

## Effect of some factors on enzymatic activity of catalase in maize (*Zea mays* L.)

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**Abstract:** Catalase (EC 1.11.1.6), an antioxidant enzyme, has been screened in different plants. The objective of this study was to evaluate the activity, kinetic behavior, and the effect of some factors : pH, temperature, and Zn on catalase activity in *Zea mays* stalks (6 day old). The results showed that catalase exhibited optimum activity at pH 7.0 using 0.05M phosphate buffer and the optimum temperature at 40°C, also the results displayed that the value of Michaelis-menten constant ( $K_m$ ) for  $H_2O_2$  in these conditions equalled 0.2 M. This study showed that kernels germination using different concentrations of zinc chloride (0; 1; 3 and 6 mM) increased the enzymatic activity and the increase was correlated with the concentration, and 9 mM caused inhibitory effect on germination, while the incubation of stalks (6 day old-distilled water was used for germination and growing) for 24h in different zinc concentration (1; 3; 6 and 9 mM) reduced the enzymatic activity; the most inhibitory effect was at 9 mM.

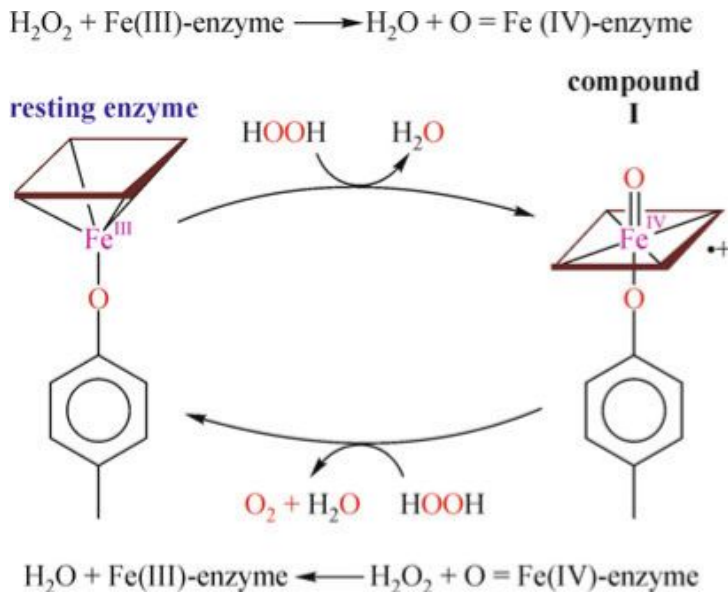
**Key words:** catalase; *Zea mays*; pH; temperature; kinetic;  $K_m$ ; zinc.

### Introduction

Maize (*Zea mays* L.) is an important worldwide cereal crop [1]. It is used as a raw material for food and feed processing and recently as a bio-fuel [2]. Thus, the demand for maize plants and grains is steadily increasing.

Catalase (EC 1.11.1.6) was the first antioxidant enzyme to be discovered and characterized [3]. It plays an important role in defending the cell against oxidative stress and is distributed in almost all aerobic and facultative anaerobic organisms [4].

The reactions it catalyses are crucial to life. The enzyme is found in high concentrations in a organelle in cells called the peroxisome in addition to glyoxysome; cytosol; and mitochondria [5]. This enzyme catalyses dismutation of two molecules of  $H_2O_2$  to water and molecular oxygen.  $H_2O_2$  is a powerful and potentially harmful oxidizing agent that generated in plants during mitochondrial electron transport, oxidation of the fatty acids and most importantly photorespiratory oxidation. Catalase (CAT) also uses hydrogen peroxide to oxidize toxins; such as phenols, formic acid, formaldehyde and alcohols [6, 7, 8, 9]. CAT is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme groups that allow the enzyme to react with the hydrogen peroxide (Figure 1)[10]. Although some bacterial catalases use manganese as the redox-active co-factor, all known eukaryotic forms are haem-based [11].



**Figure 1.** Reaction of CAT with  $\text{H}_2\text{O}_2$ .

Numerous studies have shown that catalase plays an important role in plant defense, senescence and aging [12, 13]. Previous studies have shown that catalase existed in multiple forms in several plants such as tobacco, saffron, cotton, mustard, maize, wheat, sunflower, castor bean, spinach, pepper, loblolly pine and kohlrabi [14, 16, 17, 18, 19].

Heavy metals; such as Zn are essential for normal plant growth and development since they are constituents of many enzymes and other proteins. However, elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity symptoms and growth inhibition in most plants [20, 21]. In addition, a heavy metal excess may stimulate the formation of free radicals and reactive oxygen species (ROS); such as superoxide anion ( $\text{O}_2^-$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), hydroxyl radicals ( $\text{OH}^\cdot$ ), and peroxy radicals, perhaps resulting in oxidative stress [22, 23, 24]. ROS inactivate enzymes and damage important cellular components, and are responsible for protein, lipid and nucleic acid modification and are thought to play a major role in ageing and cell death [25]. To avoid the accumulation of these compounds to toxic level, plants evolved several strategies to regulate the level of ROS in plant cells, one of this strategies is activate ROS-scavenging enzymes such as catalase, superoxide dismutase and peroxidases [26, 27].

The goals of this study were to investigate the optimum pH and temperature for catalase activity, and the effect of zinc on this activity in *Zea mays* plants.

## Materials and Methodes

### Plant Materials

*Zea mays* kernels (Ghuta-82) were obtained from General Organization for seed Multiplication (Aleppo - Syria).

### Reagents

Sulfuric Acid (10%) was obtained from Panreac.  
 Buffers (citrate buffer, phosphate buffer, Tris-HCL buffer).  
 $\text{KH}_2\text{PO}_4$  was obtained from CDH,  $\text{K}_2\text{HPO}_4$  from Fluka.  
 Potassium Permanganate from BHD.  
 Hydrogen Peroxide (1%) from Panreac.  
 $\text{ZnCl}_2$ . Distilled water was used for all assays.

## Equipment

Sensitive scales (Sartorius Bl60s), Germany.  
Vortex –MSI (USA).  
Incubator (GFL 3031), Germany.  
Cooler centrifuger (Boeco - U32R), Germany.  
Vis-Spectrophotometer (Vis- 7220).

## Plants culture

Kernels were selected for size and shape, had their surface sterilized with 1% sodium hypochlorite for 5 min to avoid fungal contamination, and rinsed with distilled water. Kernels were cultured in the dark at 25°C, moistened with distilled water for 6 days with continuous ventilation. After 6 days stalks were harvested.

## Extraction and determination of crude catalase activity

### Extract preparation

Extracts were prepared from stalks after 6 days. 1g of stalks were homogenized with 25ml of ice-cold potassium phosphate buffer (0.05 M, pH 7) in cold pestle and mortar. After centrifugation at 5000 × rpm for 20 min at 4°C, transparent supernatant termed “crude extract” was obtained and used for our studies. All experiments were done at 4°C and within 24 hours [28].

### Enzyme assays

CAT activity was measured by measuring the consumed amount of H<sub>2</sub>O<sub>2</sub> by titrating with potassium permanganate (0.1N). Assays were carried out at room temperature; extract were added to 0.05 M phosphate buffer, containing H<sub>2</sub>O<sub>2</sub> in final concentration of 0.023 to 0.18 M (for K<sub>m</sub> determination) and 0.135 mM for other experiments; then the reaction was stopped using 5ml sulfuric acid (10%). Reaction velocity was computed from linear slopes of [H<sub>2</sub>O<sub>2</sub>]-time curve. One unit of activity was defined as the amount of enzyme necessary for reducing 1 μmol of H<sub>2</sub>O<sub>2</sub> per minute.

### Effect of pH on enzyme activity

To determine the optimum pH for catalase, activity measurements were carried out over a pH range of 3.0 to 12.0. The three buffers used were citrate phosphate (pH 3.0 and 5.0), potassium phosphate (pH 6.0 and 7.0), and Tris-HCL (pH 8.0; 9.0; and 12.0). CAT activity was measured according to the method described above.

### Effect of temperature on catalase activity

Effect of temperature on catalase activity was studied at constant temperature between 4 and 90°C at atmospheric pressure. Samples were placed in the indicated temperatures for the time specified; and the reaction stopped using sulfuric acid.

### Enzymatic Kinetics

In order to determine the kinetic properties, CAT initial velocity was evaluated toward H<sub>2</sub>O<sub>2</sub> concentrations ranging from 0.023 to 0.18 M, each concentration had its separate control. The Michaelis–Menten constants (K<sub>m</sub>) were determined from Lineweaver–Burk plots at optimum pH and temperature conditions.

### Effect of Zn on CAT activity

We study the effect of Zn in tow cases. First, *Zea mays* kernels were germinated and grown under different ZnCl<sub>2</sub> concentration (0-1-3-6-9 mM) then we took stalks after 6 days and assayed CAT activity.

In another case the kernels were germinated and grown using distilled water then we took stalks after 6 days and incubated them in different solutions of ZnCl<sub>2</sub> (0; 1; 3; 6 and 9 mM) for 24hours, then we assayed CAT activity.

## Statistical analysis

Three replicates for each experiment were carried out. One-way analysis of variance was determined using the Tukey test. Differences between means were considered to be significantly different at  $P < 0.05$ .

## Results and Discussion

### Effect of pH on catalase activity

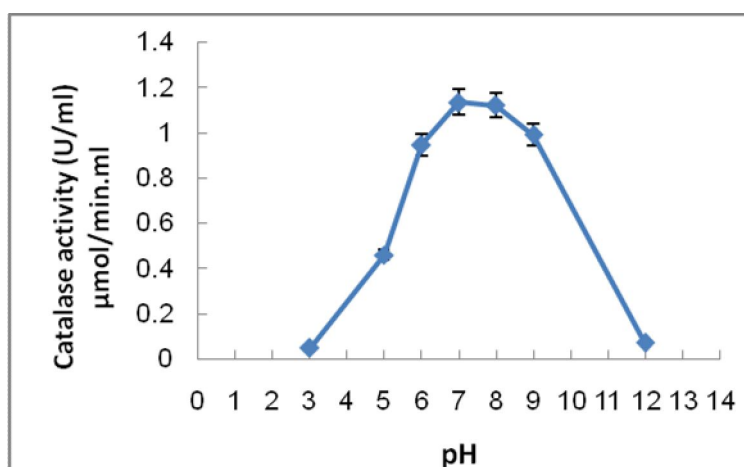
The optimum pH is a key factor in the expression of enzyme activity, using citrate phosphate, potassium phosphate, and Tris-Hcl as buffer solutions, the assay of CAT activity was carried out. It was found that the optimum catalase activity was at pH 7.0 - 8.0 with lower activity below pH 6 or above pH 8 (Figure 2, Table 1).

This result was similar to other studies [4] that found that the optimum pH of CAT in *Phyllanthus reticulatus* was pH 7. While the highest activity for CAT from *Van apple* was observed at pH 5.0 [29].

**Table 1.** Effect of pH on catalase activity (each value represents the average 3 replicates  $\pm$  standard deviation)

pH	Catalase activity ( $\mu\text{mol}/\text{min.ml}$ )
3	0.0474 <sup>a</sup> $\pm$ 0.009
5	0.4587 <sup>b</sup> $\pm$ 0.033
6	0.9459 <sup>c</sup> $\pm$ 0.072
7	1.1330 <sup>c</sup> $\pm$ 0.103
8	1.1214 <sup>c</sup> $\pm$ 0.113
9	0.9900 <sup>c</sup> $\pm$ 0.064
12	0.0697 <sup>a</sup> $\pm$ 0.006

<sup>abc</sup> Means with different superscripts are significantly different, according to Tukey test ( $p < 0.05$ ).



**Figure 2.** Effect of pH on catalase activity (means  $\pm$  standard error).

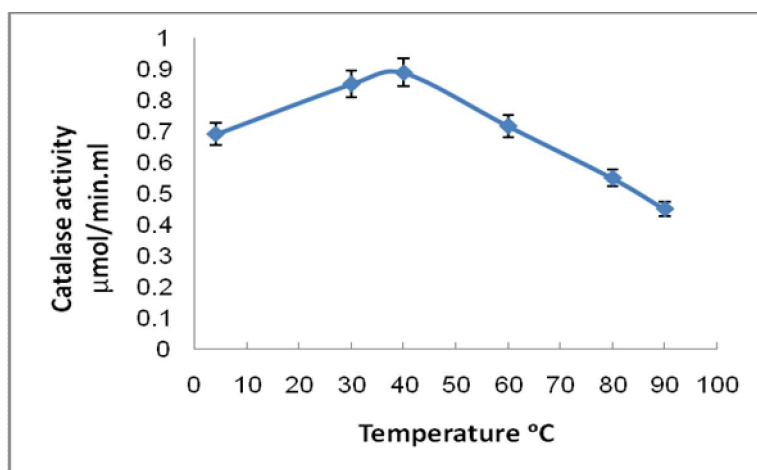
### Effect of temperature on catalase activity:

The effect of temperature on catalase activity was studied by assaying the enzyme activity at different temperatures ranging from 4°C to 90°C. Catalase activity increased gradually from 20°C to 40°C and then declined due to the denaturation of enzyme. The results obtained indicate that the catalase is maximally active at 40°C (Table 2, Figure 3). Catalase from *Phyllanthus reticulatus* and *Zantedeschia aethiopica* also showed similar optimum temperature [4].

**Table 2. Effect of temperature on catalase activity (each value represents the average 3 replicates  $\pm$  standard deviation).**

Temperature $^{\circ}\text{C}$	Catalase activity ( $\mu\text{mol}/\text{min}.\text{ml}$ )
4	$0.6925 \pm 0.004$
30	$0.8540 \pm 0.004$
40	$0.8901 \pm 0.002$
60	$0.7183 \pm 0.002$
80	$0.5503 \pm 0.003$
90	$0.4509 \pm 0.012$

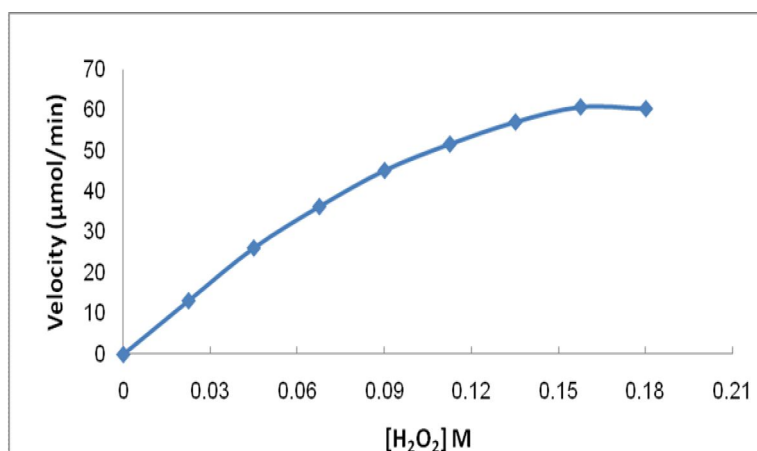
Means are significantly different ( $p=0.000 < 0.05$ )



**Figure 3. Effect of temperature on catalase activity.**

#### Kinetic studies of the catalase:

We studied the effect of different concentrations of  $\text{H}_2\text{O}_2$  (0.023; 0.045; 0.068; 0.09; 0.113; 0.135; 0.158; 0.180 M) on reaction velocity using phosphate buffer (0.05M, pH 7.0) and 6ml of crud enzyme extract. The results obtained were depicted in Figure 4 which shows that the velocity was enhanced with increasing  $\text{H}_2\text{O}_2$  concentrations.  $K_m$  for  $\text{H}_2\text{O}_2$  was found to be 0.2 M (Figure 5).



**Figure 4. Effect of  $\text{H}_2\text{O}_2$  concentration on reaction velocity.**

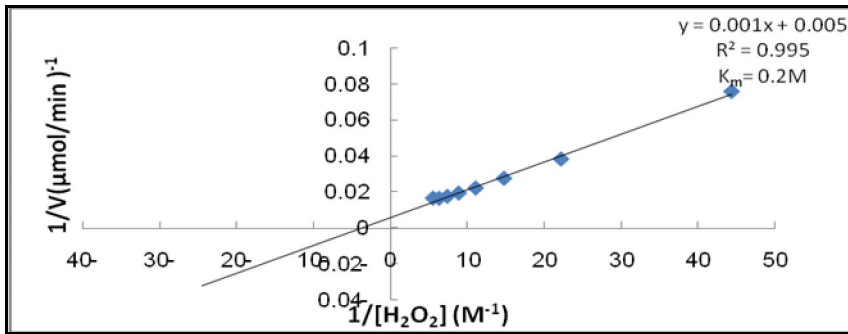


Figure 5. Lineweaver–Burk plots of CAT activity in presence of different concentrations of H<sub>2</sub>O<sub>2</sub>.

**Effect of Zn on growth**

The results showed that low concentration of ZnCl<sub>2</sub> (1mM) caused a small decrease in seedling length about 1.18%, while high concentrations (3, 6 mM) led to significant decrease in seedling length (59.53 and 66.12% respectively), and 9mM prevent seedling growth (Table 3, figure 6 and 7). From this result we can say that high concentrations of ZnCl<sub>2</sub> caused inhibitory effect on growth.

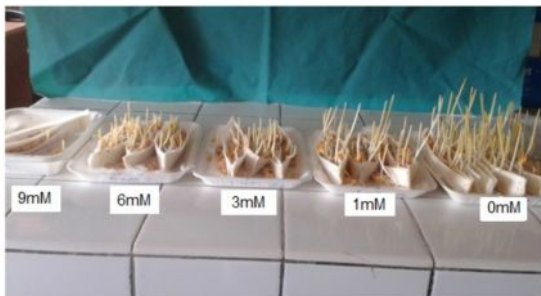


Figure 6. Effect of Zn on *Zea mays* seedling growth.

Table 3. Effect of Zn on seedling Length (each value represents the average 10 replicates ± standard deviation).

Concentration of Zn (mM)	seedling Length(cm) ± SD
0	12.75 <sup>a</sup> ± 2.01
1	12.60 <sup>a</sup> ± 3.69
3	05.16 <sup>b</sup> ± 0.77
6	04.32 <sup>b</sup> ± 0.80
9	00.00 <sup>c</sup> ± 0.02

<sup>abc</sup> Means with different superscripts are significantly different, according to Tukey test ( $p < 0.05$ ).

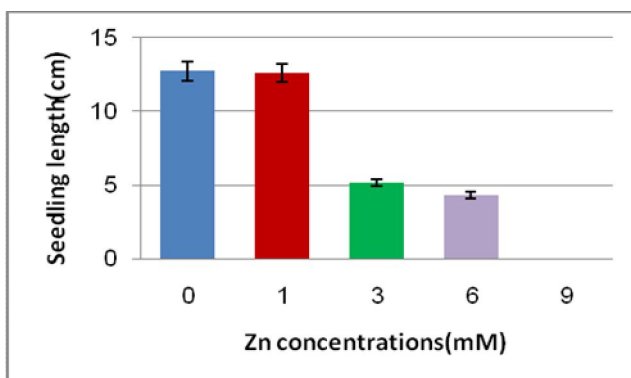


Figure 7. Effect of Zn on *Zea mays* seedling length (means ± SD).

Our result was similar to other studies that show when zinc exist in low concentrations it increases the growth, while in high concentrations it inhibits the growth as discus [30] when they showed that low zinc concentrations (0.25Mm, 0,5mM) increased the growth, while high concentrations decreased the growth.

Zinc inhibited growth by interfering with normal cellular metabolic events and inducing visible injuries and physiological disorder, as are reported by some workers [31], [32]. Also it induced nutrient deficiency e.g. that of iron and by the substitution of  $Mg^{+2}$  [33], [34]. In addition zinc caused the enhancement of ROS production, which in turn led to the oxidative damage to plant cells and blocked the growth [30].

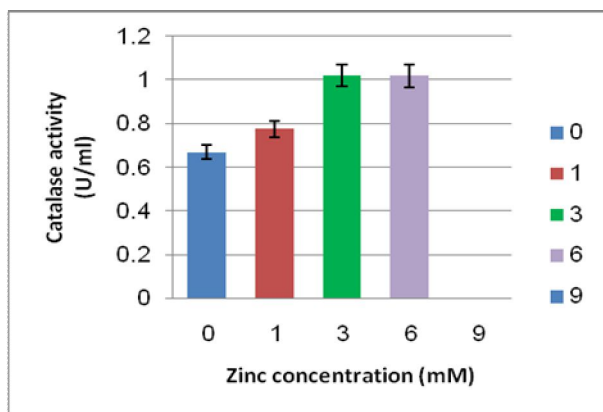
#### Effect of Zn on CAT activity

When kernels were germinated and grown using different concentrations of  $ZnCl_2$ , CAT activity increased and the increase was correlated with the concentration. The results showed non-significant increase in the activity using 1mM  $ZnCl_2$ , while there was significant increase in the activity using 3 and 6 mM. The activity increased about 2.03% at 1 mM, 34.34% at 3 mM and 34.17% at 6 mM in comparing to 0 mM (Table 4, figure 7).

**Table 4. Effect of Zn on CAT activity (we used dH2O and different Zn concentration for kernels germination and growth- each value represents the average 3 replicates  $\pm$  standard deviation ).**

Concentration of $ZnCl_2$ (mM)	catalase activity ( $\mu\text{mol}/\text{min.ml}$ ) $\pm$ SD	Percent of activity
0	0.6796 <sup>a</sup> $\pm$ 0.015	100%
1	0.7750 <sup>b</sup> $\pm$ 0.009	102.03%
3	1.0205 <sup>c</sup> $\pm$ 0.003	134.34%
6	1.0192 <sup>c</sup> $\pm$ 0.005	134.17%
9	0.00	0%

<sup>ab</sup> Means with different superscripts are significantly different, according to Tukey test ( $p < 0.05$ ).



**Figure 8. Effect of Zn on CAT activity (we used dH2O and different Zn concentration for kernels germination and growth).**

We can say that zinc caused oxidative stress to the plant so the plant responded by increasing the activity of antioxidant enzymes like catalase.

The induction of this enzyme under zinc stress indicated that it helps in inhibiting the oxygen radical accumulation. Similar to the present study, an increase in CAT activity has been reported in other plant species exposed to zinc stress [30], [35], [36]. Based on the above results, our findings provide evidence that CAT may provide an additional protection against the oxidative damage induced by zinc stress.

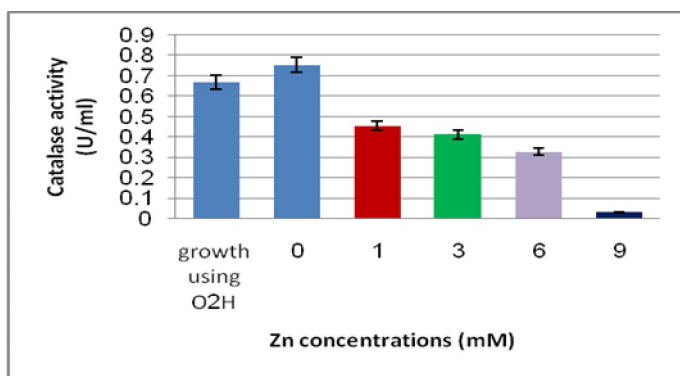
In another case, when kernels were germinated and grown using distilled water and then stalks were cut and incubated in different solutions of  $ZnCl_2$  (0; 1; 3; 6 ; and 9 mM) for 24 hours, CAT activity decreased, when

we increased the concentration the activity more decreased. The decrease was significant ( $p < 0.05$ ) (Table 5, Figure 8).

**Table 5. Effect of Zn on CAT activity after incubation (each value represents the average 3 replicates  $\pm$  standard deviation ).**

Concentration of ZnCl <sub>2</sub> (mM)	Catalase activity ( $\mu\text{mol}/\text{min.ml}$ ) $\pm$ SD
0	0.7550 $\pm$ 0.001
1	0.4561 $\pm$ 0.008
3	0.4137 $\pm$ 0.021
6	0.3282 $\pm$ 0.007
9	0.0319 $\pm$ 0.004

Means are significantly different ( $p < 0.05$ )



**Figure 9. Effect of Zn on CAT activity (after incubation).**

The results showed that when we removed the stalks from the plant and incubated it in different zinc solution, catalase activity had reduced and it was less than activity when incubated or germinated using distilled water, so stalks alone couldn't activate antioxidant enzymes when it cut from the plant and incubated in zinc solutions. The decrease in CAT activity might be due to inhibition of enzyme synthesis or due to a change in the assembly of enzyme subunits [37].

## Conclusion

Enzymes are generally sensitive to reaction conditions such as pH and temperature, so it is very important to control the reaction conditions for each enzyme. In this study we evaluated the best activity conditions for one of most important antioxidant enzymes (catalase) and studied the effect of Zn on this activity. Heavy metals are essential and important for normal growth and development of plants being an essential component of many enzymes and proteins. On the other hand, From our experiments we concluded that Zn in the used concentrations caused an inhibitory effect on growth and led to oxidative stress, so the *Zea mays* responded by increasing catalase activity to remove the accumulation of ROS and oxidative damage.

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## Abbreviations

CAT :catalase, ROS : reactive oxygen species.



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