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RESEARCH ARTICLE

BIOCHEMICAL CONTENTS AND YIELD PARAMETERS OF COWPEAVARIETIES (*VIGNA UNGUICULATA* (L.) WALP.) ON THE APPLICATION OF COPPER

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ABSTRACT

High concentrations of metals exert a negative influence on the development of plants, their use of nutrient and metabolism. The heavy metals can cause a major ecological crisis since they are non-degradable and often accumulate in plant parts, biologically magnified through trophic levels and causes deleterious effects on plants and animals. The present investigation deals with the effect of copper on the biochemical and yield parameters of Cowpea *(Vignaunguiculata* (L.) Walp.) under field condition. Copper was treated with Copper sulphate ($CuSO_4$. $5H_2O$) from two Cowpea varieties (CO 7 and CO 6) of various concentrations (0, 5, 10, 25, 50, 100, 150 and 200 mgl^{-1}) were prepared and were used for biochemical and yield studies. It is evident from the results obtained that the increase in copper concentration affected all the biochemical and yield parameters such as chlorophylls, carotenoids, total sugar, protein, amino acids, seed output, shelling percentage 100 seed weight, pod yield and harvest index were observed.

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INTRODUCTION

Heavy metal contamination of the soil is the main environmental problem in many countries. Heavy metals are basically characterized by their toxicity, persistence and difficulty to be removed. Some of them participate in a number of biochemical reactions in living beings, raising the attention regarding the toxic effects upon the plants. The toxicity of heavy metals is a problem for ecological, evolutionary and environmental reasons (Nagajyoti*et al.*,2008). Of the various sources of pollutants industrial effluents containing heavy metals pose a threat to the ecosystem. These metals are present in the waste water of different industries such as metal cleaning, plating baths, refineries, mining, electroplating, paper and pulp, textile and tanneries (Mistry*et al.*, 2010).Heavy metals are integrated components of biosphere and thus occur naturally in soil and plants. Five heavy metals (Fe, Zn, Cu, Mn and Mo) are essential for all higher plants. The presence of heavy metals in the environment causes deleterious effects to human beings, particularly at certain levels of exposure and absorption. Metals like copper, iron, manganese, zinc are essential for life processes whereas others like cadmium, nickel and mercury have no physiological function but often results in harmful disorders at a higher concentration (Kavitha, 2010). Soils with high heavy metal concentrations, including copper, have generally been contaminated due to the close proximity to natural metalliferous ore out crops, or as a result of mining, smelting or other industrial activities. Early studies of plants growing under stress environmental conditions posed intriguing questions about the nature, scale, and mechanisms of adaptation involved (Masaka and Muunganirwa, 2007). Our study here was confined to copper as it is probably one of the most common contaminants of soils. Moreover, copper is also one of the essential micronutrients for plant growth. It is involved in numerous physiological functions as a component of several enzymes, mainly those which participate in electron flow, catalyze redox reactions in mitochondria and chloroplasts (Hansch and Mendel, 2009). However, in excessive quantities copper becomes toxic as it interferes with photosynthetic and respiratory processes, protein synthesis and development of plant organelles (Upadhyay and Panda, 2009). Specifically excess copper can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability, leading to ion leakage (Bouazizi*et al.*, 2010). The present investigation was executed with an objective to study the effects of copper stress on biochemical constituents of Cowpea (*Vignaunguiculata* (L.) Walp.) with specific emphasize on yield parameters which are the defense mechanism to any type of abiotic stress.

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MATERIALS AND METHODS

Preparation of copper sulphate solution

To study the effect of Copper toxicity, different concentrations of Copper solution in were prepared by dissolving known amount of Copper sulphate salt ($CuSO_4$, $5H_2O$) in distilled water. 4.279 gm of $CuSo_4$, $5H_2O$ was dissolved in one litre of distilled water for 1000 mgl^{-1} of Copper concentration. From this stock solution, the various concentrations of Copper solution (5, 10, 25, 50, 75,100, 150 and 200 mg/l^{-1}) were prepared by dilution and used for both germination studies and field experiments.

Yield and yield components

Five plants were used for recording the various yield parameters like number of pods per plant, dry weight of the pods, seed output, weight of the 100 seeds, pod yield and shelling percentage were estimated.

Biochemical constituents

Besides certain biochemical constituents such as chlorophyll '*a*' and '*b*' (Arnon, 1949), carotenoids (Kirk and Allen, 1965), total sugars (Nelson *et al*., 1944), aminoacids (Moore and Stein, 1948) and protein (Lowry *et al*., 1951) plant materials were analyzed were determined adopting the methods mentioned earlier in the germination studies.

Table 1.Effect of different concentrations of copper on the pigment contents of (mg g^{-1} fresh weight) of cowpea(*Vignaunguiculata*(L.)Walp) seedlings

Concentration in mgl¬	CO-7				CO-6			
	Chlorophyll '*a*'	Chlorophyll '*b*'	Total chlorophyll	Carotenoid	Chlorophyll '*a*'	Chlorophyll '*b*'	Total chlorophyll	Carotenoid
Control	0.649	0.592	1.241	0.360	0.631	0.572	1.203	0.344
5	0.621	0.559	1.180	0.341	0.587	0.535	1.122	0.327
	(-4.314)	(-5.574)	(-4.915)	(-5.278)	(-6.973)	(-6.469)	(-6.733)	(-4.942)
10	0.601	0.540	1.141	0.334	0.578	0.510	1.088	0.316
	(-7.396)	(-8.784)	(-8.058)	(-7.222)	(-8.399)	(-10.839)	(-92.685)	(-8.140)
25	0.564	0.496	1.060	0.315	0.530	0.461	0.991	0.294
	(-13.097)	(-16.216)	(-14.585)	(-12.500)	(-16.006)	(-19.406)	(-17.623)	(-14.535)
50	0.526	0.450	0.976	0.293	0.495	0.413	0.908	0.265
	(-18.952)	(-23.986)	(-21.354)	(-18.611)	(-21.553)	(-27.797)	(-24.522)	(-22.965)
100	0.478	0.402	0.880	0.265	0.442	0.366	0.808	0.239
	(-26.348)	(-32.095)	-(29.089)	(-26.389)	(-29.952)	(-36.014)	(-32.835)	-(30.523)
150	0.430	0.351	0.781	0.239	0.391	0.312	0.703	0.211
	(-33.744)	(-40.709)	(-37.067)	(-33.611)	-(38.035)	(-45.455)	(-41.563)	(-38.663)
200	0.367	0.290	0.657	0.206	0.336	0.264	0.600	0.186
	(-43.451)	(-51.014)	(-47.059)	(-42.778)	(-46.751)	(-53.846)	(-50.125)	(-45.930)

	F1	F2	F3	F4
F- Test value for the variance between the varieties	834.3**	970.1**	2.270**	417.6**
F-test value for the variance between the treatment	155.9**	158.4**	1.711**	124.27**

** Significant at 1 per cent level.
Percentage of reduction over control values are given in parentheses.

RESULTS

Photosynthetic pigments

The effect of different concentrations of copper on the photosynthetic pigments contents of Cowpea seedlings are represented in Table- 1. The highest chlorophyll 'a', chlorophyll 'b', total chlorophyll (viz., (0.649, 0.592, 1.241) and carotenoids (0.360) contents were recorded at 0 mgl^{-1} concentration in the CO-7 variety. The lowest values of chlorophyll á', chlorophyll 'b', total chlorophyll (viz., 0.336, 0.264, 0.600) and carotenoids (0.186) contents were recorded at 200 mg^{-1} concentration in the CO-6 variety. The highest percentage of reduction over control value was observed (46.751%, 53.846%, 50.125% and 45.930%) at 200 mgl^{-1} concentration in CO-6 variety of chlorophyll 'a',chlorophyll 'b', total chlorophyll and carotenoid contents respectively. The lowest percentage of reduction over control value was observed (4.314%, 5.574%, 4.915% and 5.278%) at 5 mgl^{-1} concentration in CO-7 variety for chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content respectively. The F-test value calculated for the variance between varieties and between treatment were found to be highly significant.

Total sugars

Changes in total sugar content of the root, stem and leaf of Cowpea seedlings due to copper treatment are represented in Table – 2. The highest total sugar content of the root, stem and leaf (viz., 3.015, 3.869, 4.636) was recorded at 0 mgl^{-1} in CO-7 variety. The lower content of sugar in root, stem and leaf (*viz*., 1.284, 1.959, 2.598) in CO-6 variety at 200 mgl^{-1} concentration. The highest reduction over control value was observed (54.757%, 45.080%, 40.847%) in CO-6 variety at 200 mgl^{-1} concentration*viz*., for sugar content of root, stem and leaf. The lowest percentage of reduction over control value was observed (5.141%, 6.384%, 3.430%) at 5 mgl^{-1} in CO-7 for root stem and leaf sugar content respectively. The F-test values calculated for the variance between the varieties and between the treatments were highly significant.

Amino acids

Amino acids content of the root, stem and leaf Cowpea seedling are represented in Table- 3. The highest amino acid content of the root, stem and leaf were recorded at 0 mgl^{-1} (6.082, 6.984 and 8.071) of CO-7 variety. The lowest amino acid content was recorded at 200 mgl^{-1} (3.079, 3.835 and 4.889) of CO-6 variety of copper treatment. The highest percentage of reduction over control value (47.412%, 42.957 and 38.043%) was observed for root, stem and leaf of co-6 variety at 200 mgl^{-1}. The lowest percentage reduction over control values (2.581%, 2.806% and 0.991%) was observed for root, stem and leaf of CO-7 variety at 5 mgl^{-1} concentration.

Table 2.Effect of different concentrations of copper on total sugar contents (mg g^{-1} fresh weight) of cowpea (*Vignaunguiculata*(L.)Walp) seedlings

Concentration in mgl¬	CO-7			CO-6		
	Root	Stem	Leaf	Root	Stem	Leaf
Control	3.015	3.869	4.636	2.838	3.567	4.392
5	2.860	3.622	4.477	2.681	3.331	4.265
	(-5.141)	(-6.384)	(-3.430)	(-5.532)	(-6.616)	(-2.892)
10	2.771	3.501	4.394	2.535	3.290	4.113
	(-8.093)	(-100.000)	(-5.220)	(-10.677)	(-7.766)	(-6.352)
25	2.608	3.471	4.116	2.351	3.106	3.905
	(-13.499)	(-10.287)	(-11.217)	(-17.160)	(-12.924)	(-11.088)
50	2.383	3.277	3.947	2.120	2.868	3.669
	(-20.962)	(-15.301)	(-14.862)	(-25.300)	(-19.596)	(-16.462)
100	2.145	3.032	3.714	1.855	2.590	3.341
	(-28.856)	(-21.633)	(-19.888)	(-34.637)	(-27.390)	(-23.930)
150	1.860	2.694	3.373	1.590	2.270	3.014
	(-38.308)	(-30.370)	(-27.243)	(-43.975)	(-36.361)	(-31.375)
200	1.583	2.308	2.981	1.284	1.959	2.598
	(-47.496)	(-40.346)	(-35.699)	(-54.757)	(-45.080)	(-40.847)

F- Test value for the variance between the varieties	514.9**	189.4**	292.01**
F-test value for the variance between the treatment	225.4**	159.6**	137.36**

** Significant at 1 per cent level.

Percentage of reduction over control values are given in parentheses.

Table 3.Effects of different concentrations of copper on amino acids content (mg g^{-1} fresh weight) of cowpea (*Vignaunguiculata*(L.)Walp) seedlings

Concentration in mgl¬	CO-7			CO-6		
	Root	Stem	Leaf	Root	Stem	Leaf
Control	6.082	6.984	8.071	5.855	6.723	7.891
5	5.925	6.788	7.991	5.711	6.541	7.602
	(-2.581)	(-2.806)	(-0.991)	(-2.459)	(-2.707)	(-3.662)
10	5.713	6.576	7.698	5.409	6.233	7.370
	(-6.067)	(-5.842)	(-4.621)	(-7.617)	(-7.288)	(-6.602)
25	5.356	6.251	7.332	5.067	5.895	7.057
	(-11.937)	(-10.495)	(-9.156)	(-13.459)	(-12.316)	(-10.569)
50	4.868	5.787	6.884	4.602	5.458	6.507
	(-19.961)	(-17.139)	(-14.707)	(-21.401)	(-18.816)	(-17.539)
100	4.432	5.342	6.392	4.111	4.901	5.990
	(-27.129)	(-23.511)	(-20.803)	(-29.787)	(-27.101)	(-24.091)
150	4.005	4.792	5.862	3.573	4.380	5.440
	(-34.150)	(-31.386)	(-27.370)	(-38.975)	(-34.851)	(-31.061)
200	3.588	4.289	5.301	3.079	3.835	4.889
	(-41.006)	(-38.588)	(-34.320)	(-47.412)	(-42.957)	(-38.043)

	F1	F2	F3
F- Test value for the variance between the varieties	365.01**	685.2**	627.6**
F-test value for the variance between the treatment	79.456**	169.3**	138.6**

** Significant at 1 percent level.

Percentage of reduction over control values are given in parentheses.

Table 4.Changes in protein content (mg g^{-1}fresh weight) of cowpea (*Vignaunguiculata*(L.)Walp) seedlings treated with different concentration of copper

Concentration in mgl¬	CO-7			CO-6		
	Root	Stem	Leaf	Root	Stem	Leaf
Control	14.282	20.779	25.561	12.164	17.366	22.820
5	14.015	20.577	25.123	11.864	17.021	22.373
	(-1.869)	(-0.972)	(-1.714)	(-2.466)	(-1.987)	(-1.959)
10	13.424	20.017	24.706	11.330	16.339	21.810
	(-6.008)	(-3.667)	(-3.345)	(-6.856)	(-5.914)	(-4.426)
25	12.611	18.872	23.585	10.626	15.636	20.722
	(-11.700)	(-9.178)	(-7.731)	(-12.644)	(-9.962)	(-9.194)
50	11.848	18.003	22.464	9.837	14.690	19.833
	(-17.042)	(-13.360)	(-12.116)	(-19.130)	(-15.409)	(-13.089)
100	10.850	16.663	21.195	8.802	13.588	18.414
	(-24.030)	(-19.808)	(-17.081)	(-27.639)	(-21.755)	(-19.308)
150	9.789	15.204	19.543	7.848	2.299	16.984
	(-31.459)	(-26.830)	(-23.544)	(-35.482)	(-86.761)	(-25.574)
200	8.830	14.108	18.051	6.763	11.002	15.064
	(-38.174)	(-32.105)	(-29.381)	(-44.402)	(-36.646)	(-33.988)

	F1	F2	F3
F- Test value for the variance between the varieties	3159.0**	4.401**	1543.3**
F-test value for the variance between the treatment	6803.7**	14.33**	3160.8**

** Significant at 1 per cent level.

Percentage of reduction over control values are given in parentheses.

Table 5.Effect of different concentrations of copper on yield parameter of cowpea cultivars(*Vignaunguiculata*(L.)Walp)

Concentration in mgl⁻¹	CO-7					CO-6				
	Seed output	Shelling percentage	Weight 100 seeds(g)	Pod yield (kg/ha-1)	Harvest index	Speed output	Shelling percentage	Weight 100 seeds(g)	Pod yield (kg/ha-1)	Harvest index
Control	70	97	56.32	2108	0.55	67	88	49.316	2017	0.53
5	65 (-7.143)	91 (-6.186)	55.145 (-2.086)	2039 (-3.273)	0.53 (-3.636)	62 (-7.463)	82 (-6.818)	45.168 (-8.411)	1901.1 (-5.746)	0.50 (-5.660)
10	61 (-12.857)	87 (-10.309)	53.851 (-4.384)	1963.1 (-6.874)	0.51 (-7.273)	58 (-13.433)	78 (-11.364)	45.205 (-8.336)	1799.6 (-10.778)	0.47 (-11.321)
25	58 (-17.143)	79 (-18.557)	50.672 (-10.028)	1820 (-13.662)	0.48 (-12.727)	54 (-19.403)	70 (-20.455)	37.215 (-24.538)	1721.8 (-14.636)	0.45 (-15.094)
50	41 (-41.429)	73 (-24.742)	48.751 (-13.439)	1751.5 (-16.912)	0.45 (-18.182)	39 (-41.791)	64 (-27.273)	35.742 (-27.525)	1623.3 (-19.519)	0.40 (-24.528)
100	37 (-47.143)	68 (-29.897)	46.214 (-17.944)	1641.3 (-22.139)	0.42 (-23.636)	35 (-47.761)	58 (-34.091)	33.516 (-32.038)	1501.2 (-25.573)	0.31 (-41.509)
150	33 (-52.857)	59 (-39.175)	44.12 (-21.662)	1522.5 (-27.775)	0.39 (-29.091)	31 (-53.731)	50 (-43.182)	31.831 (-35.455)	1435.5 (-28.830)	0.26 (-50.943)
200	28 (-60.000)	55 (-43.299)	41.055 (-27.104)	1367 (-35.152)	0.34 (-38.182)	24 (-64.179)	46 (-47.727)	27.942 (-43.341)	1185 (-41.249)	0.23 (-56.604)

	F1	F2	F3	F4	F5
F- Test value for the variance between the varieties	1480.1**	7347.2**	28.54**	232.8**	17.74**
F-test value for the variance between the treatment	94.9**	5329.0**	173.4**	110.6**	17.39**

** Significant at 1 per cent level. Percentage of reduction over control values are given in parentheses.

The F-test values calculated for the variance between the varieties and between the treatments were highly significant.

Protein

The effect of different concentrations of copper on the protein content of the root, stem and leaf of Cowpea variety is in Table- 4. The highest protein content of Cowpea was higher in root (14.282), stem (20.779) and leaf (25.561) at 0 mgl^{-1} in the CO-7 variety. The lowest protein content of root (6.763), stem (11.002) and leaf (15.064) was observed in CO-6 variety at 200 mgl^{-1} copper concentration. The maximum percentage of reduction over control value was observed for root (44.402%), stem (36.646%) and leaf (33.988%) in CO-6 variety at 200 mgl^{-1} concentration. The minimum percentage of reduction over control value was observed for root (1.869%), stem (0.972%) and leaf (1.714%) at 5 mgl^{-1} concentration in CO-7 variety. The F-test values calculated for the variance between the varieties and between the treatments were significant at 1 percent level.

Harvest parameters

Table – 5 represents the harvest parameters of two varieties of Cowpea variety grown under copper treatment. Among the varieties tested, the highest seed output per plant (70), shelling percentage of pod (97), 100 seeds weight (56.32g), seed yield (2108 kgs ha^{-1}) and harvest index (0.55) were recorded at 0 mgl^{-1} variety CO-7. The variety CO-6 showed the lowest seed output per plant (24), shelling percentage of pod (46), 100 seeds weight (27.942 g), seed yield (1185 kgs ha^{-1}) and harvest index (0.23) were recorded at 200 mgl^{-1} variety of CO-6. The highest percentage of reduction over control value for seed output per plant (64.179%), shelling percentage of pod (47.727%), 100 seeds weight (43.341%), seed yield (41.249%) and harvest index (56.604%) were recorded at 200 mgl^{-1} of CO-6. The lowest percentage of reduction over control value of seed output per plant (7.143%), shelling percentage of pod (6.186%), 100 seeds weight (2.086%), seed yield (3.273%) and harvest index (3.636%) were recorded at 5 mgl^{-1} of CO-7. The F-values for the variance between the varietys and between the treatments were significant.

DISCUSSION

In higher concentrations, many heavy metals inhibit plants biochemical production and this has been extensively studied and reviewed (Sharma *et al*., 2010; Rastgoo and Alemzadeh, 2011 and Rastgoo*et al*., 2014). The various biochemical changes in Cowpea plant brought about by copper concentrations on the physiological effect of different constituents like pigments, total sugar, amino acids and protein have been analyzed.

Photosynthetic pigments

The photosynthetic pigments such as chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll and carotenoid contents of cowpea decreased with increasing copper level in the soil. Chlorophyll is an integral component of plant pigments and plays a vital role in the process of photosynthesis. Chlorophyll estimation is one of the important plant parameters which are used as an index of production capacity of the plant. Chlorophyll is an integral component of plant pigments and plays a vital role in the process of photosynthesis. Chlorophyll estimation is one of the important plant parameters which are used as an index of production capacity of the plant.The chlorophyll ‘a’, ‘b’ and total chlorophyll and carotenoid contents of seedlings were significantly affected by copper treatment. They showed a gradual decline with increase in copper concentrations. Similar results were noted in the earlier studies of Balashouri and Prameeladevi (1995), Bonnet *et al*. (2000), Sharma *et al*. (2009) and Umebese and Motajo (2008) in various crops. The results of the present study also confirmed these views. The decrease in chlorophyll content may be due to destabilization and degradation of proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll content in most plants under chromium stress was reported (Shanker*et al*.,2005).

Total sugar

Sugars content of Cowpea plants showed a decreasing trend with progressive increase in copper level in the soil. The inhibition of sugar content was higher with progressive increase in copper concentrations. The 200 mgl^{-1}copper treatment showed adverse effect on sugar at higher concentrations. It may be due to imbalance in nitrogen uptake and assimilation and which might eventually led to depletion of carbohydrate (Murata *et al*., 1969).

Amino acids and protein

In the present study significantly reduced the amino acid and protein contents in leaves of cowpea plants. Amino acid transport is thought to be of primary importance in organic nitrogen acquisition, and might be involved in processes such as direct uptake of amino acids from the soil, phloem and xylem loading, phloem to xylem exchange, and retrieval of amino acids that "leak" from the cells. Soluble protein is an important indicator of reversible and irreversible changes in metabolism, and it is known to respond to a wide variety of stress or such as natural and xenobiotic (Singh and Tewari, 2003).Similar observation were observed the reductions in amino acids and protein content due to cadmium treatments were registered by Costa and Moral (1994) in *Lettus*, Dinakar*et al*. (2008) in *Arachishypogaea,* Rolli*et al*. (2011) in green gram, Merve (2013) in soybean (*Glycine max*) and Mayahi (2014) in *Phoenix dactylifera*.Free amino acids and other nitrogenous compounds due to the exposure to heavy metals may represent a metal-detoxification mechanism (Callahan *et al*., 2007).

Yield components

In the present study, the various yield parameters like number of pods, seed output, shelling percentage, hundred seed weight, pod yield and harvest index varied significantly between cultivars and copper concentrations. When the plants were treated with copper, much variation was recorded among the varieties studied. Similar results were obtained by several authors in various plants such as *Vignaungiculata* (James and Sharavanan, 2010) due to chromium, *Oryza sativa* (Vijayarengan, 2012) due to cadmium and *Cymopsistetragonolaba* (Vijayarengan, 2013) due to zinc. Agarwal and Mishra (2008) also suggested that the reduction in yield is a typical index of sensitivity of plants to various stresses, as it represents the cumulative effects of damaged or inhibited physiological function.

Conclusion

Application of Cu in excess may induce the deficiency of biochemical and yield. Hence, judicious and adequate amendment of Cu can contribute to a great deal in enhancing the yield of Cowpea crop, especially in Cu-responsive alkaline alluvial soils of India. The present investigation reveals that the various concentration of copper has drastic effects on biochemical and yield parameters. The growth of the crop plants has been highly reduced at higher concentrations. It can be concluded that the CO 7 variety of Cowpea was proved to be tolerant and CO 6 varieties was sensitive to copper treatment. Hence, the variety CO 7 can be cultivated in the soil irrigated with industrial effluent and also in contaminated area.

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