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Changes in growth parameters and pigment content of radish (*Raphanus sativus*) under the influence of copper toxicity

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Abstract

The effect of copper stress on the growth, leaf area, dry weight and photosynthetic pigments content in *Raphanus sativus* L. was studied. The plants were grown in earthen pots containing soil amended with different concentrations of Cu (50, 100, 150,200, 250 mg kg^{-1}) as $CuSO_4$. Growth parameters viz., root and shoot length, total leaf area, dry weight of root and shoot and photosynthetic pigments content (chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid) increased at a concentration of 50 mg kg^{-1}. However, further increase in the copper level in the soil (100-250 mg kg^{-1}soil) showed a negative effect on these parameters.

Keywords: Copper, growth, pigments, *Raphanus sativus* .

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INTRODUCTION

Heavy metals and metalloids are increasing the environmental problem worldwide. Agricultural soil is contaminated with heavy metals mainly by human-induced activities and creating severe problems (Ahmad *et al.*, 2011; Ali *et al.*, 2011). The research works on the toxicity of copper on growth and biochemical responses of plants are very meager and are needed to be investigated. The present study aims to fulfill these aspects. Several studies were undertaken by several workers with different types of metals to study their effects on many kinds of plants. Saravanan *et al.* (1996) studied the effect of copper sulphate on germination of greengram. Senguttuvel *et al.* (1998) studied the effect of copper, nickel and mercury on *Arachis hypogaea*. Leaf area of blackgram (*Vigna mungo*) and greengram (*Vigna radiata*) was reduced by nickel (Vijayarengan and Lakshmanachary, 1995; Vijayarengan, 2004). The effect of increasing concentration (10-50 mg kg^{-1}) of cadmium on growth and leaf variations in radish plants was analysed by Vijayaragavan *et al.* (2007). McBarik and Chandel (2001) investigated the dry matter yield with various levels of copper application in *Glycine max*. The decreasing growth and dry matter yield of tomato plants were reported to be due to the toxic effects of higher concentration of zinc (Vijayarengan and Mahalakshmi, 2013). The present investigation deals with the effect of copper on growth parameters and pigment content of radish.

MATERIALS AND METHODS

Raphanus sativus (Cultivar Pusa Chetki) belongs to the family Cruciferae (Brassicaceae), and is one of the important vegetable crops of the world. Certified seeds of radish 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 sulphate was added at the concentration of 50, 100, 150, 200 and 250 mg kg^{-1} soil. The inner surfaces of pots were lined with polythene sheet. Each pot contained 6 kg of air dried soil. The copper as $CuSO_4 \times 7H_2O$ 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 on 7th day after germination. Each treatment including the control was replicated seven times.

SAMPLE COLLECTION

The plant samples were collected at fifteen days intervals *viz.*, 15, 30 and 45 days after Cu treatment for the measurement of various morphomatric growth parameters. The pigment contents of the plants were estimated on 45th day after Cu treatment. Five plants were randomly chosen from each treatment and were used for measuring various morphological parameters and for the estimation of pigments and the average was calculated.

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MORPHOLOGICAL PARAMETERS

The various morphological parameters such as root length, shoot length, total leaf area and dry weight of root and shoot per plant were determined for each and every sample. The leaf area was calculated by measuring the length and breadth of the leaf and multiplied by a correlation factor (0.69), derived from the method of Kalra and Dhiman (1977).

PIGMENTS ANALYSIS

Chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids contents were estimated by the following methods.

(i) Estimation of chlorophyll (Arnon, 1949)

Two-hundred milligram of fresh leaf was ground in a mortar and pestle with 10 ml of 80 per cent acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was saved. The pellet was reextracted with 5 ml of 80 per cent acetone each time, until it become colourless. All the supernatants were pooled and utilized for chlorophyll determination. Absorbance was measured at 645 and 663 nm using spectrophotometer. The chlorophyll content was determined by using the following formula.

$$\text{Chlorophyll 'a' } (\text{mg g}^{-1}) = \frac{12.7A_{663} - 2.69A_{645}}{a \times 1000 \times w} \times V$$

$$\text{Chlorophyll 'b' } (\text{mg g}^{-1}) = \frac{22.9\ A_{645} - 4.68\ A_{663}}{a \times 1000 \times w} \times V$$

$$\text{Total chlorophyll } (\text{mg g}^{-1}) = \frac{20.2\ A_{645} + 8.02\ A_{663}}{a \times 1000 \times w} \times V$$

Where,

a = length of light path in the cell (1 cm)

v = volume of the extract in ml and

w = fresh weight of sample in gram

(ii) Estimation of carotenoids (Kirk and Allen, 1965)

The same chlorophyll extract was measured at 480nm, using spectrophotometer to determine the carotenoids content.

$$\text{Carotenoids content } (\text{mg g}^{-1}) = \frac{(A_{480} + 0.114A_{663}) - 0.638\ A_{645}}{a \times 1000 \times w} \times V$$

RESULTS

MORPHOLOGICAL PARAMETERS

Root length (cm plant^{-1})

The root length of radish plants at different stages of growth under copper stress is represented in Table 1. Root length of radish increased at 50 mg kg^{-1} (36.32 cm plant^{-1}) on 45th day and decreased further with the increase of copper level in the soil. The root length of radish increased with various sampling days and decreased with an increase in the concentration of copper in the soil. F-test values were significant at 1 per cent for copper levels and sampling days.

Shoot length (cm plant^{-1})

Shoot length of radish at different stages of growth under copper stress is presented in Table 2. The highest shoot length was recorded on 45th day at 50 mg kg^{-1} (19.72 cm plant^{-1}) plants of radish. The lowest shoot length (9.25 cm plant^{-1}) was at 250 mg kg^{-1}of copper treatment on 15th day. F-test values for the difference between copper levels and sampling days were significant at 1 per cent.

Total leaf area (cm^2 plant^{-1})

Total leaf area of radish under copper stress recorded at different stages of growth is presented in Table 3. Total leaf area of radish plants on 15th day was 50.21, 60.81, 42.02, 38.92, 36.51 and 26.62 cm^2 plant^{-1} for control, 50, 100, 150, 200 and 250 mg kg^{-1} of copper treated plants respectively. It increased in the subsequent sampling periods and decreased at high levels (100-250 mg kg^{-1}) of copper in the soil. ANOVA values were significant at 1 per cent for copper levels and sampling days.

DRY MATTER PRODUCTION (g plant^{-1})

Root

The root dry weight of radish plants in various levels of copper at different stages of growth is presented in Table 4. When compared to the control, copper at 50 mg kg^{-1} levels in the soil increased the dry weight of root and decreased the root dry weight at high levels (100-250 mg kg^{-1}) in all the sampling days. Statistical analysis revealed significant (1 per cent) F-test values for the copper levels and sampling days.

Shoot

The results showed in Table 5 indicated that maximum shoot dry weight of radish (0.582 g plant^{-1}) at 50 mg kg^{-1} on 45th day. Minimum dry weight of shoot (0.143 g plant^{-1}) was observed on 15th day at 250 mg kg $^{-1}$ of copper treated plants. F-test values calculated for the copper levels and sampling days were significant at 1 per cent.

Pigments (mg g^{-1} fresh wt.)

The effect of copper on the pigments contents of radish is represented in Table 6. Pigments viz., chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content of radish leaves increased at lower concentration (50 mg kg^{-1}) and decreased further with an increase in the copper level (100-250 mg kg^{-1}) in the soil. Total chlorophyll content of the control is 1.245 mg g^{-1} fresh wt. and increased at 50 mg kg^{-1} (1.418 mg g^{-1} fresh wt.) and decreased 43.93 per cent

Table 1. Effects of copper on root length (cm plant^{-1}) of radish

Copper added in the soil	Sampling days (After treatment)		
(mg kg^{-1})	15	30	45
Control	24.18	27.42	30.12
50	28.11 (+16.25)	33.23 (+21.18)	36.32 (+20.58)
100	22.45 (-7.15)	25.14 (-8.31)	27 (-10.35)
150	21.12 (-12.65)	22.12 (-19.32)	24.15 (-19.82)
200	20.31 (-16.00)	21.15 (-22.86)	21.7 (-27.95)
250	17.4 (-28.03)	19.02 (-30.63)	20.11 (-33.23)

Comparison of significant effects

	F-test	CD P=0.05
Copper levels	**	0.1847
Sampling days	**	0.3451

Averages of five replications

Per cent over control values are given in parentheses

Table 2. Effect of copper on shoot length (cm plant^{-1}) of radish

Copper added in soil	Sampling days (After treatment)		
(mg kg^{-1})	15	30	45
Control	12.11	15.08	18.02
50	14.25 (+16.59)	16.57 (+9.88)	19.72 (+9.43)
100	12.01 (-0.82)	14.82 (-1.72)	16.86 (-6.43)
150	11.23 (-7.26)	14.09 (-6.56)	15.23 (-15.48)
200	10.15 (-16.18)	13.18 (-12.59)	14.08 (-21.86)
250	9.25 -23.69	10.25 (-32.02)	11.92 (-33.85)

Comparison of significant effects

	F-test	CD P=0.05
Copper levels	**	0.2730
Sampling days	**	0.3887

Averages of five replications

Per cent over control values are given in parentheses

Table 3. Effect on copper on leaf area (cm^2 plant^{-1}) of radish

Copper added in the soil	Sampling days (After treatment)		
(mg kg^{-1})	15	30	45
Control	50.21	84.72	108.51
50	60.81 (+21.11)	105.31 (+24.30)	132.42 (+22.03)
100	42.02 (-16.64)	66.57 (-21.47)	90.8 (-16.32)
150	38,92 (-22.48)	61.85 (-26.99)	84.02 (-22.56)
200	36.51 (-27.28)	57.02 (-32.69)	78.41 (-21.73)
250	26.62 (-46.98)	45.13 (-46.73)	63.13 (-41.82)

Comparison of significant effects

	F-test	CD P = 0.05
Copper levels	**	6.8768
Sampling days	**	8.4540

Averages of five replications

Per cent over control values are given in parentheses

Table 4. Effect of copper on root dry weight (g plant^{-1}) of radish

Copper added in the soil	Sampling days (After treatment)		
(mg kg^{-1})	15	30	45
Control	0.065	0.134	0.2
50	0.08 (+23.07)	0.16 (+19.40)	0.241 (+20.50)
100	0.048 (-26.15)	0.1 (-25.37)	0.153 (-23.50)
150	0.045 (-30.76)	0.092 (-31.34)	0.137 (-31.5)
200	0.042 (-35.38)	0.081 (-39.55)	0.125 (-37.50)
250	0.031 (-52.30)	0.059 (-55.97)	0.092 (-54.00)

Comparison of significant effects

	F-test	CD P=0.05
Copper levels	**	0.0012
Sampling days	**	0.0007

Averages of five replications

Per cent over control values are given in parentheses

Table 5. Effect of copper on shoot dry weight (g $plant^{-1}$) of radish

Copper added in the soil	Sampling days (After treatment)		
($mg\ kg^{-1}$)	15	30	45
Control	0.261	0.435	0.582
50	0.311 (+19.15)	0.528 (+21.37)	0.689 (+18.38)
100	0.21 (-19.54)	0.338 (-22.29)	0.451 (-22.50)
150	0.192 (-26.43)	0.311 (-28.50)	0.426 (-26.80)
200	0.173 (-33.71)	0.292 (-32.87)	0.394 (-32.30)
250	0.141 (-45.97)	0.221 (-49.19)	0.319 (-45.18)

Comparison of significant

effects	F-test	CD P=0.05
Copper levels	**	0.0824
Sampling days	**	0.0758

Averages of five replications

Per cent over control values are given in parentheses

Table 6. Effect of copper on pigments content (mg g^{-1} fresh wt.) of radish (45^{th} day after treatment)

Copper added in the soil (mg kg-1)	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Carotenoid
Control	0.708	0.598	1.245	0.271
50	0.858 (+2118)	0.612 (+2.34)	1.418 (+13.89)	0.311 (+14.76)
100	0.639 (-9.74)	0.511 (-14.54)	1.137 (-8.67)	0.253 (-6.64)
150	0.571 (-19.35)	0.408 (-31.77)	0.918 (-26.26)	0.228 (-15.86)
200	0.458 (-35.31)	0.338 (-43.47)	0.797 (-35.98)	0.181 (-33.21)
250	0.399 (-43.64)	0.298 (-52.81)	0.698 (-43.93)	0.149 (-45.01)

Average of five replications

Per cent over control values are given in parentheses

at 250 mg kg^{-1}. The carotenoid content in the leaves of radish in the control, 50, 100, 150, 200 and 250 mg kg^{-1} of copper treated plant was found to be 0.271, 0.311, 0.253, 0.228, 0.181 and 0.149 mg g^{-1} fresh wt. respectively.

DISCUSSION

MORPHOLOGICAL PARAMETERS

Root and shoot length

Root and shoot length of radish plants decreased with an increase in copper level in the soil. Root and shoot length of radish were found to be higher at 50 mg kg^{-1}. Similar decrease in plant height was observed by Roth *et al.* (1971) (copper), Sharma and Sharma (1993) (chromium), Kalyanaraman and Sivagurunathan (1993) (zinc), Vijayarengan and Lakshmanachary (1995) (nickel), and Vijayarengan (2012) in radish. The results of the present study also confirmed these views.

Leaf area

Leaf area of radish decreased with increase in copper level of the soil. However it increased at 50 mg kg^{-1} soil. Similar reduction in total leaf area due to cadmium and manganese has been reported (Terry *et al.*, 1975), The total leaf area showed a gradual decline when there was a further increase in copper level in the soil in all the sampling days. Similar observations were made by Mocquot *et al.* (1996) and Mc Bride (2001) in maize and Saravanan *et al.* (2001) in soybean under copper treatment.

Dry matter production

Dry matter production in various parts of radish varied according to copper level. Dry matter of root and shoot was the highest at 50 mg kg^{-1}, but showed a gradual decline from 100 mg kg^{-1} level onwards. Similar results were obtained by several authors in a number of plants such as Mocquot *et al.* (1996) in maize, Mc Barik and Chandel (2001) in soybean under copper treatment and Vijayarengan (2012) in radish under zinc treatment.

Pigments

The photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of radish decreased with increasing copper level in the soil. In excess levels, copper (100-250 mg kg^{-1}) become toxic to plants and a decrease in photosynthetic pigments were observed. These results are comparable with the reports of Mysliwa-Kurdziel and Strazalka (2002) under copper treatment. It has been proposed that Cu at toxic concentration interferes with enzymes associated with chlorophyll biosynthesis and protein composition of photosynthetic membranes (Lidon and Hendriques, 1991; Singh *et al.*, 2007).

The overall decrease in growth, dry weight and photosynthetic pigment parameters of radish was due to the toxic effect of higher concentration of copper. 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

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their overall growth processes. This led to the decrease in the overall growth of the stressed plants.

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