



## Antioxidant enzyme activity in response to iron and copper in *Cuminum cyminum* L.

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### Abstract

In this study, the interaction between iron and copper on growth parameters (plant height, fresh weight of roots, stem's length, fresh weight of shoots, fresh and dry weight of seeds), total proteins and the antioxidant enzymes activity in *Cuminum cyminum* L. were investigated. This experiment was carried out in completely randomized blocks with 9 treatments in 3 repetitions. Plants were treated with Fe fertilizer (0, 3, 6 kg<sup>h</sup><sup>-1</sup>) together with CuSO<sub>4</sub> · 5H<sub>2</sub>O (0, 100, 200 μM). Maximum level of stem length and shoot fresh weight was observed in Fe<sub>3</sub>Cu<sub>100</sub>. In the treatment of Fe<sub>6</sub>Cu<sub>100</sub>, root weight was significantly increased. Fresh and dry weights of seeds were significantly increased under Fe<sub>3</sub> treatment. High concentration of Fe fertilizer with low concentration of CuSO<sub>4</sub> decreased catalase and peroxidase enzymes activities. However, by increasing concentration of Cu in both Fe levels, the activity of ascorbate peroxidase was decreased.

**Keywords:** *Cuminum cyminum* L.; copper; iron; antioxidant enzymes

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### Introduction

Consecutive and simultaneous cultivation of fields, cultivation of high yielding varieties with high nutritional requirements and uncontrolled and unbalanced use of fertilizers, especially nitrogen and phosphorus fertilizers, has caused micronutrients deficiency symptoms and the yield loss due to this deficiency. Not only has the balanced use of main fertilizers and micronutrients increased the production of high

quality agricultural products, but also it ensures the health of consumer's society. *Cuminum cyminum* L. is an annual and a flowering plant in the family Apiaceae, native from the east Mediterranean to India. *C. cyminum* is widely used by people as a carminative, anti-worm and seizure, eliminator of cramps in children, effective for eye health and eye bleeding and redness or sticky eyelids, and helpful for Rickets disease and stomach pains (Zargari, 1989).

The most important task of iron is its presence in enzymatic systems of plant. The presence of iron is necessary for Chlorophyll synthesis, because its shortage causes yellowing

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and chlorosis in the leaves. The importance of iron enzymes, especially cytochromes, is obvious in plant respiration. Iron is absorbed by plants as  $Fe^{2+}$ . Although the volume of this element in soil is very high, its deficiency symptoms is frequently reported (Ramesh, 2001; Taiz and Zieger, 2010).

Copper ( $Cu^{+2}$ ) is an essential trace element needed for the normal growth and development of plants. Cu participates in membrane structure, metalloproteinase enzymes, and plastocyanin in chloroplasts membrane (Marschner, 2005). Both absence and excess of copper inhibits the plant growth and impairs important cellular processes. Therefore, optimum copper concentration ensures the normal growth and development of plants (Jain et al., 2009). On the other hand, at high concentrations, copper was shown to inhibit plant growth by hampering important cellular processes such as photosynthesis and respiration (Fariduddin et al., 2009). Copper is one of the heavy metals that contribute in soil pollution. Kashirad (1970) reported the beneficial effect of using manganese, zinc and copper in improving performance and increasing the concentration and absorption of these elements in grains.

Although both iron and copper are essential for the plants growth, their excess can produce reactive oxygen species (ROS) and increase the number of free radicals. Induction of oxidative stress from toxicity of metals causes toxicity and decreases the growth (Rahimizadeh, et al., 2007; Azooz et al., 2012). One way to deal with these stresses is to increase the activity of antioxidant enzymes such as catalase, peroxidase and ascorbate peroxidase. Murugan and Harish (2007) and Sztrum et al. (2010) suggested that plants and alga responded to heavy metals effectively by antioxidant compounds and scavenging enzymes. The purpose of this study is to survey the effect of iron and copper on growth and activity of antioxidant enzymes.

## Materials and Methods

### Planting and treatment

The experiment was designed in completely random blocks and performed with 9 treatments, each with 3 replicates. For this

purpose, 27 plots with an area of 1 m<sup>2</sup> were prepared.

A week before planting the seeds, iron fertilizer Omex (0, 3, 6 kg h<sup>-1</sup>) was added to the soil of plots. Then, the seeds were planted and irrigated frequently. After the stage of four-leaves, they were treated by spraying  $CuSO_4 \cdot 5H_2O$  (0, 100, 200  $\mu$ M). Spraying was performed once a week.

### Assay and protein extraction

Frozen leaves (0.5 g fresh weight) were homogenized in 5 ml Tris- Glycine buffer (pH 8.3). The homogenate was centrifuged at 12000 g for 10 min. All operations were performed at 4 °C. Protein contents were determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard.

### Measurement of catalase activity

Activity of catalase was measured in a reaction mixture consisting Tris-Glycine buffer (50 mM, pH 7.5),  $H_2O_2$  (10 mM) and enzyme extract. The decomposition of  $H_2O_2$  was followed by the decline in absorbance at 240 nm (Pereira et al., 2002).

### Measurement of peroxidase activity

Peroxidase activity was measured in a reaction mixture consisting acetate buffer (0.2 mM, pH 4.8), hydrogen peroxide (0.1 mM), benzidine (0.04 M) and enzyme extract. Enzyme activity was measured at 530 nm (Koroi, 1989).

### Measurement of ascorbate peroxidase activity

Ascorbate peroxidase activity was measured according to the method of Nakano and Asada (1981). The reaction mixture consisted of enzymatic extract, L-1 sodium phosphate buffer (50 mM, pH 7), ascorbate (0.5 mM), hydrogen peroxide (0.1 mM), EDTA (0.1 mM) and enzyme extract. The reaction started after addition of the hydrogen peroxide, and the absorbance was measured by a spectrophotometer at 290 nm.

## Results

Four days after the third copper spraying, samples were harvested to study the growth parameters. The maximum root fresh weight (RFW) was recorded in the treatment of Fe<sub>6</sub>Cu<sub>100</sub>. High concentrations of iron (6 kg h<sup>-1</sup>) and copper (200 μM) decreased the RFW. Among treatments of the study, the minimum RFW was observed in iron-less treatments (Table 1).

The maximum level of shoot fresh weight (SFW) was obtained from treatment of Fe<sub>3</sub>Cu<sub>100</sub> (56.33 g). Using high concentration of Fe (6 kg h<sup>-1</sup>) decreased the SFW in all copper treatments. Using high concentration of copper also decreased this growth parameter in all iron treatments (Table1).

The result showed that the maximum stem length (SL) was obtained in Fe<sub>3</sub>Cu<sub>100</sub> treatment (25.33 cm). The minimum SL on the other hand, was observed at Fe<sub>0</sub>Cu<sub>0</sub> (Table 1).

The maximum seed fresh weight (SFW) (46.53 g) and seed dry weight (SDW) (13.33 g) were obtained from Fe<sub>3</sub>Cu<sub>0</sub> treatment. Simultaneous application of high concentration of iron (6 kg h<sup>-1</sup>) and copper (200 μM) decreased SFW and SDW. Among 9 treatments, the minimum of them were observed in Fe<sub>0</sub>Cu<sub>0</sub> treatments (Table1).

Four days after the third copper spraying, samples were harvested for protein and the activity of antioxidant enzymes assay (Table 2). Catalase activity increased in Fe<sub>0</sub>Cu<sub>100</sub> and Fe<sub>0</sub>Cu<sub>200</sub> treatments. The minimum of catalase and peroxidase activities were observed in Fe<sub>6</sub>Cu<sub>200</sub>. However at this treatment the activity of ascorbate peroxidase increased. In the presence of Fe, with increasing of Cu

concentration the level of ascorbate peroxidase increased.

## Discussion

Results of the present study indicated that growth parameters increased in the Fe<sub>3</sub>Cu<sub>100</sub> treatment. But high level of seed production was obtained in the treatment of Fe<sub>3</sub>Cu<sub>0</sub>. In all three treatments of copper, high concentration of iron caused a decrease in SFW and SL while simultaneous use of copper and iron fertilizers in low concentrations increased the growth parameters. Surveying growth parameters in different treatments revealed an interaction between copper and iron. Yeritsyan and Economakis (2002) demonstrated that, using iron with high concentration can cause a decrease in growth.

Increasing iron and copper concentration decreased the protein level because free radicals produced from application of these elements deteriorate proteins. The stress produced by microelements and heavy metals increase the activity of antioxidant enzymes. In both concentrations of iron in the presence of low concentration of copper, the activity of peroxidase and catalase increased and by increasing the concentration of copper, their activity decreased. Azooz et al. (2012) in their study on wheat showed copper treatments exhibited a non-significant change in the activities of catalase and ascorbate peroxidase up to 10 mM Cu<sup>2+</sup>. However, when we used copper and iron, by increasing the copper concentration, the activity of ascorbate peroxidase increased. This result was consistent with those reported by Li et

Table 1

Mean stem length (SL), shoot fresh weight (SFW), root fresh weight (RFW), seed fresh weight (SFW), seed dry weight (SDW) in response to different concentrations of Fe and Cu (grouped by Duncan test (p ≤ 0.05). Different letters indicate significant differences between the means.

Treatment	Fe(kg/h)	Cu(μmol)	SL(cm)	SFW(g)	RFW (g)	SFW(g)	SDW(g)
Fe <sub>0</sub> Cu <sub>0</sub>	0	0	20.26(e)	23.33(e)	0.97(d)	14.13(f)	4.23(e)
Fe <sub>0</sub> Cu <sub>100</sub>	0	100	22.33(cd)	32.00(d)	0.85(d)	15.47(e)	5.43(d)
Fe <sub>0</sub> Cu <sub>200</sub>	0	200	22.33(cd)	25.33(e)	1.23(d)	18.37(d)	9.40(b)
Fe <sub>3</sub> Cu <sub>0</sub>	3	0	25.33(ab)	36.00(c)	1.30(cd)	46.53(a)	13.33(a)
Fe <sub>3</sub> Cu <sub>100</sub>	3	100	27.00(a)	56.33(a)	1.60(c)	23.93(b)	9.43(b)
Fe <sub>3</sub> Cu <sub>200</sub>	3	200	21.53(cd)	24.00(e)	2.20(b)	15.33(e)	6.26(e)
Fe <sub>6</sub> Cu <sub>0</sub>	6	0	22.33(cd)	25.33(e)	2.43(ab)	22.83(c)	7.27(cd)
Fe <sub>6</sub> Cu <sub>100</sub>	6	100	23.30(cd)	20.33(ef)	2.73(a)	24.80(b)	7.70(c)
Fe <sub>6</sub> Cu <sub>200</sub>	6	200	23.24(cd)	25.30(e)	1.30(cd)	13.23(f)	3.00(e)

Table 2

Mean of protein content ( $\text{mg}\cdot\text{g}^{-1}\cdot\text{FW}$ ), catalase, peroxidase and ascorbate peroxidase activity ( $\text{OD}\cdot\text{min}^{-1}\cdot\text{g}^{-1}\cdot\text{FW}$ ) in response to different concentrations of Fe and Cu (grouped by Duncan test ( $p \leq 0.05$ )). Different letters indicate significant differences between the means.

Treatment	Fe(kg/h)	Cu( $\mu\text{mol}$ )	Protein	Catalase	Ascorbate peroxidase	Peroxidase
Fe <sub>0</sub> Cu <sub>0</sub>	0	0	25.53(a)	6.05(ab)	2.28(ab)	6.52(ab)
Fe <sub>0</sub> Cu <sub>100</sub>	0	100	21.50(a)	7.60(a)	1.43(cd)	6.58(ab)
Fe <sub>0</sub> Cu <sub>200</sub>	0	200	23.30(a)	7.51(a)	1.55(cd)	5.40(b)
Fe <sub>3</sub> Cu <sub>0</sub>	3	0	26.30(a)	5.67(ab)	1.21(d)	6.15(b)
Fe <sub>3</sub> Cu <sub>100</sub>	3	100	23.80(a)	6.63(ab)	1.66(bcd)	6.14(ab)
Fe <sub>3</sub> Cu <sub>200</sub>	3	200	22.53(a)	6.06(ab)	2.44(a)	6.39(ab)
Fe <sub>6</sub> Cu <sub>0</sub>	6	0	26.26(a)	6.40(ab)	1.10(d)	5.89(b)
Fe <sub>6</sub> Cu <sub>100</sub>	6	100	22.53(a)	7.30(ab)	2.08(abc)	7.53(a)
Fe <sub>6</sub> Cu <sub>200</sub>	6	200	25.63(a)	4.68(b)	2.19(ab)	5.51(b)

al. (2009) on two cultivars of *Brassica campestris*. They showed that by increasing copper concentration, the activities of catalase and peroxidase decreased while the activity of superoxide dismutase increased.

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