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ORIGINAL ARTICLE

Effect of iron and zinc limiting conditions on the growth behaviour of *Coriandrum sativum*

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ABSTRACT
Micronutrients are very important for the overall growth of plants. These micronutrients play different roles. One way, they are part of the enzymes required for photosynthesis and other pathways, while other ways they are involved in synthesis of molecules itself. Iron and zinc, both plays their roles for the plant growth and increase nutritional value of plants. Coriander is an annual herb, used widely for culinary and medicinal purposes in almost all parts of the world. Benefits provided by this plant is very high, they are also loaded with medicinal properties. In this paper we study the effect of iron and zinc removal, on the growth behaviour of coriander plant. Results showed that both iron and zinc are important for growth of the plants as well as chlorophyll and carotenoid content of the plant. Interestingly, total carbohydrate content was unaffected by removal of iron from the growth medium, in fact it showed a positive effect on carbohydrate content.
Keywords: *Micronutrient, Coriander, Iron, Zinc*

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INTRODUCTION

Micronutrients are very important for plant growth and their nutritional value. These nutrients are often an integral part of the enzyme system and play roles in redox reactions. They also play important roles in N_2 fixation, photosynthesis, and protein synthesis processes. Iron and zinc are two such micronutrients, which are important for plants. Iron is the fourth most important heavy metal found in the earth's crust and is taken up by plants either in ferrous (Fe2+), or ferric (Fe3+) form. It is required for the formation of chlorophyll in plant cells and also serves as an activator for respiration, photosynthesis, etc. Iron deficiency is present in presence of high levels of manganese and/or high lime content in soil (1). It is reported that iron (Fe) deficiency reduces the quality of plant products and yield can also be severally compromised. Generally, to fight with iron deficiency, plants store iron as ferritin (a protein that encapsulated protein iron) (2).

Similar to iron, zinc is another important micronutrient. Zinc acts as a metal component of enzymes or as a functional, structural, or regulatory cofactor of a large number of enzymes. It is an essential component of RNA polymerase as well as it is essential for the structural integrity of ribosomes. The rate of protein synthesis and the protein content of zinc-deficient plants are drastically reduced (3). Large application of phosphorous fertilizer can lower the availability of zinc in soil, which induces zinc deficiency (4).

Coriandrum sativum L. (Coriander) is an annual plant from Apiaceae family. It is used for culinary and medicinal purposes, in almost all parts of the world. The whole plant is beneficial for human health. Fresh green leaves are used as a flavouring agent, while dried seeds are used as a spice. The green leaves of immature coriander are one of the world's most widely used culinary herbs and are also used for medicinal purposes (5).The coriander plant is also a good source of essential oils, used for this purpose in many

countries (6). Potential benefits of the coriander herb include Analgesic, Anti-asthmatic, Anti –HIV,Anti-agingg, Anti-cancer effects etc (7). In addition, coriander herbal extract (Aqueous and ethanol) and essential oil have also been shown to function as natural antioxidant and antimicrobial agents (8). Coriander herbs is very low in calories and contain no cholesterol, however its deep green leaves possess good amounts of antioxidants, essential oils, vitamins, and dietary fibre, which may help to reduce LDL or bad cholesterol levels in the blood. Coriander is also one of the richest herbal sources for vitamin K. It also has an established role in the treatment of Alzheimer's disease patients by limiting neuronal damage in their brain. Cilantro leaves provide only 23 calories/100 g, but their phyto-nutrients profile is no less than any superfoods around us (https://www.nutrition-and-you.com/cilantro.html). It is also a good source of minerals like potassium, calcium, manganese, iron and magnesium. It is rich in many vital vitamins including folic acid, riboflavin, niacin, vitamin –A, and beta-carotenes etc. (9). 100g of coriander leaves provide 30% of vitamin C and 225% of Vitamin A of daily recommended levels of these vitamins.
Coriander is well suited to growing on a range of soils, but it performs best on well-drained loam and sandy-loam soils. Suitable pH ranges are from 4.5 to 8.0, with an optimum of 6.3-6.5. Although the crop is heat loving, it has an optimum growing temperature of 18° C. Production quality and quantity both get affected by altering the growth conditions (10)

MATERIAL AND METHODS

Coriendrum sativum was used to test iron and zinc stress at different stages of development, during the month of March and April (15-25°C).
This experiment was conducted on autoclaved sand because after proper leaching the sand particles become almost free from micro and macronutrients and this will ensure the proper amount of micro/macronutrients addition during the experiment.
All the glassware's was dipped overnight in chromic acid mixture followed by washing in flowing tap water and finally, they were rinsed with double distilled water and dried in a hot air oven, before use.
To grow the plants in pots, Hogland medium was used as described by Hogland and Arnon (11). The medium was modified for iron and zinc content, as and when required. Fresh medium (normal or modified) was applied in each pot every 3 days intervals during the experiments. Experimental conditions were described in Table 1.
Coriander seeds were placed in 10 pots (20 seeds in each), after adding the medium and allowed to grow. Samples were collected at different time intervals, i.e. 10 days, 20 days, and 30-days post-planting. Morphological changes (in terms of fresh weight, root length, shoot length) and physiological (biochemical)changes (protein content, carbohydrate content, chlorophyll and carotenoids) under different levels of IRON and ZINC exposure were analysed. To take these observations, two plants for chlorophyll, two plants for protein and two for carbohydrate analysis were taken from each experimental set for each interval of observation.

Growth Estimation:

Fresh weight: - plants were uprooted from different pots; cleaned with tap water, and blotted with filter paper and then the weight of the sample was taken by using an electronic weighing machine.
Root and Shoot length: - After taking the fresh weight, the same plants were used for measuring the root length and shoot length, by using a scale and graph paper. The average length is reported.
Estimation of Photosynthetic pigments (chlorophyll and carotenoids): - The coriander plants were taken from pots, cleaned with water and blotted dry on filter paper. Samples were prepared in 5 ml of acetone with the help of mortar and pestle, kept in dark o/n to allow better extraction, followed by centrifugation. Amount of chlorophyll in the supernatant was determined according to Arnon (12). Absorbance were taken at 645 nm and 663 nm for total chlorophyll and 420 nm for carotenoids.
Estimation of Protien and Carbohydrate Content:- Protein was estimated by the method of Lowry et al., (13). The freshly collected plants were crushed with the help of mortar and pestle. 0.5 ml of sample (crushed plants) was used for protein estimation. A calibration curve of protein was prepared by using lysozyme as standard. Total protein is expressed in terms of mg (g fresh wt)$^{-1.}$
Carbohydrate was estimated by the method of Dubois et al. (14). The freshly collected plants were crushed adding with distilled water (1ml) and sample was used for carbohydrate estimation.

RESULTS

Growth of *Coriendrum sativum* was studied at different concentrations of Iron and Zinc (Set 1-5) in terms of fresh weight, root and shoot Length, chlorophyll, carotenoid, protein, carbohydrate content up to 30 days of growth period.

Effect of iron and zinc on the growth behaviour of *Coriendrum sativum*

According to table 2 fresh weight of the coriander, plant was 980mg in set 1 which was considered a control sample. The lowest weight was observed at 367 mg in set 2 (Zn 100%+Iron 0%) followed by set 4 (Zn 0% +Iron 100%). It was also observed that fresh weight had been increased in 10 days, 20 days, and 30 days duration which followed in every set of samples

Experimental studies reveal the Root and shoot length in table 3. The highest root length (7.8 cm) in the control set was followed by set 5 (Zn 50%+Iron 100%) (whereas the minimum length (4.3 cm) was observed in set 2 **(**Zn 100%+Iron 0%) followed by set 4.

Effect of iron and zinc on the biochemical components of *Coriendrum sativum*

Maximum Chlorophyll concentration was found in control conditions than other set of samples. In set 2 **(**Zn 100%+Iron 0%) minimum chlorophyll content has been observed followed by set 4 (Zn 0%+Iron 100%) (Fig 1a), meaning both iron and zinc are important for chlorophyll content of the plant.

Optimum synthesis of carotenoids (85.6mg/100mg) were also seen in control conditions than other concentrations of Iron and Zinc. In set 2 (Zn 100%+Iron 0%) minimum carotenoid (38.6 mg/100mg) content has been observed. Carotenoid content has shown the same trend as chlorophyll content. Carotenoid synthesis increased with the growing period in each set of samples. (Fig 1b).

Same pattern was observed in case of total Protein content of the plant, only that effect were more severe in this case (Fig 1c). When total carbohydrate was estimated in the plants, it showed an interesting pattern, maximum amount was seen in Set 2 (without iron). It looks that iron does not play an important role for carbohydrate synthesis, while in absence of zinc, total carbohydrate concentration is dropped in comparison to control (Fig 1d).

Table 1: Concentrations of Iron and Zinc, used for the experiments

Condition	Iron Concentration	Zinc Concentration
Set 1 (Control)	100% (5ppm)	100% (0.05ppm)
Set 2	0%	100%
Set 3	50%	100%
Set 4	100%	0%
Set 5	100%	50%

Table 2: Fresh Weight of plants after different time intervals in different experimental sets

	10 Days	20 Days	30 Days
Set 1	272 mg	568 mg	980 mg
Set 2 (Zn 100%+Iron 0%)	124 mg	265 mg	367 mg
Set 3 (Zn 100%+iron 50%)	185 mg	386 mg	665 mg
Set 4 (Zn 0%+Iron 100%)	143 mg	325 mg	472 mg
Set 5 (Zn 50%+Iron 100%)	227 mg	467 mg	789 mg

Table 3: Root Length and Shoot Length of plants after different time intervals in different experimental sets

Experimental Conditions	Root Length			Shoot Length		
	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
Set 1	1.8 cm	4.3 cm	7.8 cm	4.8 cm	6.5 cm	9.7 cm
Set 2 (Zn 100%+Iron 0%)	0.7 cm	2.3 cm	4.3 cm	3.1 cm	4.2 cm	7.3 cm
Set 3 (Zn 100%+iron 0%)	1.3 cm	3.9 cm	5.9 cm	3.9 cm	5.8 cm	8.8 cm
Set 4 (Zn 0%+Iron 100%)	0.9 cm	2.8 cm	4.8 cm	3.4 cm	4.7 cm	7.8 cm
Set 5 (Zn 50%+Iron 100%)	1.5 cm	4.1 cm	6.4 cm	4.5 cm	6.2 cm	9.4 cm

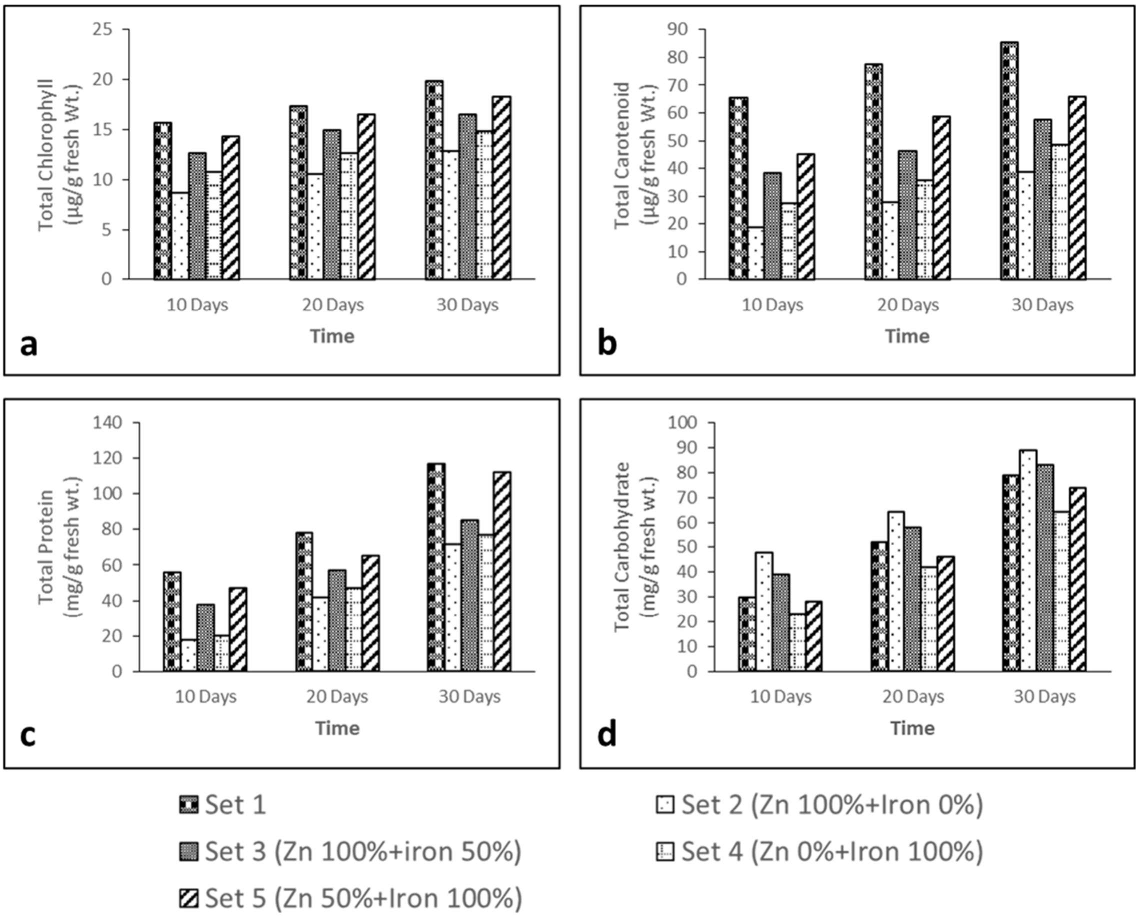

Figure 1(a-d)- Different biochemical growth parameters were evaluated to see the effect of iron and zinc concentration on *Coriandrum sativum* plant at different time intervals.

DISCUSSION

Iron and Zinc plays a very significant role in physiological processes in plants. Iron is best known for its role in nitrogen fixation. Iron is taken up by plants as ferrous (Fe2+) and / or ferric (Fe3+) form. It is also required for the formation of chlorophyll in plant cells. Plants store iron as ferritin, for use in iron deficient conditions.

Zinc acts as cofactor of large number of enzymes. It is an essential component of RNA polymerase as well as ribosomes and is essential for their activity and structural integrity respectively. There are More than 80 zinc containing protein have been reported. The rate of protein synthesis and the overall protein content of plants in zinc deficient conditions are drastically reduced (3).

When *Coriendrum sativum* was grown with different concentration of Iron and Zinc, obvious changes with respect to growth has been observed. Similar findings have been also reported in other plants in Iron and Zinc replete conditions. As growth rate, metabolic processes and cellular pathways are strongly inhibited by presence or absence of these micronutrients (15, 16).

Other growth parameters like fresh weight, photosynthetic pigments concentration, carbohydrate and protein contents were also changed considerably.

As expected, variation in fresh weight and other physical parameters were affected by change in micronutrient concentration. Results showed that role of iron is more important than zinc, as removal of iron from media has greater effect then zinc removal.

In chlorophyll and carotenoid synthesis, it was observed that both Iron and Zinc play important role in synthesis. Concentrations of these photosynthetic pigments were maximum in control condition, followed by in zinc deficient condition and was lowest in iron limiting conditions. Effect of these variations was more drastic in carotenoid concentration in comparison to chlorophyll content.

Similar trend was also seen in total protein content of the cell. In this case also control conditions showed maximum number of proteins then other conditions, while minimum concentration was reported in iron deficient condition.

When total carbohydrates concentration in the cell was compared, an interesting result was seen. It looks that for carbohydrate production / metabolism in the cell, iron and zinc play opposite roles. On one hand increase in iron concentration leads to oxidation of carbohydrates, while on the other hand zinc plays role greater in synthesis of carbohydrates. So, for carbohydrate, maximum amount was reported in iron deficient condition. In control conditions, both zinc and iron were present and due to opposite role in carbohydrates metabolism, intermediate amount of carbohydrate is reported.

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Author contributions
VNT conceptualize the idea for the manuscript. AM and RG performed the experiments. AM and VNT wrote the paper and finalize it.

Competing interests
The authors have declared that no competing interest exists.

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