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Research Article
Effect of Micronutrients on Growth and Antioxidant Activity of *Corchorus olitorius*

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Abstract

Background and Objective: *Corchorus olitorius* is a leafy vegetable and medicinal plant rich in antioxidants and also cultivated worldwide for its stem fibers. This work studied the influence of micronutrients fertilization on growth and antioxidant activity of this plant. The study aimed to help improvement of this crop yield as well as study the impact of fertilization on its characteristic antioxidant activity. **Materials and Methods:** In this work, micronutrients fertilization by Cu, Zn, Ni and Fe were studied for their effect on fresh weight, stem length, leaf number and leaf areas of *C. olitorius*. Antioxidant activity was estimated by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. A number of non-enzymatic antioxidants were estimated including free and wall-bound phenolics, total flavonoids, total anthocyanins and ascorbic acid. **Results:** The results recorded increased growth and reduced antioxidant activity as a result of all metal treatments, except Ni. Iron treatment resulted in the best growth and least antioxidant activity. After a second dose of the tested metals, growth rate decreased with increasing metal concentrations and this was accompanied by elevated antioxidant activity. These results record the positive impact of micronutrient fertilization on crop production of jute. **Conclusion:** However, the negative impact on its antioxidant value suggests a limitation on micronutrients fertilization of this plant if cultivated for consumption as a vegetable or for medicinal purposes.

Key words: *Corchorus olitorius*, antioxidants, fertilization, Jute, leafy vegetables, micronutrients

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jute (*Corchorus olitorius* L.) is an annual herb used as a green leafy vegetable in many tropical areas. This plant is a very good source of proteins, carotenoids, vitamins (A, B1, B2, C and E) and is rich in mineral nutrients like Ca, K, Cu, Zn and Mn. It is also cultivated for fibers production from its stems in most parts of the world. The jute fibers have many industrial and commercial applications which may be applied to make use of jute plants grown under metal-contaminated soils[1]. Jute leaves are particularly known to contain a number of strong antioxidants, including phenolic compounds, tocopherols and ascorbic acid. There is a lack of information on the qualitative improvement of foliage yield of this plant[1,2].

Micronutrients, including copper (Cu), zinc (Zn), nickel (Ni) and iron (Fe) elements are essential in minute amounts and toxic if exceeded certain levels[3]. Low soil fertility is a factor responsible for low productivity of vegetables[4]. Responses of many crops to micronutrients have been reported[5,6]. Adding micronutrients as fertilizers could contribute to lowering their deficiency in humans and in improving crop production[7].

The impact of heavy metals contamination on jute has been studied[8,9]. Only a few studies have investigated the role of heavy metals as micronutrients for jute[10]. Soil amended with organic or inorganic fertilizers improve growth and yield performance of *C. olitorious*[2,6,11]. Information on effect of fertilization on antioxidant activity of other plants is contradictory[12, 13]. The effect of micronutrients fertilization on jute and the influence on antioxidant activity is not clear. This study aimed to assess the effect of four micronutrients (Cu, Zn, Ni and Fe) on the growth and non-enzymatic antioxidant activity of jute.

MATERIALS AND METHODS

Study area: The experiment was carried out at the Botanical Garden of the Faculty of Sciences, Minia University in the duration from August-September, 2019.

Research protocol: Seeds of jute were obtained from the Agricultural Research Unit, Minia, Egypt. They were sown in 3.6 kg pots filled with clay soil obtained from the Botanical Garden of the Faculty of Sciences, Minia University. The experiment was conducted in the middle of July, 2019 for 45 days. Ten seeds were sown per pot and irrigated by normal tap water. After germination, seedlings were thinned to 3 per pot. Fifteen-day-old seedlings were subjected to levels of Cu (as $CuSO_4.5H_2O$), Zn (as $ZnSO_4.7H_2O$), Ni (as $NiCl_2.6H_2O$) and Fe (as $FeSO_4.7H_2O$). Solutions of concentrations 0.02, 0.05 and 0.1 g L^{-1} were prepared from each of the metals and were chosen after preliminary seed germination experiments. Concentrations 0.02, 0.05 and 0.1 g $L^{-1}$are referred to as T_1, T_2 and T_3, respectively. Three pots were irrigated to the field capacity (330 mL kg^{-1} soil) with the appropriate solution. The elements concentrations added to the soil were 6.6 mg kg^{-1} soil in T_1 pots, 16.5 mg kg^{-1} soil in T_2 pots and 33 mg kg^{-1} soil in T_3 pots. Pots were kept outdoors and irrigated with tap water for 15 days. A second dose, equal to the first dose, of the micronutrients solutions was added to each pot. Plants, either treated or control, were irrigated with normal tap water until the end of the experiment. No additional fertilizer was added. Samples were collected at 15 days after treatment with the first dose of the tested metals, plants were 30 days old. Samples were collected again at the end of the experiment, 15 days after treatment with the second dose of tested metals solutions, plants were 45 days old.

Measuring growth parameters: Shoot lengths were measured and leaves counted at both stages. Leaf area was measured using the disk method[14] and expressed as cm^2/plant leaf. Plant stems and leaves were harvested and fresh weights determined. Leaves were washed thoroughly with deionized water and forced-air oven dried at 65°C for 48 hrs until a constant weight was reached. Dried leaves were ground into a fine powder, passed through a 2 mm sieve and kept in paper bags for analysis. For protocols that required fresh tissues, plant leaves were kept in freezer till used.

Soil analysis: A soil sample was taken, air-dried, ground and passed through a 2 mm sieve and kept for analysis. The pH was measured in 1:2.5 soil-water suspensions by pH-meter[15]. Five gram of dried soil was subjected to wet digestion[16]. The soil sample was mixed with 10 mL of $HCl:HNO_3$ (3:1 molar ratio). The mix was digested on a hot plate at 95°C for 1 h and allowed to cool to room temperature (20-25°C). The sample was diluted to 50 mL with deionized distilled water and left to settle overnight. The supernatant was filtered through Whatman no. 1 filter papers and used for determining concentrations of Cu, Zn, Ni and Fe with an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 400) and compared to the maximum allowable limits of heavy metals in soils[17] and to critical limits[18,19].

Estimation of total antioxidant activity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay: Dried powdered plant leaves were used for extraction of total flavonoids and for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay[20]. A weight of 0.05 g of each dried plant sample was extracted in 5 mL of

50% methanol overnight. The samples were centrifuged at 14000 rpm for 15 min and the supernatants used for determining total flavonoids content and for DPPH assay.

Free radical scavenging activity measurement using DPPH: A 0.1 mM solution of DPPH in methanol was prepared and 1 mL mixed with 1 mL of methanol extract of each sample. The mix was incubated for 30 min in dark and absorbance measured at 517 nm. The DPPH solution without sample was the control and the 50% methanol was used as a blank. A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. The purple color generally disappears when an antioxidant is present in the medium, antioxidant molecules quench DPPH free radicals and convert them to a colorless product (2,2-diphenyl-1-picrylhydrazine), resulting in a decrease in absorbance. The percentage (%) scavenging activity was calculated using the following equation[21]:

$$SA\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where, SA (%) is the percentage scavenging activity, A_0 is the absorbance of control and A_1 is the absorbance of sample. The higher SA (%) indicates higher antioxidant activity.

Estimation of ascorbic acid content: The ascorbic acid content was determined based on Jagota and Dani[22]. A fresh weight of 0.1 g of plant leaves was homogenized with 2 mL of 0.75 M metaphosphoric acid. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was collected and 200 µL of 3% meta-phosphoric acid and 200 µL of Folin reagent (1:5) were added to 400 µL of the supernatant and brought to a total volume of 2 mL with distilled water. The content was mixed for 10 min and the absorbance read at 760 nm. The ascorbic acid concentration was calculated using the standard curve constructed with known concentrations of ascorbic acid (50-1000 µg mL^{-1}).

Estimation of Total Anthocyanins Content (TAC): Total anthocyanins content was determined according to Strack and Wary[23]. A weight of 0.1 g of fresh frozen leaves was ground in liquid nitrogen and extracted with 2 mL of 1% HCl acidified methanol [1% (v:v) HCl in methanol] for 2 hrs at room temperature (20-25°C). Samples were centrifuged at 12000 rpm for 15 min and the supernatant used for measuring anthocyanins content by reading absorbance at 535 nm. Acidified methanol was used as a blank. The dilution factor and the cyanidin 3-galactoside coefficient of extinction (98.2) were taken into account in the anthocyanins concentration calculations[24] according to the equation:

$$\text{Total anthocyanins (mg cyanidin /g fresh plant material)} = \frac{\text{Absorbance} \times \text{Dilution factor}}{98.2}$$

Estimation of free and wall-bound Total Phenolic Compounds (TPC): The total phenolic compound concentration was determined according to Kofalvi and Nassuth[25]. Fresh leaves (0.25 g) were extracted in 5 mL of 50% methanol (1:1 v/v) for 90 min at 80°C. The extract was centrifuged at 14000 rpm for 15 min. The supernatant was collected and used for the determination of free phenolics. The pellet was used for the extraction of bound phenolics. The pellet was mixed with 2 mL of 0.5 N NaOH for 24 hrs at room temperature (20-25°C) to release bound phenolics, neutralized with 0.5 mL of 2 N HCl and centrifuged at 14000 rpm for 15 min. The supernatant was used for the estimation of bound phenolics.

Free and bound phenolics were determined using the Folin Ciocalteu method. One hundred µL of each sample was diluted to 1 mL with deionized water and mixed with 0.5 mL of 1 N Folin Ciocalteu reagent (1:1 diluted commercial 2 N reagent). After 5 min, 2.5 mL of 20% Na_2CO_3 was added to each sample. Samples were incubated at room temperature (20-25°C) for 20 min and absorbance measured at 735 nm against a blank. A standard curve was prepared with gallic acid of known concentrations (100-1000 µg mL^{-1}) prepared in 50% methanol. Total phenolic content was expressed as Gallic Acid Equivalents (GAE) in mg g^{-1} fresh plant material.

Estimation of Total Flavonoids Content (TFC): Estimation of total flavonoids in plant extracts used the method of Ordonez *et al.*[26]. Five hundred µL of 2% $AlCl_3$ in methanol was added to 0.5 mL of samples extracted in methanol. After 1 h incubation at room temperature (20-25°C), the absorbance was measured at 420 nm. A standard curve was prepared using quercetin of known concentrations (20-200 µg mL^{-1}) prepared in 50% methanol. Total flavonoids content was expressed as Quercetin Equivalents (QE) in mg g^{-1} dried plant material.

Statistical analysis: All data were subjected to 1-way analysis of variance (ANOVA) using SPSS (ver. 21.0). Vertical bars in fig indicate ± SD based on 3 determinations. Least significant difference (LSD) was used to separate means of control and treated plants.

RESULTS

Table 1 represents soil concentrations of the four metals under examination in this work. Their concentrations are within the normal range in cultivated soils as compared to their corresponding maximum permissible limits as well as critical limits in cultivated soils.

Table 2 represents the data of measured stems and leaves growth parameters for control and treated jute plants in the first stage of growth. Most of the measured growth parameters were highly significantly stimulated as a result of different micronutrients treatments, particularly fresh weights of stems and leaves. The tested micronutrients concentrations resulted in doubling growth parameters of *C. olitorius* plants in many cases. For example, Plants treated by T_1 and T_2 Cu recorded leaves fresh weights of 211 and 206% in comparison with control, respectively and Zn-treated stems recorded fresh weights of 248 and 237%, respectively, compared to their control. Nickel was the only tested micronutrient whose treatment resulted in a reduction of some growth parameters (number of leaves and leaves fresh weight at higher treatment). The highest stimulation of leaves fresh weights in this stage was due to Cu and Fe treatments while the highest stimulation in stems resulted from Fe and Zn treatments.

Table 3 showed the results of growth parameters for *C. olitorius* stems and leaves in the second stage of growth. The measured growth parameters were in most cases significantly higher than control. The best growth in this stage was recorded by Fe-treated plants, particularly under T_2 treatment. T_2 Fe-treated *C. olitorius* stems and leaves recorded fresh weights of 282 and 180%, respectively, in relation to their controls. However, the positive impact of micronutrients treatments in this stage was less significant than the first stage, particularly under T_3 treatments (the highest tested metal concentration) and the negative impact

Table 1: Concentrations of Cu, Zn, Ni and Fe in the studied soil

Element (μg g^{-1})	Cu	Zn	Ni	Fe
Maximum permissible level in soil	100	300	50	50000
Critical limit	0.2	0.6	0.2	4.5
Concentration in studied soil	1.57	6.92	6.62	6.03

Table 2: Effect of Cu, Zn, Ni, Fe and control on growth of *Corchorus olitorius* plants [fresh weights of stems and leaves (g), stem length (cm), no. of leaves and leaf area (cm^2)] in the first growth stage

Metal	Treatment	Stem fw (g)	Control (%)	Leaves fw (g)	Control (%)	Stem length (cm)	Control (%)	No. of leaves	Control (%)	Leaf area (cm^2)	Control (%)
Control		1.72±0.18	100	2.62±0.24	100	16.13±0.33	100	24.00±1.01	100.00	5.50±0.58	100.00
Cu	T_1	3.36±0.22*	195.35	5.52±0.72*	210.69	18.66±0.33	115.69	32.00±2.16*	133.33	9.07±1.11*	164.91
	T_2	3.66±0.12*	212.79	5.4±0.06*	206.11	21.66±0.88*	134.28	29.00±3.15	120.83	10.30±1.12*	187.27
	T_3	4.00±0.60*	232.56	5.02±0.58*	191.60	23.00±1.73*	142.59	31.00±1.53	129.17	12.23±1.08*	222.36
Zn	T_1	4.26±0.26*	247.67	3.88±0.48	148.09	27.33±1.34*	169.44	23.33±2.33	97.20	11.73±0.32*	213.27
	T_2	4.08±0.52*	237.21	4.08±0.42*	155.73	26.66±1.2*	165.28	26.00±2	108.33	10.20±0.95*	185.46
	T_3	2.84±0.20*	165.12	3.42±0.42	130.53	23.00±1*	142.59	21.00±3.79	87.50	7.38±0.98	142.36
Ni	T_1	2.08±0.06	120.93	2.68±0.16	102.29	20.00±0.17*	123.99	20.50±2.19	85.42	8.68±0.85*	157.82
	T_2	2.58±0.34*	150.00	3.14±0.1	119.85	19.83±1.2*	122.94	18.33±0.87*	76.38	10.65±0.25*	193.64
	T_3	1.92±0.30	111.63	2.25±0.08*	85.88	19.33±0.88*	119.84	15.33±1.76*	63.88	8.05±0.3*	146.36
Fe	T_1	3.24±0.30*	188.37	4.60±0.2*	175.57	20.33±0.33	126.04	28.00±3.04	116.67	9.95±1.19*	180.91
	T_2	4.04±0.12*	234.88	5.16±0.22*	196.95	26.00±1.15*	161.19	36.00±2*	150.00	13.23±0.80*	240.55
	T_3	3.36±0.18*	195.35	5.36±0.50*	204.58	22.83±1.57*	141.54	31.00±3.79	129.17	13.65±0.99*	248.18

Values are represented as means of three replicates±SD. Value with (*) are statistically different from their control at $p<0.05$, f w: Fresh weight

Table 3: Effect of Cu, Zn, Ni, Fe and control on growth of *Corchorus olitorius* plants [fresh weights of stems and leaves (g), stem length (cm), no. of leaves and leaf area (cm^2)] in the second growth stage

Metal	Treatment	Stem fw (g)	Control (%)	Leaves fw (g)	Control (%)	Stem length (cm)	Control (%)	No. of leaves	Control (%)	Leaf area (cm^2)	Control (%)
Control		1.94±0.12	100	2.90±0.18	100	20.83±1.31	100	25.00±2.72	100	6.10±0.68	100
Cu	T_1	2.96±0.48*	152.58	3.60±0.34*	124.14	23.83±1.19	114.4	38.67±3.79*	154.68	12.20±1.02*	200
	T_2	4.08±0.18*	210.31	3.26±0.06*	112.41	32.83±2.78*	157.61	32.00±2.55*	128	11.37±1.13*	186.39
	T_3	2.82±0.30*	145.36	2.56±0.24	88.28	26.83±2.32*	126.64	28.67±2.90	114.68	16.63±1.82*	272.62
Zn	T_1	3.22±0.26*	165.98	3.72±0.02*	128.28	29.50±3.01*	141.62	31.67±3.15*	126.68	15.23±0.93*	249.67
	T_2	4.06±0.44*	209.28	3.56±0.28*	122.76	33.50±3.46*	160.83	29.33±1.09	117.32	18.45±1.12*	302.46
	T_3	3.04±0.20*	156.70	3.02±0.22*	104.14	32.67±2.19*	156.84	30.00±2.36	120	11.83±1.25*	193.93
Ni	T_1	2.48±0.28*	127.84	3.06±0.34	105.52	29.83±3.14*	143.21	31.50±3.02*	126	8.85±0.86*	145.08
	T_2	3.32±0.12*	171.13	2.54±0.18*	87.59	30.83±2.56*	148.01	25.50±1.55	102	9.28±0.72*	152.13
	T_3	2.30±0.24*	118.56	1.98±0.18	68.28	29.50±2.71*	141.62	17.33±1.14*	69.32	8.40±0.51	137.71
Fe	T_1	3.70±0.64*	190.72	3.54±0.12*	122.07	32.33±1.99*	155.21	38.33±4.05*	153.32	11.15±0.97*	182.79
	T_2	5.48±0.70*	282.47	5.22±0.24*	180	36.33±4.05*	174.41	52.00±4.39*	208	17.50±1.98*	286.89
	T_3	3.48±0.30*	179.38	3.66±0.12*	126.21	30.00±3.89*	144.02	40.00±3.98*	160	14.40±1.12*	236.07

Values are represented as means of three replicates±SD. Value with (*) are statistically different from their control at $p<0.05$. fw: Fresh weight

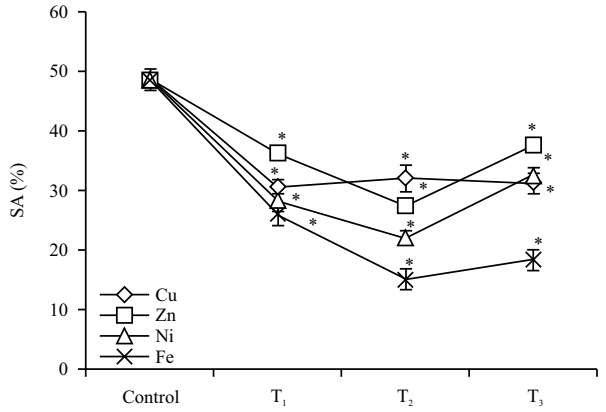

Fig. 1: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on percentage DPPH scavenging activity (SA %) of jute plants in the first growth stage

Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

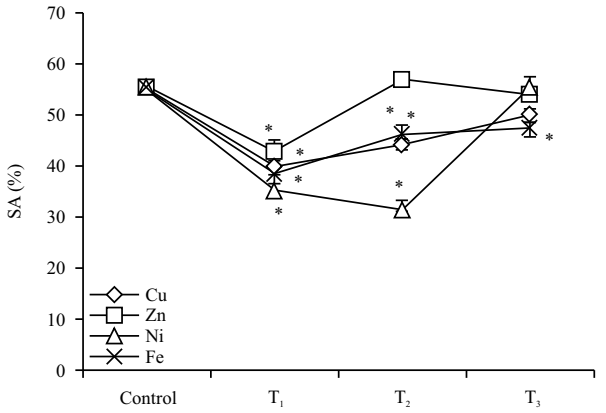

Fig. 2: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on percentage DPPH scavenging activity (SA %) of jute plants in the second stage

Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

of Ni was more significant. For example, leaves fresh weights in relation to control in the first stage were 192, 131, 86 and 205% in plants treated by T_3 concentrations of Cu, Zn, Ni and Fe, respectively (Table 2). In the second stage, the corresponding values of leaves fresh weights under T_3 treatments of Cu, Zn, Ni and Fe were 88, 104, 68 and 126% compared to control, respectively (Table 3).

The total antioxidant activity of young *C. olitorius* leaves in the first stage of growth as estimated by the DPPH assay

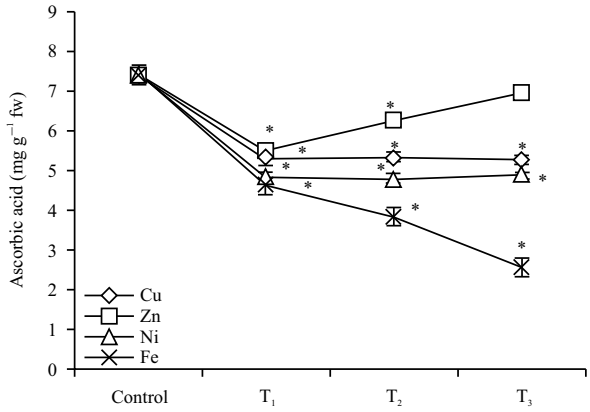

Fig. 3: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on ascorbic acid content in mg g^{-1} fresh weight of jute plants in the first stage

Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

is represented in Fig. 1. Antioxidant activity was highest in control plants (48% SA) while different metal treatments resulted in significant reduction in antioxidant activity which was minimum in Fe-treated plants, particularly under T_2 and T_3 Fe treatments (16 and 18% SA, respectively). Figure 2 represents antioxidant activity of plants in the second stage in which control plants also recorded the highest antioxidant activity (56% SA) while T_1 and T_2 Ni-treated plants recorded the least values (33 and 30% SA, respectively). Generally, antioxidant activity of plant leaves in this stage was significantly reduced in response to low and middle metal treatments of the different tested metals (T_1 and T_2). On the other hand, leaves treated by the highest metal concentration (T_3) recorded values of antioxidant activity comparable to control plants, except for Fe-treated T_3 plants which had antioxidant activity that was significantly lower than control (45% SA).

Ascorbic acid contents of *C. olitorius* leaves in the first stage of growth are shown in Fig. 3. Control plants recorded the highest content of ascorbic acid (7.5 mg ascorbic acid g^{-1} fresh weight). Different metal treatments resulted in reduced ascorbic acid contents. The lowest results of ascorbic acid were recorded in Fe-treated plants (4.5, 3.9 and 2.5 mg ascorbic acid g^{-1} fresh weight in T_1, T_2 and T_3 Fe-treated plants, respectively). Figure 4 shows ascorbic acid contents in the second stage of growth where almost no significant differences were recorded among different treatments and control plants. Most values of control and metal-treated plants ranged around 10 mg ascorbic acid g^{-1} fresh weight.

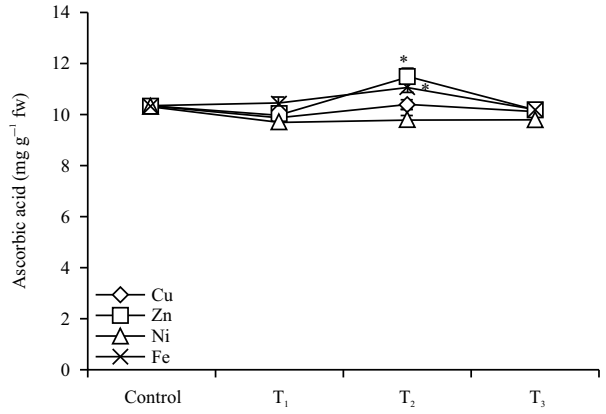

Fig. 4: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on ascorbic acid content in mg g^{-1} fresh weight of jute plants in the second stage

Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

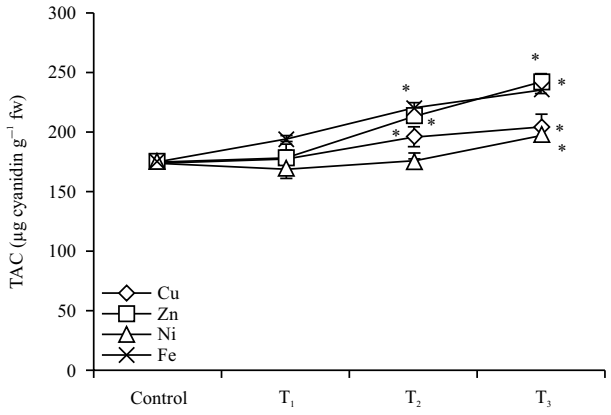

Fig. 5: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on total anthocyanins content (TAC) in µg cyanidin g^{-1} fresh weight of jute plants in the first stage

Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

Anthocyanins contents of jute plants in their first stage of growth in Fig. 5 gradually increased in response to metal treatments. Control plants recorded the least anthocyanins content (174 µg cyanidin g^{-1} fresh weight) while the highest values were recorded in plant leaves treated by T_3 concentration of the different tested metals. Zn T_3-treated plants recorded the highest content of anthocyanins (228 µg cyanidin g^{-1} fresh weight). Anthocyanins contents of plants in

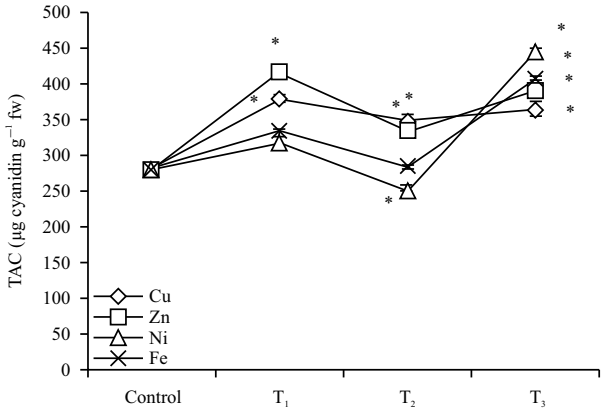

Fig. 6: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on total anthocyanins content (TAC) in µg cyanidin g^{-1} fresh weight of jute plants in the second stage

Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

the second stage represented in Fig. 6 reveal similar trend to the first stage. Control plants also recorded the lowest value (260 µg cyanidin g^{-1} fresh weight) while metal treatment resulted in irregular increase of anthocyanins contents. The highest values were recorded in T_3-treated plants under different metals treatments (ranging between 360 and 450 µg cyanidin g^{-1} fresh weight). A general increase in the values of anthocyanins was observed in the second stage compared to the first. For example, control plants recorded 270 µg cyanidin g^{-1} fresh weight in the second stage compared to 174 µg cyanidin g^{-1} fresh weight in the first. Similarly, the general range of anthocyanins was higher in metal treated plants in the second stage as compared to the first.

Figure 7 shows the contents of free phenolic compounds in young plants where values were more or less comparable to control with slight reduction under Zn and Ni treatment and a significantly sharp reduction under Fe treatment. T_3 Fe-treated leaves recorded the least contents of free phenolic compounds in the first stage (2.1 mg GAE g^{-1} fresh weight) while control plants recorded 4.5 mg GAE g^{-1} fresh weight. Only Cu-treated plants had non-significantly higher values of free phenolics compared to control in this stage.

Free phenolic compounds in the second stage of growth are shown in Fig. 8. Metal treatment reduced free phenolics in jute leaves in this stage, except for Cu and Ni T_2 concentrations which stimulated free phenolics to record 16.5 and 17 mg GAE g^{-1} fresh weight, respectively, which is significantly higher than control values (11.2 mg GAE g^{-1} fresh weight). The least values resulted from Zn and Fe T_3 treatments

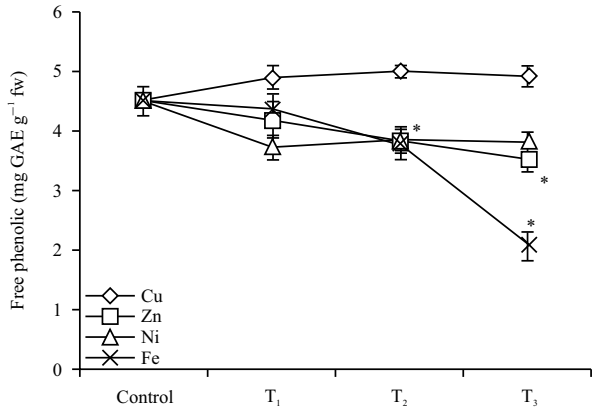

Fig. 7: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on free phenolic compounds content in mg GAE g^{-1} fresh weight of jute plants in the first growth stage.
Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

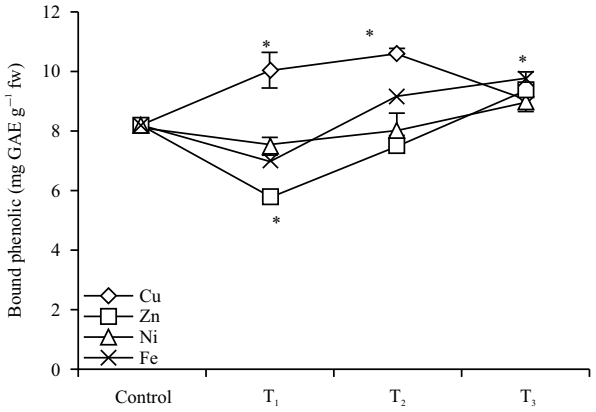

Fig. 9: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on wall-bound phenolic compounds content in mg GAE g^{-1} fresh weight of jute plants in the first growth stage
Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

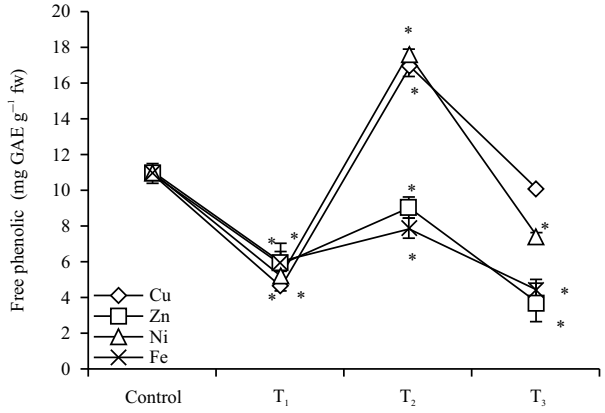

Fig. 8: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on free phenolic compounds content in mg GAE g^{-1} fresh weight of jute plants in the second growth stage
Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

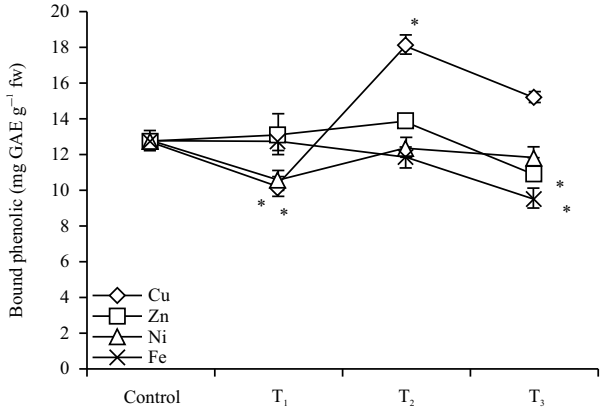

Fig. 10: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on wall-bound phenolic compounds content in mg GAE g^{-1} fresh weight of jute plants in the second growth stage
Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

(around 4 mg GAE g^{-1} fresh weight). Figure 9 showed the contents of wall-bound phenolics in the first stage where a similar trend to free phenolics in the same stage (Fig. 7) was observed. Most values were also comparable to control (8 mg GAE g^{-1} fresh weight) except for Cu-treated plants which recorded higher values up to 10 and 10.2 mg GAE g^{-1} fresh weight in T_1 and T_2 Cu-treated plants, respectively, which were the highest values of wall-bound phenolics in the first stage.

Wall-bound phenolics in the second stage are represented in Fig. 10 and also record the prevalence of Cu treatment in bound phenolics where T_2 recorded the highest value (18 mg GAE g^{-1} fresh weight) compared to 12.2 mg GAE g^{-1} fresh weight for control while the least value was recorded by T_3 Fe-treated plants (9.1 mg GAE g^{-1} fresh weight). Cu treatment generally stimulated the contents of free and wall-bound phenolics in both the first and second

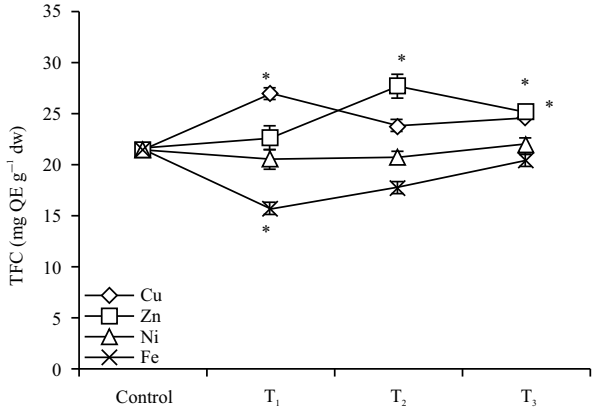

Fig. 11: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on total flavonoids content (TFC) in mg QE g^{-1} dry weight of jute plants in the first growth stage
Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

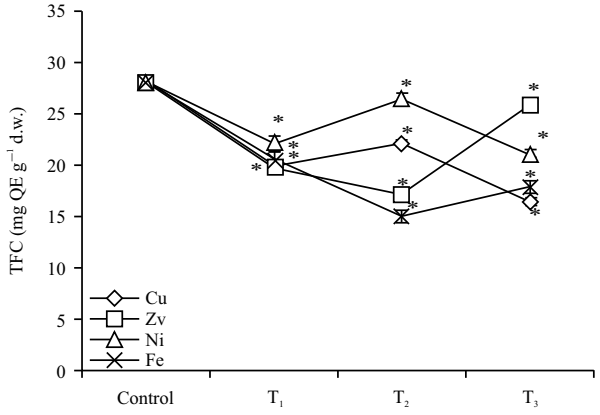

Fig. 12: Effect of control, T_1, T_2 and T_3 of Cu, Zn, Ni and Fe on total flavonoids content (TFC) in mg QE g^{-1} dry weight of jute plants in the second growth stage
Results are means±SD of three replicates. Vertical bars indicate standard deviations. Bars with (*) are significantly different from control at $p<0.05$. T_1: 0.02 g L^{-1} (solution concentration), T_2: 0.05 g L^{-1} (solution concentration), T_3: 0.1 g L^{-1} (solution concentration)

tested stages (Fig. 7-10). Like the other tested antioxidants parameters in this work, values of phenolic compound, free or wall-bound, increased by increasing plant age in both control and metal-treated plants. For example, at the level of control, free phenolics were 4.5 and 10.5 mg GAE g^{-1} fresh weight in the first and second stages, respectively. Similarly, bound phenolics recorded 8 and 12.2 mg GAE g^{-1} fresh weight in the first and second stages, respectively.

Total flavonoids contents for jute leaves in the first stage in Fig. 11 showed values that are mostly similar to control value (21.5 mg QE g^{-1} fresh weight) under most metal treatments. The least flavonoids content was under Fe treatment, particularly T_1 (15.5 mg QE g^{-1} fresh weight) while the highest was under T_1 Cu and T_2 Zn treatments (27 and 28 mg QE g^{-1} fresh weight, respectively). Flavonoids contents in the second stage in Fig. 12 were significantly inhibited in response to metal treatment. Control plants had the highest value (28 mg QE g^{-1} fresh weight) while T_2 Fe-treated leaves had the lowest (14.2 mg QE g^{-1} fresh weight).

DISCUSSION

This work studied the potential fertilization of *Corchorus olitorius* by the four micronutrients Cu, Zn, Ni and Fe and the influence of these metal treatments on the non-enzymatic antioxidants of this plant. Growth parameters were measured for both stems and leaves as the economically important organs of this plant. The four investigated metals are known to have a biphasic effect on plants depending on their concentration[27]. All tested metals, except Ni, resulted in a considerable enhancement in plant growth after the first dose of metals. A second dose of metals was added to further experiment the plant tolerance against increased levels of tested metals. Increasing metal concentrations in the second stage of this work also resulted in stimulated growth. Therefore, micronutrient fertilization could be applied for increasing crop productivity of *C. olitorius*. However, the stimulated growth parameters were accompanied by general reduction in antioxidant activity of jute leaves, the edible plant organ. This result needs to be taken into consideration in the light of the production of healthy food; particularly that nutritional value of jute leaves is distinguishable by the strong antioxidant activity. When environmental conditions are favorable, vegetative growth generally receives resource priority over secondary metabolites, decreasing the relative availability of carbon for the support of secondary metabolism. Since phenols and other antioxidants are mainly stress components, stressed plants are expected to stimulate their antioxidant defense system while in non-stressed environments they are not[28].

The metal toxic effect on jute plants was only recorded in plants treated by the highest Ni concentrations in both stages of this study, more pronounced after adding the second dose of Ni. This may be attributed to the very low concentrations of Ni as a micronutrient needed by plants[29]. Nickel toxicity was only recorded in growth parameters of leaves but not in stems, which may suggest the suitability of jute to cultivation in soils with relatively high Ni levels for stem fibers production.

The highest general growth in this work was recorded in plants treated by Fe. This could be related to the high plant requirements of Fe in comparison with other micronutrients. The range of soil critical levels of Fe, Zn and Cu are 4.7, 0.7 and 1.4 mg kg^{-1}, respectively[30]. Average plant contents of Fe, Zn, Cu and Ni are 100, 10, 6 and 0.0001 mg kg^{-1}, respectively[31]. It is estimated that about one-third of Earth's soil can be considered to be Fe deficient[32]. The Fe-treated plants were recorded to have the lowest antioxidant activity in most cases. This was observed in the DPPH scavenging assay, contents of ascorbic acid and total flavonoids (particularly in the first stage) as well as free phenolic compounds in both stages.

Copper and Zn were more or less comparable as micronutrients for jute plants. Both enhanced growth at all tested metal concentrations. Cu was superior to other treatments after adding the first dose. Both metals also showed similar patterns in most of the tested antioxidant parameters. Zinc treatment was only characterized by remarkable reduction in total phenolic compounds, but not in flavonoids.

The absolute values of most measured antioxidants in this study increased significantly after increasing plant age even for the absolute control plants, which may be attributed to increased exposure to normal stresses of the environment[33] and may also be related to the end of the vegetative stage and preparation for the flowering and fruiting stages of plant life which are characterized by increasing antioxidant activity[34,35]. However, the increased antioxidant activity and the general reduction in growth rates with extending plant life were more pronounced in metal-treated plants rather than control. This might be due to increasing soil concentrations of the tested metals after the second dose and to increasing the duration of exposure. Therefore, adjusting the concentrations of micronutrient fertilizers is crucial.

The effect of fertilizers on phenol contents is different in different plants and types of fertilizers. Inorganic fertilizers are reported to reduce antioxidants levels, while organic fertilizers mostly enhance plants antioxidant content[36,37]. In addition, low fertilization levels increase the phenols contents while heavy fertilization tends to decrease these contents[28]. This different behavior of phenolics production in response to different treatments was recorded in this study. Free phenolic compounds in the second stage, attributed a particular trend (Fig. 8) where T_1 treatment decreased free phenolic contents, T_2 treatment sharply stimulated their accumulation and T_3 significantly dropped their contents. This pattern was observed in the four investigated metals and it suggests that T_2 metal concentration was stimulator to free phenolics production in response to the accumulative stress resulting from increased metal concentration. The sharp drop at T_3 concentration might be explained by impairment of the antioxidative system responses due to increased metal concentrations which limited plant ability to synthesize new phenols[38].

The results of this work confirm the positive impact of fertilization by the micronutrients Cu, Zn and Fe on jute crop production. However, the stimulated growth might have adverse effects on the benefits of this plant as a strong source of antioxidants.

CONCLUSION

Growth of *Corchorus olitorius* plants was significantly stimulated by the tested concentrations of Cu, Zn and Fe. The *C. olitorius* plant leaves were sensitive to high concentrations of Ni. Enhanced growth of *C. olitorius* under metal treatment was accompanied by inhibited antioxidant activity. Iron-treated plants recorded the best growth and least antioxidant activity while control plants recorded the least growth and highest antioxidant activity.

SIGNIFICANCE STATEMENT

This study recorded the highly significant impact of adding micronutrients to soil cultivated by *C. olitorius* on growth stimulation. These results could be applied for the quantitative improvement of this crop. However, the recorded suppression of antioxidant activity that accompanied stimulated growth is to be taken into consideration as it negatively affects the remarkable value of this plant as a source of antioxidants. Micronutrients fertilization can thus be applied when *C. olitorius* is cultivated for its stem fibers or for its biomass rather than being consumed as a vegetable or medicinal plant. This study also spots more light on the effect of different fertilizers on the nutritional and medicinal value of cultivated crops, a study area that requires more work to explore.

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