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Effects of Ferrous Iron Toxicity on Growth, Biochemical Alterations and Enzymatic Antioxidant Status in *Capsicum annuum* L

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Abstract **Original Research Article**

Ferrous iron toxicity is the most important problem of heavy metal research due to its widespread environmental occurrence and its harmful effects on plant growth and metabolism. Evaluating the impact of ferrous iron induced toxicity provides valuable insights into the biochemical and physiological responses of plants, including alterations in growth parameters, nutrient content and antioxidant defense systems. This study is aimed to investigate the effects of ferrous iron toxicity on growth, biochemical alterations and enzymatic antioxidant status in *Capsicum annuum L*. Experimental *Capsicum annuum* plants were divided into 4 groups. Group 1 served as control, received normal nutrients and water, while Groups 2, 3 and 4 were treated with increasing concentrations of ferrous iron 100, 200 and 400 mg respectively. The results demonstrated that ferrous iron significantly reduced several growth parameters, including germination percentage, root length, shoot length, fresh weight, dry weight and vigour index. Moreover, higher concentrations of ferrous iron led to a decrease in carbohydrate and protein levels, as well as reduction in the activities of important enzymic antioxidants such as catalase and superoxide dismutase indicating a weakened oxidative defense system. The findings highlight the harmful effects of ferrous iron toxicity in *Capsicum annuum*, underscoring the potential risks to agricultural productivity. This research not only deepens our understanding of ferrous iron induced toxicity but also emphasizes the necessity to developing strategies to protect crops from metal toxicity, ensuring sustainable agriculture and food security in contaminated environments.

Keywords: *Capsicum Annuum*, Environment, Heavy metals, Iron, Plants, Pollution, Toxicity, Antioxidants.

INTRODUCTION

Heavy metals (HMs) contamination is the excessive accumulation of toxic HMs in the soil due to human activities. In recent years, as the global economy has developed in recent past years, both types and HMs concentrations in the soil have increased, leading to the environment degradation (Zojaji *et al*., 2014). HMs are highly hazardous to living organisms and environment. It can be accumulated through the food chain. Once soil becomes contaminated with HMs, remediation process is extremely difficult. In the recent past, contamination of soil was not considered as most important than air or water pollution, because it often occurs over a broad area and is more difficult to manage. However, in the past years, contamination of soil in developed countries has becomes a growing concern, increasing more attention and becomes most important topic in the safety of environment worldwide.

HMs contamination in soil arises from both anthropogenic and geological activities (Dembitsky, 2003). Sources of anthropogenic contamination of HMs which includes agricultural chemicals utilization, industrial effluents, mining, miliary operations, fuel production, smelting processes, small scale industries such as metal manufacturing, metal smelting, battery production, cable coating, brick kilns and coal combustion (Zhen-Guo *et al*., 2002). Enzymatic and microbial activity of the soil can be sensitively reflecting the soil quality (Lee *et al*., 1996). Soil microbial biomass is a key indicator in investigating the degree of soil contamination (Aceves *et al*., 1999) as HMs significantly inhibit the microbial activity in polluted soils.

HMs at lower to moderate concentrations in soil, generally do not affect plant growth, regardless of whether they are necessary for the plants. However, when the concentration is too high, the amount of HMs absorbed by the plants may exceed its tolerance threshold, results in poisoning and potentially it leads to

plant death. Previous literature indicates that HMs in urban soils can enter the human body through skin absorption and inhalation of dust, etc., posing direct health risks, particularly to children. Furthermore, these contaminants can degrade urban quality of the environment and indirectly affect human health by polluting water, food and atmosphere.

Recent studies have shown that the characteristics of HMs pollution in agricultural and urban soils are different (Babula *et al*., 2008). In urban soils, HMs can enter the human body directly via ingestion, skin contact, etc. In contrast, HMs in agricultural soils are absorbed and accumulated by crops, and ingestion of these HMs via the soil-crop system is a primary route for human health risks (Aelion *et al*., 2008).

Iron (Fe) is an important essential element for all the plants, playing vital roles in biological processes such as synthesis of chlorophyll, photosynthesis and the development of chloroplast (Sahrawat, 2003). Excessive Fe is typically found in waterlogged or flooded soils, where anaerobic conditions prevail. Under these circumstances, Fe3+ ions are reduced easily to the more soluble Fe2+ ions. Fe toxicity related nutritional disorders can be classified into two types such as direct and indirect toxicity (Backer *et al*., 2005). Direct toxicity occurs when plants absorb excessive Fe results in plant's cell damage. Initial symptoms appear on younger leaves as small brown dots, a phenomenon known as bronzing (Baruah and Nath, 1996). Under severe conditions, the leaves may turn chlorotic, as prolonged toxicity situations necrosis can occur. Indirect toxicity arises from limited nutrients absorption such as magnesium, calcium, phosphorus, potassium and Fe itself, due to precipitation of Fe on root epidermis. The Symptoms are often linked to the Fe deposition in the roots (Sahrawat, 2010).

Capsicum is an important crop of agriculture, widely cultivated not only for its economic worth value, but also for the vibrant color, taste and health benefits of its fruit (Al-Snafi, 2015). It has been used as a flavorant, colorant and a source of spiciness. The key compound which is responsible for the sharpness heat in peppers is capsaicinoids (CAPS), a group of alkaloids produced in the fruit. *Capsicum annuum* possess various biological properties such as anti-inflammatory (Jolayemi and Ojewole, 2013), anticancer (Xu *et al*., 2018), antidiabetic effect (Roghani *et al*., 2004), antimicrobial (De Lucca *et al*., 2006), antifungal (Shabnam and Shayesteh, 2009), antioxidant (Popovich *et al*., 2014) and antiangiogenic (Pyun *et al*., 2008) effects.

Capsicum annuum is sensitive to pollutants in the environment and its growth, development and yield which can be negatively affected by soil contamination with HMs. Investigating the effects of soil pollution provides valuable information into how it affects agricultural productivity and plant health. Therefore, the current study is aimed to investigate the effects of ferrous iron toxicity on growth, biochemical alterations and enzymatic antioxidant status in *Capsicum annuum* L.

MATERIALS AND METHODS

The experimental protocol designed to achieve the objectives that were carried out following standard procedures. Capsicum seeds were obtained from an agricultural store in Puducherry. Ferrous sulphate was used to induce Fe toxicity.

Seed Sterilization

Uniform sized seeds were selected and surface sterilized with a 0.1% mercuric chloride solution for 2-3 minutes to avoid infection of fungus. These seeds were taken out immediately and washed many times with distilled water.

Polyethylene Bag Experiment

Polyethylene bag culture experiments were conducted to investigate the effect of the HMs toxicity in *Capsicum annuum*. The growth medium in polyethylene bags consist of artificially contaminated soil at concentrations of 100, 200 and 400 mg of ferrous sulphate. Using a wooden stick, 2 cm deep holes were made, and sterilized seeds were sown in each bag. Afterwards each seed was covered with a small amount of soil for proper supplement of germination factors. Soil moisture content was adjusted regularly by its water holding capacity with tap water.

Experimental Design

After the initial phase, the *Capsicum annuum* plants were divided into four groups. Group 1, which served as the control, was grown in soil without any Fe treatment. In contrast, groups 2, 3 and 4 were treated with Fe at concentrations of 100, 200 and 400 mg respectively. The plants were cultivated under conditions of natural photoperiod, average temperature and relative humidity.

Germination Parameters

The germination percentage was calculated by dividing the number of seeds that germinated on each day by the total number of seeds, then multiplying by 100, and finally adding the total percentages over the entire period.

Germination rate = No. of Seeds germination/Total number of seeds

Germination percentage = Germination rate × 100

Root Length and Shoot Length (in cm)

The root length, measured from the soil surface to the tip of the root, and the shoot length, measured from the soil surface to the tip of the shoot, were both determined using a standard centimeter scale.

Fresh Weight and Dry Weight (in gm)

The fresh weight and dry weight of the entire plant are measured using an electronic balance.

Vigour Index

Vigour index data were recorded based on germination. The vigour index was calculated using the mean values of root length and shoot length, following the formula of Baki and Anderson (1973).

Vigour Index = (Mean Shoot length + Mean root length) × Germination %

Biochemical Estimations

Estimation of Carbohydrates

The carbohydrate content was determined using the method of Hedge and Hofreiter (1962). The absorbance of the solution was measured at a wavelength of 640 nm.

Estimation of Proteins

The protein content was determined using Lowry's method (1951). The developed colour was measured at a wavelength of 660 nm.

Enzyme Assays and Analysis

Estimation of Catalase

Catalase (CAT: EC 1.11.1.6) activity was assayed using the method of Sinha (1972). The absorbance of the resulting color solution was measured at 620 nm.

Estimation of Superoxide Dismutase

Superoxide dismutase (SOD: EC 1.15.1.1) activity was assayed using the method of Kakkar et al., 1984. The absorbance of the colored solution was measured at 620 nm.

Statistical Analysis

The results were expressed as means ± standard deviation based on six plants per group. Data were analyzed using one-way analysis of variance (ANOVA) and significant differences between treatment groups were determined using Duncan's multiple range test. Results were considered statistically significant when $P<0.05$. All statistical analyses were performed using SPSS version 15.0 software package (SPSS, Tokyo, Japan).

RESULTS AND DISCUSSION

Germination Percentage, Root Length and Shoot Length

Table 1 shows the effect of Fe stress in *capsicum annuum* plants on germination percentage (%), root length (in cm) and shoot length (in cm) of different experimental groups. Results revealed that seed germination was adversely affected by the increasing concentrations of ferrous iron. Compared to the control group, seed germination was significantly lower in plants exposed to high concentrations of Fe. Suppression of seed germination and plant growth responses is likely due to the development of higher toxic effect syndrome (HTES) caused by the higher accumulation of metallic salts in the plant biomass (Singh and Singh, 1981). Iron is essential for plant growth and also it can become toxic to plants at higher concentration. Moreover, the inhibition of seed germination in response to HMs treatments in plants can be linked to a decrease in some metabolic processes such as cell division and certain enzymes suppression which are necessary for seed germination and reserve food mobilization as well as potential damage to the tissues in seeds caused by the toxicity of HMs.

Inhibition of growth is a common response which is associated with most of the HMs (Reichman, 2002). The results also showed that as the concentrations of heavy metals increased, there was a notable reduction in both root and shoot length. At the highest concentrations of Fe, the root and shoot length was significantly decreased. This decline in root and shoot length may be attributed to the inhibition of respiration, enzymatic activities and mitochondrial transport. The inhibition is likely caused by the cellular toxicity of Fe, which affects mitochondrial enzymes and disrupts tissue respiration.

Table 1: Effect of ferrous iron on germination percentage, root length and shoot length on different experimental groups of *capsicum annuum* plants

Groups	Germination percentage (%)	Root length (cm)	Shoot length (cm)
Control (C)	90	17.07±1.43	43.90±3.6
Test (T1)	65	13.54±1.25	32.38±2.4
Test (T2)	50	10.93±0.89	26.04±2.2
Test (T3)	30	6.47±0.51	20.45±1.8

Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

Fresh Weight, Dry Weight and Vigour Index

Figure 1 shows the effect of Fe induced stress on fresh weight, dry weight, whereas figure 2 shows the vigour index values for different groups of *capsicum annuum* plants. These observations were recorded on the 30th day after sowing. The results from our study demonstrates a significant reduction in fresh weight, dry weight and vigour index in *capsicum annuum* plants subjected to Fe toxicity, when compared to the control plants. Several studies also have reported the decrease in fresh weight, dry weight and vigour index of seedlings due to higher concentrations of HMs. The inhibition

observed at elevated concentrations of ferrous iron is partially attributed to its negative impact on the rate of photosynthesis process (Metwally *et al*., 2003).

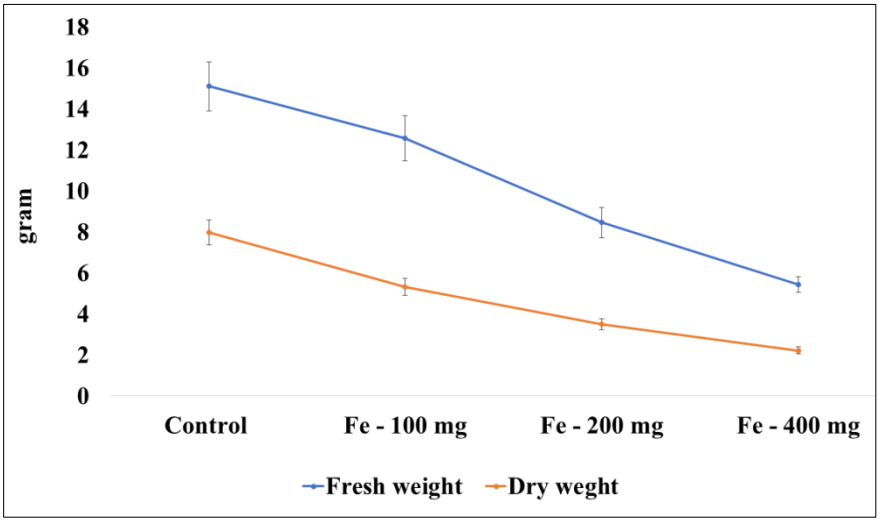

Figure 1: Effect of ferrous iron on fresh weight, dry weight on different experimental groups of *capsicum annuum* plants

Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

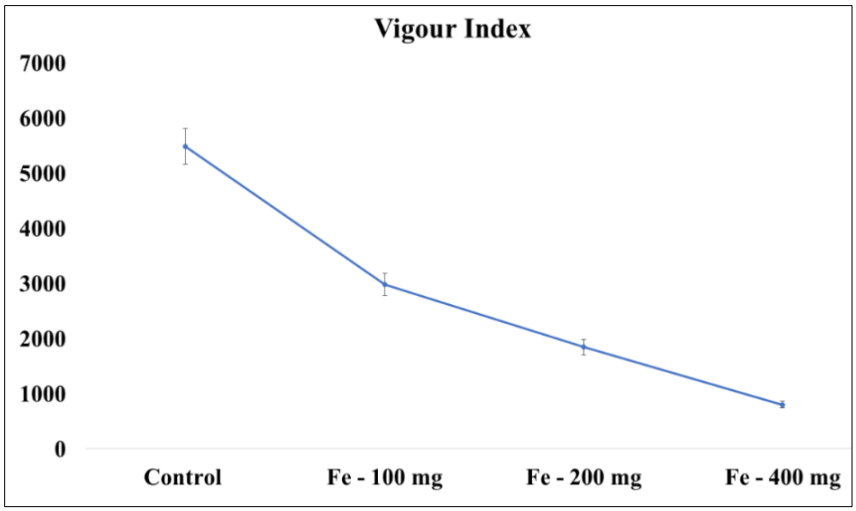

Figure 2: Effect of ferrous iron on vigour index on different experimental groups of *capsicum annuum* plants

Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

Carbohydrates and Protein Contents

Figure 3 illustrates the effect of Fe on the total carbohydrate and protein on three groups of tested *capsicum annuum* plants. These observations were recorded at 30th day after sowing the seeds. The results from our study indicate that the reduction in carbohydrates contents of *capsicum annuum* may be attributed to the decreased levels of chlorophyll contents, which in tum results in reduced photosynthesis efficiency. As a result, the toxic effects of Fe on the metabolism of carbohydrates and photosynthesis may lead to lower carbohydrate levels in higher concentrations of Fe tested plants compared to the control plants.

The results of our study also confirms that reduced protein levels in *capsicum annuum* plants when it was subjected to Fe toxicity as compared to untreated control plants. As the concentrations of Fe increases, there occurs a significant reduction in protein contents in *capsicum annum*. Several factors can contribute to this reduction in protein content under HMs stress. One possible reason is the accelerated protein degradation due to increased activity of protease enzyme (Xu *et al*., 2006) or disruptions in the metabolism of nitrogen caused by the presence of HMs. Stress conditions, such

as the presence of HMs in the growth medium, are known to elevate the activity of protease enzyme (Weihong *et al*., 2009). Additionally, HMs such as Fe at elevated concentrations, can impair the process of photosynthesis, which in turn reduces protein synthesis (Lin *et al*., 2007).

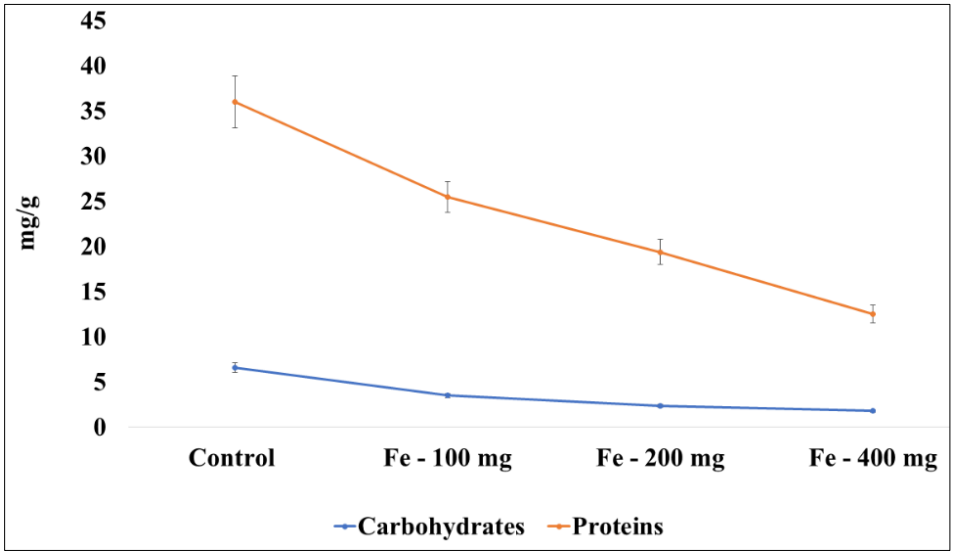

Figure 3: Effect of ferrous iron on carbohydrate and protein contents on different experimental groups of *capsicum annuum* plants

Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

Enzymic Antioxidants (Catalase and Super Oxide Dismutase)

HMs induced stress leads to free radicals production which are neutralized by the plant's antioxidative defense mechanism. Plants possess various enzymes that convert the reactive oxygen species (ROS) into less harmful products. The effect of Fe on the activity of antioxidant enzyme in *Capsicum annuum* is presented and discussed. Figure 4 illustrates the effect of Fe stress on the enzymic antioxidant status in different experimental groups of *capsicum annuum* plants.

Catalases play a crucial role in scavenging H_2O_2 which is generated during photorespiration and the β-oxidation of fatty acids (Morita *et al*., 1994). The results of the present study showed a significant difference in catalase activity in the Fe tested plants when compared to normal control plants. A gradual decline in catalase activity was observed upon increasing the concentration of Fe in *capsicum annuum* plants when compared to untreated normal control plants. This decrease in catalase activity may be attributed to the detrimental effects of excess production of H_2O_2 and other ROS radicals (Victoria *et al*., 2001). This decline in catalase function could impair the plant's ability to detoxify excess hydrogen peroxide, contributing to oxidative stress.

SOD is the primary enzyme in the detoxification process converting the superoxide radicals to H_2O_2 immediately after their synthesis. Treatment with heavy metal Fe resulted in statistically significant differences in the activity of SOD of *Capsicum annuum* plants, with treated plants showing varying levels as compared to control. Our data indicate a decrease in the activity of SOD upon increasing the Fe concentrations when compared to control plants which negatively affects the status of antioxidants. This significant reduction in the SOD activity may be attributed to the enzyme inactivation by H_2O_2, and further increases in higher HMs concentration could results in the inhibition of antioxidant activity. Several studies indicate that a reduction in the antioxidant activity can impair cellular functions, hinder growth and disrupt metabolism as plants face challenges in manage the increased oxidative stress.

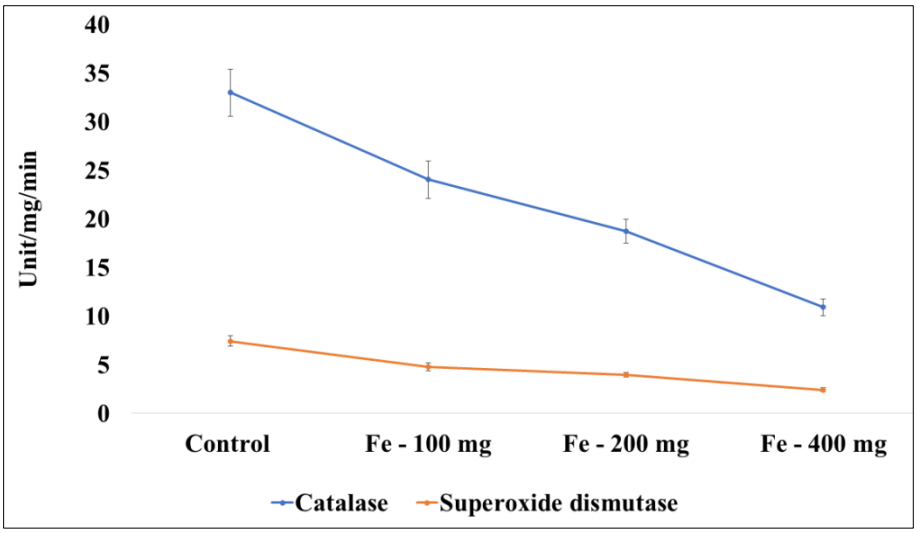

Figure 4: Effect of ferrous iron on enzymic antioxidants levels on different experimental groups of *capsicum annuum* plants.

Values are expressed as mean±SD. Groups not sharing a common superscript letter differ significantly at $p<0.05$. Duncan's multiple range test (DMRT).

CONCLUSION

In conclusion, the present findings confirm that ferrous iron exerts toxic effects on *Capsicum annuum* plants, as demonstrated by several key observations. Under ferrous iron stress, growth parameters such as germination percentage, root length, shoot length, fresh weight, dry weight, and vigor index were significantly reduced compared to control plants. Additionally, the carbohydrate and protein contents of *Capsicum annuum* plants decreased under ferrous iron exposure. Furthermore, levels of antioxidant enzymes, including catalase and superoxide dismutase, were diminished, indicating a decline in the plant's ability to counter oxidative stress caused by ferrous iron toxicity. Future research should focus on elucidating the molecular mechanisms of ferrous iron tolerance in *Capsicum annuum*, exploring its phytoremediation potential, enhancing antioxidant responses, and evaluating the synergistic effects of multiple heavy metals on plant growth, yield, and quality.

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