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EFFECTS OF COPPER ON SEED GERMINATION AND SEEDLING GROWTH PERFORMANCE OF *LENS CULINARIS* MEDIK.

Muhammad Zafar IQBAL[1], Umm-e-HABIBA[1], Sundus NAYAB[1], Muhammad SHAFIQ[1*]

[1] University of Karachi, Department of Botany, Karachi – Pakistan
[*] Corresponding author. E-mail: shafiqeco@yahoo.com

Abstract: The discharge of heavy metals in the environment due to anthropogenic, industrial and automobile activities is a worldwide environmental pollution problem. Copper is widely used in different forms in fertilizer, fungicides. Industrial effluents and for the removal algal growth in ponds. In this study the toxic effects of copper (Cu) on seed germination and seedling growth of *Lens culinaris* were investigated. Germination rate of *L. culinaris* that showed that increased in concentration of copper treatment at 25 ppm significantly ($p<0.05$) reduced germination percentage as compared to control. Seedling growth variables i.e. root and shoot length, seedling size and root/shoot ratio also declined significantly ($p<0.05$) with the treatment of copper at 25 ppm as compared to control. Seedlings dry weight of *L. culinaris* gradually reduced with increased in all treatment of copper concentration as compared to control.

Tolerance indices and seedling vigor index of *L. culinaris* also decreased with increase in concentration of copper treatment. Low percentage of reduction in tolerance indices and seedling vigor index of *L. culinaris* was recorded at 25 ppm copper treatment as compared to control. A high percentage of reduction in seedling tolerance indices of *L. culinaris* was recorded at 100 ppm of copper treatment as compared to control.

Keywords: heavy metals, phytoxicity, seed germination, seedling growth, tolerance index.

Introduction

The group of elements have a density greater than 5g/cm^3 belongs to heavy metals group [AGORAMOORTHY & al. 2008]. The ever increase of heavy metal contamination in the environment has caused a serious environmental concern among researchers community. The effects of heavy metals on plant growth varied from species to species and the level of heavy metals available in the environment which may be beneficial or toxic to the plant growth environment. The essentials element likewise Fe, Zn, Cu or Mo are required in small quantities but at higher concentrations they may be poisonous for plant growth. Heavy metal contamination of soil and water causing toxicity/stress has become one important constraint to crop productivity and quality [SINGH & al. 2016]. Among the heavy metals copper can be considered important heavy metals for ecotoxicology concern. The effects of copper upon seedling growth of *Cucumis sativus*, carrot, maize and wild plant species on bioaccumulation, yield of tomato and mineral nutrients were reported [MOUSTAKAS & al. 1997; STOYANOVA & DONCHEVA, 2002; ROUT & DAS, 2003; AN & al. 2004; SONMEZ & al. 2006; MAHMOOD & al. 2005; AN, 2006; XU & al. 2006; AUDA & ALI, 2010; MUHAMMAD & al. 2011]. Copper is an essential micronutrient and is easily absorbed by plants. Its optimal content in plants tissues is reported to be 5-20 µg g^{-1} [FERNANDEZ & HERNIQUES, 1991]. High concentrations of Cu become extremely toxic

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for root growth inhibition [FARGASAVA 2001]. Rice grain yields decreased exponentially and significantly with the increase of soil Cu levels. Rice grain yield was reduced about 10% by soil Cu level of 100 mg kg(-1), about 50% by soil Cu level of 300-500 mg kg(-1) and about 90% by soil Cu concentration of 1,000 mg kg(-1). Copper treatment at 0.5 mM inhibited the root growth of two lentil cultivars viz. cv. Krak and cv. Tina [JENAS & al. 2015].

Copper is toxic heavy metals and high concentration can be harmful for agriculture cops. The excess concentration of copper induced huge losses in agricultural crops viz wheat, iron, maize, sunflowers and cucumber production [ADREES & al. 2015]. Lentil is an edible pulse, oldest crop [SULTANA & GHAFOOR, 2006] and predominantly successfully cultivated in South East Asia and Turkey, Syria, Egypt, Iran and Pakistan. *L. culinaris* is annual leguminous popular crop due to its lens shaped seed and is a main source of vegetable protein. *L. culinaris* is a rabi legume plant and cultivated on large area in Pakistan [RAHMAN & al. 2013]. Little is known about the effects of copper on growth of an important crop lentil. Therefore, the aim of the present study was to evaluate the effects of copper on seed germination and seedling growth performance of *L. culinaris*.

Lentils (*Lens culinaris* Medik.) is an important legume pulse crop in world for millions of people as source of food. SZILAGY & al. (2011) examined the stability for seed yield in lentils (*Lens culinaris* Medik) in Romania. At present in Romania, lentils grown on the lower areas, the only lentil Romanian cultivar being 'Oana'. The temperature hydration kinetics of *Lens culinaris* was evaluated in water at different temperature [OROIAN, 2017].

Materials and methods

The healthy seeds of *Lens culinaris* Medik were collected from the local market. The seeds were surface sterilized with 0.2% dilute solution of Sodium-hypo chlorite for two minutes to prevent any fungal contamination. The seeds were washed with double distilled water. Ten seeds were placed in Petri dishes (90 mm diameter) uniformly on filter paper added at proper place and covered with lid to prevent loss of mixture through evaporation. Solutions of copper sulfate were prepared having five concentrations (0, 25, 50, 75 and 100 ppm) concentrations. At the start of the experiment, 3ml of solution of above treatment were applied to each set of respective treatment. After two days the old solution from every petri plate was sucked out and 2 ml fresh solution of respective treatment was added. The distilled water was added to each set of control treatment. The control received only 1ml of distilled water on alternate days. The experiments were designed on the basis of three replicates and the Petri dishes were kept at room temperature (32±2 °C) with 240 Lux light intensity and the experiment lasted for ten days. The experiment was completely randomized. Seed germination, root, shoot, seedling lengths, seedling dry weight and root / shoot ratio was recorded. Three seedlings having maximum from each petri plate was sampled to measure the seedling growth variable. The dry biomass was determined by placing the seedling in an oven at 80 °C for 24 hours. Seedling vigor index (S.V.I) was determined as per the formula given by BEWLY & BLACK (1982). Tolerance indices of seedlings were determined with the help of the following formula.

$$\text{Tolerance indices (T.I.)} = \frac{\text{Mean root length of treated seedlings}}{\text{Mean root length of control seedlings}} \times 100$$

The seed germination and seedling growth data were statistically analyzed by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to determine the level of significance at $p < 0.05$ on personnel computer using COSTAT version 3.

Results

The different concentrations of heavy metal treatment as copper sulfate on *Lens culinaris* indicated low percentage of seed germination, seedling growth, seedling dry weight and root/shoot ratio as compared to control. The results indicated that root is strongly affected as compared to shoot by copper treatment. An increased in concentration of Cu produced more toxic effects on seedling dry weight and root / shoot ratio of *L. culinaris* as compared to control treatments (Figure 1-2; Table 1).

Copper treatment at 25 ppm not significantly affected seed germination percentage of *L. culinaris* as compared to control (Table 1). The percentage of seed germination was significantly ($p<0.05$) reduced to 76.66, 66.66 and 56.66% at 50, 75 and 100 Cu treatment as compared to control (100%). Copper treatment showed that root growth of *L. culinaris* was decreased with increase in concentration up to 100 ppm. The treatment of Cu showed more adverse effects on shoot growth of *L. culinaris* as compared to control. Results showed that shoot growth parameters were also declined with increase in concentration from 25 to 100 ppm of Cu. The treatments of Cu at 25 ppm increased shoot length 12.72 cm of *L. culinaris* over control 8.87 cm and significantly decreased to 7.0, 5.87, and 5.07 with the treatment at 50, 75 and 100 ppm, respectively. Seedling size which consists on the length of root and shoot was recorded as 16.77 cm for control which decreased to 13.97 cm, 7.96 cm, 5.43 cm and 5.49 cm when treated with 25, 50, 75 and 100 ppm copper solution treatment. Copper treatment at all concentration influenced the seedling dry weight of *L. culinaris*. Seedling dry weights of *L. culinaris* when treated with different concentration of Cu was reduced to 0.03 g to 0.0133 g at 25 and 100 ppm concentration as compared to control. Results indicated that reduction was observed in root/shoot ratio with the increase in concentration copper particularly at 75 and 100 ppm.

Table 1. Effects of different concentration of copper on seed germination (%), seedling growth and seedling dry weight (g) of *Lens culinaris*

Treatments (Copper concentration ppm)	**Germination (%)**	**Root length (cm)**	**Shoot length (cm)**	**Seedling size (cm)**	**Seedling dry veight (g)**	**Root/shoot Ratio**
00	100.00±3.33a	9.46±0.06a	8.87±0.06a	16.77±0.06a	0.034±0.98a	1.72±0.01a
25	100.00±5.77a	1.19±0.12b	12.72±0.9b	13.93±0.20b	0.030±2.64b	0.091±0.01b
50	76.66±3.33b	0.95±0.18b	7.00±0.2bc	7.96±0.4c	0.020±3.38b	0.133±0.02b
75	61.66±0.00c	0.28±0.14b	5.87±0.18c	5.43±0.33d	0.0133±2.96b	0.033±0.00b
100	51.66±3.33d	0.40±0.03b	5.07±0.25c	5.49±0.27d	0.0133±2.88b	0.091±0.006b
L.S.D.	8.13	2.25	1.09	2.28	0.01	0.12

Number followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at <0.05 level. ± Standard error. L.S.D. Least significant difference.

The seedlings of *L. culinaris* showed lowest percentage of tolerance indices were recorded for 100 ppm of Cu treatment as compared to control (Figure 1). Similarly, Seedling Vigor Index (S.V.I.) for *L. culinaris* seedling was recorded highest in control and gradually declined with the increase in concentration of Cu treatments from 25 to 100 ppm (Figure 2).

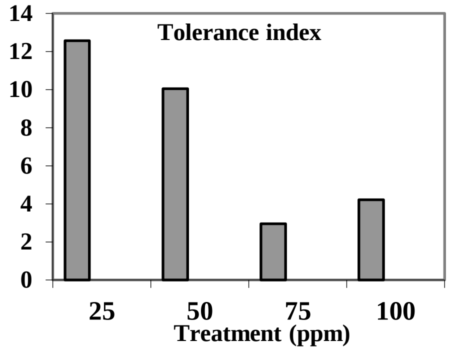

Figure 1. Percentage of tolerance in *L. culinaris* using different concentration (0, 25, 50, 75, 100 ppm) of copper (Cu)

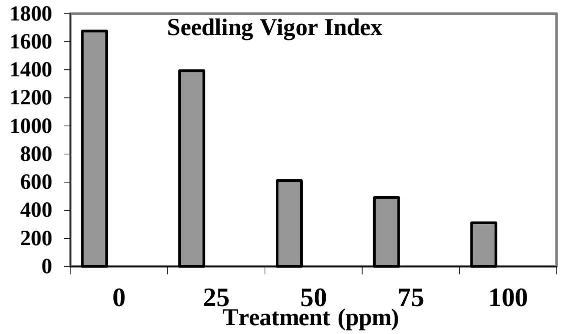

Figure 2. Seedling vigor index for *L. culinaris* using different concentration (0,25, 50, 75, 100 ppm) of copper

Discussion

Excessive concentration of copper generally produce common toxic effects on different growth variable of plants, such as low biomass accumulation, chlorosis, inhibition of growth and photosynthesis, altered water balance and nutrient assimilation, and senescence, which ultimately cause plant death. The plant under abiotic stress conditions are most likely to be adversely affected by heavy metals contamination. In present studies the toxicity and tolerance of copper on seed germination and seedling growth performances of *L. culinaris* were found significantly affected at higher concentration of copper treatment. Copper treatment at 25 ppm did not produce any significant effect on seed germination which might be due to its resistance to copper. The effect of the Cu at 25 ppm concentration on root growth of *L. culinaris* was observed and agreed with the findings of ISMAIL & al. (2013). Exposure to 25ppm concentration of Cu reduced the root length of *L. culinaris* as compared with the root length of control. In another studies, the copper treatment in resin buffered solution culture at < 1 µ M produced toxic effects on the root morphology of a pasture species, Rhodes grass (*Chloris qayana* Knuth.) observed [SHELDON & MENZIES, 2005]. Similarly, the increasing concentrations of Cu significantly inhibited the growth of young sweet potato plants (*Ipomoea batatas*) reported earlier [KIM & al. 2010].

Results also showed that seedling dry weights of *L. culinaris* were also declined with increased concentration of copper sulphate and this reduction was more prominent with increasing concentration of copper in substrate. The increase in concentration of copper upto 100 ppm was found responsible for decreased the seedling growth of *L. culinaris* as compared to control treatment. Copper treatment at all concentration none significantly affected seedling dry weight as compared to control. The results indicated that increasing concentrations of Cu in seedlings tissues significantly ($p < 0.05$) reduced the seedlings growth. In addition to growth inhibition of *L. culinaris* copper treatment reduced root / shoot ratio.

The effects of Cu on the plant growth have been reported in some studies over the past few years. At the cellular level, oxidative stress has been reported as a common mechanism in both stress situations [SMEETS & al. 2009]. The treatment of copper produced toxic effects on germination and growth of *L. culinaris.* Lowest percentage of tolerance indices for *L. culinaris* seedlings were recorded for 100 ppm copper treatment.

Conclusion

It was concluded that the copper treatment produced toxic effects on seed germination and seedling growth of *L. culinaris* along with significant reduction in seedling dry weight as compared to control treatment. Similarly, the tolerance to copper treatment decreased the tolerance indices for *L. culinaris* seedlings with the increase in metal concentration in the substrate as compared to control. The difference in tolerance and seedling vigor index in response to copper toxicity should be considered while *L. culinaris* cultivated in copper contaminated areas. There is a need to be carried out further studies on other copper tolerant species for plantation in copper contaminated areas to overcome the shortage of agriculture crops.

Notes on contributors

Muhammad Zafar IQBAL - Ph.D., Professor, designed and supervised the experiment. Umm-e-HABIBA - M.Sc., Research scientist, performed the experiment. Sundus NAYAB - M.Sc., Research scientist, performed the experiment and assisted to collect the data. Muhammad SHAFIQ - Ph.D., Research scholar statistically analysed the experimental data, reviewed the literature and draft the manuscript.

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