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Changes in Micronutrient Contents of Radish (*Raphanus sativus*) Under Copper Toxicity

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Abstract: In this experiment, we tried to explore the changes that are occurring in radish in response to copper application. A pot culture experiment was conducted and copper was applied to radish (*Raphanus sativus*) plant. Micronutrients such as, iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) contents were analyzed for treated plants. The results showed that the low concentrations of Cu level (50 mg kg^{-1}) enhanced the micronutrients (Fe, Mn and Zn) while higher concentration of Cu (100 - 250 mg kg^{-1}) in the soil decreased these parameters. The accumulation of Cu in radish is directly proportional to the applied Cu in the soil.

Key words: *Raphnus sativus* · Iron · Manganese · Zinc · Copper.

INTRODUCTION

Heavy metals such as Cu and Zn are essential for normal plant growth and development since they are constituents of many enzymes and other proteins. Plants need relatively small amounts of metals for their growth and soils harbor these metal ions either naturally or as a consequence of contamination. Soil contamination with heavy metals is now a worldwide problem, leading to agricultural losses and hazardous health effects as metals enter the food chain [1, 2]. Copper (Cu) which is relatively mild character is highly toxic to plants even at a micro molar range of exposes [3]. Plants use certain metals, such as Cu, Co, Fe, Mn and Zn, which are essential mineral nutrients. However, other metals such as Cd and Pb are thought to play a physiological role [4]. Among the myriad of heavy metals Cu occupies the prominent position, since it plays a vital role in the growth and development of plants. The present investigation deals with the effect of Cu on the micronutrient content of radish.

MATERIALS AND METHODS

The experimental plant, the radish belongs to the family Cruciferae (Brassicaceae) and is one of the important vegetables of the world.Certified seeds of radish (Cultivar Pusa Chetki) were obtained from TNAU, Coimbatore. Seeds with uniform size and weight were chosen for experimental purpose.

Pot Culture Experiments: Radish plants were grown in pots in untreated soil (control) and in soil to which copper had been applied @ 50, 100, 150, 200 and 250 mg kg^{-1} soil. The inner surfaces of pots were lined with a polythene sheet. Each pot contained 6 kg of air dried soil. The Cu as finely powdered ($CuSO_4.7H_2O$) was applied to the surface soil and thoroughly mixed with the soil. Fifteen seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of five per pot, after a week of germination. Each treatment including the control was replicated seven times.

Sample Collection: The plant samples were collected on 45^{th} day for the measurement of micronutrient contents. Iron, manganese, zinc and copper contents in plant materials were estimated by the following methods.

Estimation of Cu, Fe, Mn and Zn [5]: One mL of sulphuric acid and 15 mL of double distilled water were added to a Kjeldahl flask containing 0.5 g of dried and powdered material and incubated at 80°C for overnight. After that 5 mL of diacid mixture (nitric acid and perchloric acid) 3:1 was added and digested until the nitric acid and perchloric acid were driven off.The digest was cooled, diluted, filtered through Whatmann No. 42 filter paper and made the final volume up to 50 mL. The solution was directly, aspirated to an atomic absorption spectrophotometer (Perkin-Elmer-2280), with air/acetylene flame for estimating Cu, Fe, Mn and Zn.

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Table 1: Effects of copper on micronutrients content (μg g^{-1} dry wt.) of radish (45th day)

Copper added in the soil (mg kg^{-1})	Fe	Mn	Zn	Cu
Control	310.25	57.37	39.55	23.46
50	350.12 (+12.85)	75.99 (+53.45)	52.04 (+31.58)	36.4 (+55.58)
100	265.12 (-14.54)	52.16 (-9.08)	32.32 (-18.28)	46.68 (+98.97)
150	246.48 (-20.55)	49.07 (-14.46)	27.78 (-29.75)	52.41 (+123.39)
200	238.61 (-23.09)	46.98 (-18.11)	24.87 (-37.11)	57.38 (+144.55)
250	214.95 (-30.71)	37.84 (-34.04)	19.43 (-50.87)	65.73 (+180.20)

Average of five replications.

Percent over control values are given in parentheses.

RESULTS

Micronutrients (μg g^{-1} dry wt.)

Iron (Fe): Fe content of radish leaves increased at 50 mg kg^{-1} and decreased further with an increase in Cu level in the soil (Table 1). It was found to be 310.25, 350.12, 265.12, 246.48, 238.61 and 214.95 in the control, 50, 100, 150, 200 and 250 mg kg^{-1} copper level in the soil, respectively.

Manganese (Mn): Mn content of radish leaves increased at 50 mg kg^{-1} and further progressively declined with an increase of copper in the soil (Table 1). It was found to be 57.37, 75.99, 52.16, 49.07, 46.98 and 37.84 in the control, 50, 100, 150, 200 and 250 mg kg^{-1}Cu level in the soil respectively.

Zinc (Zn): The maximum Zn content of radish was recorded at 50 mg kg^{-1} Cu level in the soil (52.04). The minimum zinc content of radish was observed at 250 mg kg^{-1} Cu level in the soil (19.43).

Copper (Cu): Cu content of radish plants is presented in Table 1. Maximum Cu accumulation of radish plants (65.73) was recorded at 250 mg kg^{-1}Cu level in the soil. The minimum Cu accumulation of radish (23.46) was observed at control.

DISCUSSION

Iron (Fe): The Fe content of radish significantly decreased with an increase in the Cu level in the soil. However, low level of Cu 50 mg kg^{-1} increased the Fe content. In many instances, the results are in agreement with earlier results that there was a depression in the concentration of Fe in plant tissues when grown with excess supply of Cu [6], Zn [7] and Ni [8]. Cu may be dependent on a similar type of mechanism for transport within the plant and may well compete with Fe, resulting in lower translocation of Fe from root to shoot. The decrease in Fe concentration with an increase in Cu induced Fe deficiency.

Manganese (Mn): Mn content in radish under Cu treatment decreased gradually with an increase in Cu level in the soil. However, 50 mg kg^{-1} of Cu treated plants exhibited higher Mn contents. The decreased levels of Mn are similar to the findings of Madhavi and Rao [9] due to Cd and Vijayarengan [8] due to Ni. The Mn content and its uptake were depressed due to elevated level of Cu showing that these elements are synergistic [10]. Reduction of Mn content resulted in the reduction of chlorophyll content [11, 12]. Decrease in Mn content may also due to the competition of Cu with Mn for transport sites in plasma lemma.

Zinc (Zn): The Zn content of Cu treated *Raphanus sativus* gradually decreased with an increasing concentration of applied Cu in the soil. However, 50 mg kg^{-1} Cu treated plants exhibited higher Zn contents. These results are in agreement with Lidon and Henriques [13] and Agarwal and Gupta [14] in rice under Cu and Kalyanaraman and Sivagurunathan [15] in blackgram under Cd and Cu.

Copper Uptake and Accumulation: When Cu is applied to the soil in excess levels, plants uptake more amount of it. The accumulation of Cu in copper treated radish leaves is directly proportional to the applied Cu concentrations in the soil. Similar results were found by Lidon and Henriques [13] in rice, Mocquot *et al*. [16] in *Zea mays* under Cu treatment. The accumulation of metals in the plant tissues might be due to the binding of metals negative charges in various macromolecules that are either soluble or part of cellular structures [17]. It has been shown that some plants store copper in the cell walls and that aluminum can bind to specific sites in the walls of epidermis and mesophyll [18]. Organic acids (citrate, malate, oxalate, trans-aconitate) can be produced and used to bind metals [19]. Metals can also be stored in the vacuoles as free ions. Presently it has shown that metals can be stored in complexes with extracellular proteins [20]. Under lower concentrations Cu improved the root system, thereby helping the plant in better absorption of water and other nutrients dissolved in it and consequently improved the growth of different organs and the entire plant [21]. The improvement in the growth efficiency of plant organ might also be due to the beneficial effects of copper treatments on the physiological activities and other enzyme reactions in the transformation of carbohydrates and activities of hexokinase of plants, which were responsible for improving the growth of plant and its component organs, ultimately influencing the relative development of plant parts and their growth efficiency. The overall decrease in micronutrient contents of radish was due to the toxic effect of higher concentration of Cu. It might also be due to the reason that the stressed plants spent more energy for their survival in the hostile environment, which otherwise would be available for their overall growth processes. This led to the decrease in the micronutrient contents of the stressed plants.

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