 2% of the hydrogen peroxide, it increased significantly by 133.51% compared to the non-working spray and the concentration of hydrogen peroxide itself, For triangular interference 5.05 absorption units. mL^{-1} at 2% of the concentration of hydrogen peroxide and when spraying with a concentration of 50 and 100 $mg.L^{-1}$ for the humic fulvic acid and the zinc element while the lowest value was 1.18 absorption units. mL^{-1} at zero concentration of hydrogen peroxide and non-spray catalyst.

DISCUSSION.

The increased concentration of hydrogen peroxide increased the efficiency of the enzymatic oxidation system, which includes the enzymes of superoxide oxide dismutase, peroxidase and catalase with a concentration of 2% for all enzymes. This may be due to the fact that low concentration hydrogen peroxide encourages plant tolerance to stress [21]. hydrogen peroxide sends chemical signals that stimulate the transport mechanisms of stress types. It also regulates the control of defensive genes of enzymatic antioxidants, defensive proteins, and genetic cloning factors. Hydrogen peroxide is more stable in the cell And has a role in the release of chemical signals that cause plant resistance to stress and this signal works on the so-called gene expression These genes are working in the development of the defense system through the induction of systemic acquired resistance or systemic acquired acclimation [22]. or perhaps back The cause of the increase in the contribution of the enzyme POD in many resistance mechanisms, it works to strengthen the cell wall

by the formation of lactin and this compound is important because it is a means of defense against pathological injuries [23]. The root of the single oxygen and hydrogen peroxide that accumulates in the plastids, mitochondria, the internal endoplasmic network and the peroxysomes and their evolution from the activation stage to the interactions between the free radicals, stimulates the plant to activate the enzyme system against these roots [24].

The increased concentration of zinc concentrations has led to an increase in the effectiveness of SOD, POD, and catalase. The amino acid, is the essential building block for oxins. It also stimulates the enzymes of carbonic peptidase, proteinase, enolase, and anhydrase. It has a role in building protein, starch, and cytochrom b, cytochrom, cytchrom oxidase and maintains the stability of the ribosome parts [25]. Aging is a by-product of oxidation of the plant's cells, especially in organelles in which transfers of electrons, such as plastids and mitochondria, occur in the form of oxidative damage [26]. Zinc An anti-oxidant agent that has the role of diphtheria and systemic antioxidant in the cell membrane by increasing the activity of antioxidant defense in the plant cells of oxidative enzymes, superoxide oxide dismutase, Catalase peroxidase Ascorbi and also affects the increase in the content of antioxidant ascorbic acid Saw poison roots 0_2 and H_2O_2 [27].

The increased concentration of humic fulvic acid concentrations has led to an increase in the effectiveness of SOD, POD and catalase enzymes. This may be due to the addition of Folic acid which encouraged the absorption of elements by the plant and increased concentrations of iron, zinc, manganese, nitrogen, phosphorus and potassium which have a correlation Close to antioxidant enzymes [28].

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