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The Potential Role of Cu^{2+} and Combined Action with IAA on Tolerance Strategy of Two Broad Bean Cultivars

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Abstract

The present work was conducting to study the strategy response of two broad bean cultivars Assiut 84 and Assiut 125 to different Cu^{2+} concentrations 100 ppm, 200 ppm, 300 ppm and 350 ppm in addition to control and interactions with IAA treatments. The dry matter exhibited the biphasic effect of Cu^{2+} on the growth criteria of the two broad bean cultivars. While the lower and moderate doses of Cu^{2+} (100 ppm and 200 ppm) stimulated the growth of the two cultivars, the higher doses revealed the opposite event where the growth dropped in both cultivars. This effect was more pronounced in cv. Assiut 84 than in cv. Assiut 125 and also at the higher Cu^{2+} concentration the growth dropped slightly in cv. Assiut 84 and highly significantly in cv. Assiut 125. The percent of increase in dry matter at 200 ppm in stem and leaf of cv. Assiut 84 was 120.45% and 155.31%, otherwise this percent of increase in these organs of cv. Assiut 125 was 114.29% and 131.41%. However the percent of reduction at 350 ppm Cu^{2+} in root and stem of cv. Assiut 84 was 74.13%, 79.23% and in root, stem and leaf of cv. Assiut 125 was 59.27%, 70.91%, 70.76% compared with control plants. Soluble carbohydrate in cv. Assiut 84 and cv. Assiut 125 was markedly increased while soluble protein was decreased in root, stem and in leaves at lower Cu^{2+} concentration. Also while Cu^{2+}-stressed cv. Assiut 84 maintained potassium and magnesium levels around the control values and some promotion occurred especially in roots and stems, these cations dropped markedly in cv. Assiut 125 as a result of Cu^{2+} treatments. While Cu^{2+} had a marked stimulatory effect in the absorption and accumulation of calcium in the different organs of cv. Assiut 84, it, on the other hand, significantly inhibited the accumulation of this cation in the different organs of cv. Assiut 125. Treatments broad bean cultivars with Cu^{2+} plus IAA induced an increase in growth parameters, soluble suga, soluble protein, K^+, Ca^{++} and Mg^{++} in different parts of two tested cultivars. The uptake, translocation and distribution of mineral ions are affected by various growth regulators among others by IAA. This strategy might be important in heavy metals tolerance mechanisms of crop plants.

Keywords

Broad Bean Cultivars, Sensitive, Tolerant, Copper, IAA

1. Introduction

Heavy metals as Cu, Fe, Mn, Zn, Ni and AS include elements with densities above 5 $g \cdot cm^{-3}$, but the term was extended to a vast range of metals and metalloids [1] [2] [3]. High contents of trace elements have been found in numerous soils, which may become toxic for plants and other components of terrestrial biota [4] [5] [6]. Essential micronutrients are required in low contents to develop plant normally, but are toxic in high contents [5] [7] [8]. These can lead to physiological alterations, which are widely documented [9] [10]. Cu^{++} plays an essential role in cell wall metabolism, signaling to the transcription and protein trafficking machinery, oxidative phosphorylation, iron mobilization and the biogenesis of molybdenum cofactor [11] [12] [13]. On the other side, Cu interferes in several physiological processes and therefore, potentially inhibits plant growth, resulting in a decrease in performance, delays in leaf and root growth, as well as anatomical and ultra structural alterations that often lead to the formation and accumulation of reactive oxygen species (ROS) [14] [15]. Neto *et al.* (2017) discuss the role of plant growth hormones abscisic acid, auxin, brassinosteroid and eythylene in signaling pathways, defense mechanisms and alleviation of heavy metal toxicity. Ochao *et al.* (2018) [16] try to evaluate the nutritional components in seeds of green pea 9 *Pisum sativum* cultivated in soil amended with nCuO at 50 or 100 mg/kg with 100 μM IAA. Thus, the present work was conducted to compare the Cu^{2+} tolerance of the two selected broad bean cultivars Assiut 84 and Assiut 125 and ameliorating effect of IAA during vegetative growth of two broad bean cultivars.

2. Materials and Methods

2.1. Experimental Sites and Cu^{2+} Treatments

Broad bean seeds cv. Assiut 84 and Assiut 125 were obtained from one of the active breading programs directed by Prof. Dr. Esmat Waly and Prof. Dr. Saeyd Abdellah, Faculty of Agriculture, Assiut University, Egypt. Broad bean plant is important economic crop plants and consider the first plant food for Egyptian people because it contains highly benefit protein and other essential elements for man healthy, which has several common names (broad bean, fava bean, faba bean, horse bean, field bean, tic bean), is a species of bean (Fabaceae) native to

North Africa and southwest Asia and is extensively cultivated elsewhere. In much of the world, the name broad bean is used for the large-seeded cultivars grown for human food. In Egypt, faba beans are the most common fast food item in the Egyptian diet, eaten by rich and poor alike. Egyptians eat faba beans in various ways; the most popular way of preparing faba beans is taking cooked beans, mashing them and adding oil, lemon, salt and cumin. The prepared beans, called fulmedames, are then eaten with bread. Faba bean is an excellent source of protein (20% - 25%), calcium (0.15%), phosphorus (0.50%), lysine (1.5%) and methionine-cystine (0.5%) in dry weight. It is also an excellent source of complex carbohydrates, dietary fiber, choline, lecithin, minerals and secondary metabolites (phenolics and levo-dihydroxy-phenylalanine (L-DOPA), which is the precursor of the neurotransmitter dopamine and naturally found in seedlings, green pods and beans) [17] [18]. Broad bean seeds were surface sterilized by immersion in a mixture of ethanol 96% and H_2O_2 (1:1) for 3 minutes, followed by several washings with sterile distilled water. The concentrations of Cu^{2+} were chosen after preliminary experiments in which the seeds were subjected to different concentrations of Cu^{2+}. The chosen concentrations caused slight stimulation and moderate inhibition of germination. Cu^{2+} was added as Cu^{2+} sulphate ($CuSO_4$). Ten seeds were sown per pot. Each pot contained 3.6 kg of garden clay soil. The clay soil comprise four components minerals and soil organic matter make up the solid fraction, whereas air and water comprise the pore space fraction. A typical agricultural soil is usually around 50% solid particles and 50% pores (Adapted from Brady and Weil, 2002 [19]). Soil particle of clay is <0.002 invisible to naked eye. Considerations of working in controlled environments were followed by Tibbitts & Langhans (1993) [20]. All pots were irrigated with tap water for four weeks until full germination. In preliminary experiments explained that low concentration is 100 ppm $CuSo_4$ and the high concentration is 350 ppm. The seedlings were then irrigated by different concentrations of $CuSO_4$ solutions (0, 100 ppm, 200 ppm, 350 ppm) and were classified into two groups.

2.2. Cu^{2+} Treatment and Combined with IAA

From two of previous groups one was sprayed by 200 ppm IAA. In order to maintain the osmotic potential, the soil moisture content was kept near the field capacity using tap water. The seedlings were left to grow in natural conditions under these conditions for 150 days. At the end of the experimental period (5 months) yields of the different organs (roots, stems and leaves) were determined.

2.3. Laboratory Analysis for Metabolities

To determine the dry matter yields of roots, stems and leaves they were dried in an oven at 80˚C. Successive weighting was carried out until the constant dry weight of each sample was reached. The leaf area was measured using the disk method [21] and was expressed as cm^2/plant. The photosynthetic pigments, chlorophyll a, chlorophyll b and carotenoids, were determined using the spectrophotometric method recommended by Metzner *et al.* (1965) [22]. The soluble carbohydrates were determined by the method of anthronesulphoric acid which was stated by Fales (1951) [23]. The soluble proteins were determined according to the method adopted by Lowery *et al.* (1951) [24]. Calcium and magnesium determination by Schwarzenbach and Biedermann, 1948 [25] was employed. Potassium, Flamephotometer method using Carl Zeiss Flamephotometer was used by Williams and Twine, (1960) [26].

3. Statistical Analysis

The triplicate sets of the experimental data for the different tested parameters were subjected to the one way analysis of variances (ANOVA) test in accordance with the experimental design using the SPSS program, version 13.0 and the means were compared using the least significant differences, L.S.D. at P levels of 0.05 and 0.01 [27].

4. Results

In cv. Assiut 84 the dry matter of stems and leaves increased as Cu^{2+} increased in the soil up to 200 ppm, then while a slight stimulation was obtained in leaves (about 4%), a slight reduction was recorded in stems (20%) (**Table 1**). The dry matter of roots remained more or less unchanged up to 200 ppm Cu, then about 24% reduction was recorded, which means that the three plant organs responded differently to Cu^{2+} treatments. In cv. Assiut 125 the dry matter of stems and leaves increased by Cu^{2+} treatment up to 200 ppm, which was more pronounced in leaves, then a marked reduction was recorded (about 30% in both). In roots, a gradual reduction was exhibited by increasing the Cu^{2+} concentration in the soil (**Table 1**). This inhibitory effect was more obvious at the level of 350 ppm Cu^{++} (about 40% reduction). In cv. Assiut 125, an increase in leaf area was obtained up to the level of 100 ppm Cu^{++}, and then a highly significant reduction was obtained which was much more pronounced at the level of 350 ppm Cu^{++}. At this level, the percentage of reduction was about 30%, as compared with that of control (**Figure 1**). Leaf area in Assiut 84 was increased up to 200 ppm Cu^{++} treatment, then a reducion was recorded compared with uncupper application (**Figure 1**). Of special interest in this work is that the concentration of the photosynthetically active pigments in cv. Assiut 84 seemed to be increasing by the increasing concentration of Cu^{++} in the soil (**Figure 2(a)**). Consequently, the highest concentration of the different fractions of the photosynthetic pigments was registered at the highest dose of Cu^{++}. At this level, the percent of increase in chlorophyll a, chlorophyll b and carotenoids was 29.7%, 32.014% and 54.35% respectively when compared with the control values. It is also noticeable that the highest increase was obtained in the values of carotenoids when compared with

Table 1. Effect of $CuSO_4$ and $CuSO_4$ plus IAA on the dry matter yield (g. plant^{-1}) of the different organs of the broad bean cultivars Assiut 84 and Assiut 125.

Teatment	$CuSO_4$ (ppm)	Root	%	Stem	%	Leaves	%
Cv. Assiut 84	0	0.43	100	1.57	100	0.62	100
	100	0.39	90.53	1.65	105.10	0.78 *	125.305
$CuSO_4$	200	0.38	87.07	1.89	120.45	0.97**	155.305
	350	0.32	74.13	1.24	79.23	0.65	104.50
	0	0.49	112.01	2.03	129.78	1.10**	177.33
	100	0.55	126.1	2.44**	155.66	1.55**	249.36
$CuSO_4$ + IAA	200	0.69**	158.89	2.49**	158.85	1.52**	244.70
	350	0.38	88.68	1.93	123.45	1.29**	208.36
L.S.D. 0.05%		0.12		0.43		0.26	
Cv. Assiut 125	0	0.63	100	1.41	100	0.55	100
$CuSO_4$	100	0.49**	79.71	1.53	108.89	0.67	120.34
	200	0.41**	65.18	1.61	114.29	0.73	131.41
	350	0.37**	59.27	0.99*	70.91	0.39	70.76
$CuSO_4$ + IAA	0	0.61	96.65	1.89**	134.07	1.27**	229.78
	100	0.64	102.72	2.02**	143.39	1.32**	238.45
	200	0.53*	84.51	2.09**	148.36	1.22**	220.76
	350	0.51*	81.95	1.92**	136.34	0.72	129.24
L. S. D. 0.05%		0.08		0.27		0.37	

**Highly significance compared with control plants.

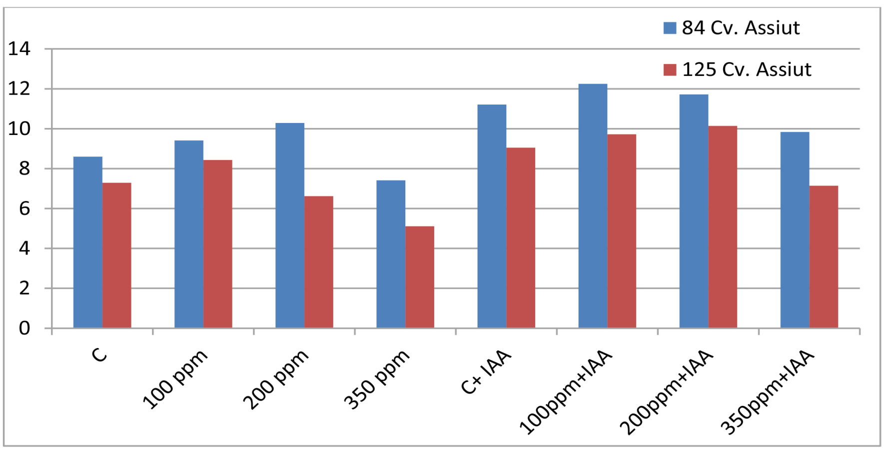
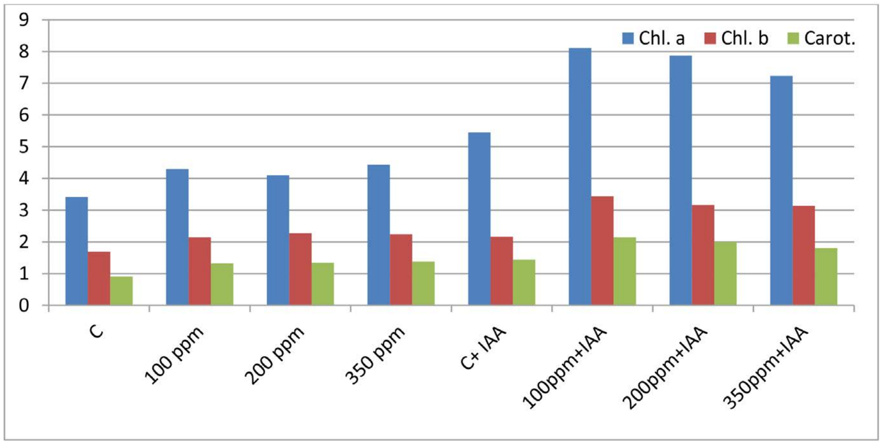
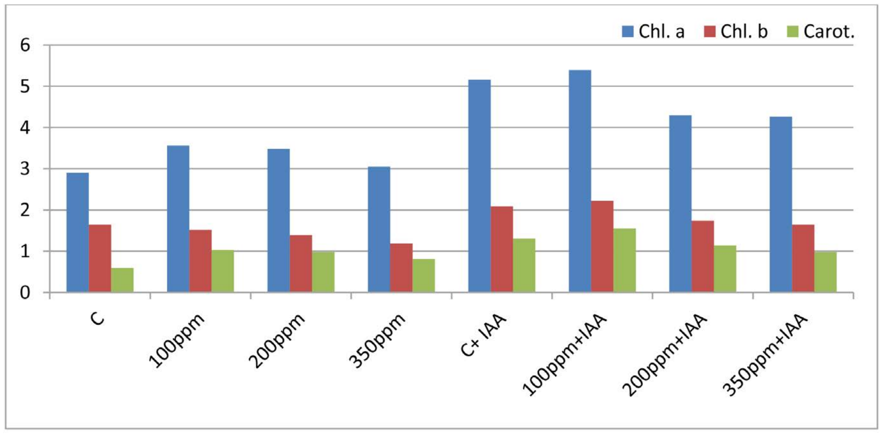


Figure 1. The effect of $CuSO_4$ and $CuSO_4$ plus IAA treatments on the leaf area of the two broad bean cultivars Assiut 84 and Assiut 125 (Cm^2/leaf). L.S.D. 0.05%: 0.46 in cv. Assiut 84 and 0. 0.37 in cv. Assiut 125.

chlorophyll a and chlorophyll b whatever the concentration of Cu^{++} used. The data of cv. Assiut 125 exhibited that an increase in chlorophyll a was reported as a result of Cu^{2+} treatment. There is a gradual dropping off in chlorophyll b



(a)



(b)

Figure 2. Effect of $CuSO_4$ and $CuSO_4$ plus IAA on chlorophyll a, chlorophyll b and carotenoids contents (mg. g.$^{-1}$ d. m.) in leaves of the broad bean cultivars 84 a and cv. Assiut 125 b. (a) L.S.D. 0.05%: Chl. a, 0.27 Chl. b, 0.14 Carot., 0.27; (b) L. S. D. 0.05%: Chl. a, 0.17, Chl. b, 0.23 Carot., 0.08.

content by increasing the dose of Cu^{++} in the soil (**Figure 2(b)**). At the level of 350 ppm Cu^{++} the chlorophyll b content decreased to about 70%. On the other hand, there is an unexpected increase in carotenoids at all the levels of Cu^{++}. The percent of increase in carotenoids fluctuated from 36% to 73% over those of control values. In roots, stems and leaves of cv. Assiut 84 the production of the soluble carbohydrates was stimulated by the various levels of Cu^{2+} (**Table 2**). Soluble carbohydrate in cv. Assiut 84 was markedlys increased in root, stem and in leaves at lower Cu^{++} concentration (100 ppm Cu^{++}, the percent of increase was 142.09% compared with untreated plant). It is worthy to note that the high value was recorded at 200 ppm in root and stem, the percent of accumulation in soluble carbohydrate was 133.33% and 143.35% in root and stem (**Table 2**). Soluble carbohydrate was accumulated with elevating Cu^{++} level in cv. Assiut 125, the high value was recorded at 200 ppm Cu^{++} level in root and stem, the percent of increase was 210.68% and 130.14% (**Table 2**). While a reduction was exhibited in leaf organ, the percent of reduction at 350 ppm Cu^{++} level was 65.75% compared

Table 2. The effect of $CuSO_4$ and $CuSO_4$ plus IAA treatments on soluble sugar contents (mg. g.$^{-1}$ d. m.) in the roots, stem and leaves of the broad bean cultivars Assiut 84 and Assiut 125.

Treatment	$CuSO_4$ (ppm)	Soluble sugar					
Cv. Assiut 84		Root	%	Stem	%	Leaves	%
$CuSO_4$	0	10.09	100	11.19	100	20.44	100
	100	12.35**	122.43	12.23*	109.25	29.05**	142.09
	200	13.45**	133.33	16.04**	143.35	21.02	102.85
	350	11.13**	110.25	13.13**	117.35	15.788*	77.22
$CuSO_4$ + IAA	0	8.54**	84.61	8.86**	79.20	10.74**	52.53
	100	8.60**	85.25	9.77**	87.28	13.07**	63.92
	200	11.97**	118.58	9.83*	87.86	15.01**	73.42
	350	11.26**	111.54	13.97**	124.86	16.95**	82.91
L. S. D. 0.05%		0.59		0.82		0.97	
Cv. Assiut 125	0	4.85	100	5.37	100	8.73	100
$CuSO_4$	100	6.86*	141.33	6.79*	126.53	7.96	91.11
	200	10.22**	210.68	6.99*	130.14	6.99**	80.01
	350	4.33	89.34	6.66	124.11	5.74**	65.75
$CuSO_4$ + IAA	0	10.67**	220.02	11.64**	216.90	14.04**	160.74
	100	12.16**	250.67	11.26**	209.67	16.30**	186.67
	200	12.03**	248.01	12.35**	230.14	14.36**	164.45
	350	7.44*	153.35	9.19**	171.11	9.25	105.93
L. S. D. 0.05%		1.59		1.09		0.82	

**Highly significance compared with control plants.

with uncoper treatments (Table 2). Soluble protein in cv. Assiut 84 and cv. Assiut 125 was significantly decreased as increasing Cu^{++} concentration in three different organs, this effect was pronounced in root organs than stem and leaf (Table 3). The percent of reduction at 350 ppm Cu^{++} level was 46.63%, 78.2%, and 58.11% in root, stem and leaf whereas in cv. Assiut 125 this percent was 66.28%, 85.53% in root and stem. Except of this trend soluble protein in leaf of cv. Assiut 125 was markedly accumulated, the percent of increase at 350 ppm Cu^{2+} level was 180% compared with uncopper treatment (Table 3). In cv. Assiut 84 a slight increase in potassium content in roots and stems was exhibited by Cu^{2+} treatment, while in leaves a slight reduction was recorded (Table 4). This reduction did not exceed 18% at the level of 350 ppm. In cv. Asiut 125 potassium in roots, stems and leaves decreased gradually by increasing the Cu^{2+} concentration in the soil (Table 4). This inhibitory effect was more pronounced at the highest dose of Cu^{2+} and in stems than in roots or leaves. Potassium content in leaves seemed to be less affected by copper treatments. At the level of 350 ppm

Table 3. The effect of $CuSO_4$ and $CuSO_4$ plus IAA treatments on soluble proteins contents (mg. g.$^{-1}$ d. m.) in the roots, stem and leaves of the broad bean cultivars Assiut 84 and Assiut 125.

Treat.	$CuSO_4$ (ppm)	Soluble protein					
Cv. Assiut 84		Root	%	Stem	%	Leaves	%
$CuSO_4$	0	60.82	100	49.63	100	116.79	100
	100	34.33**	56.44	45.15**	90.98	117.54	100.64
	200	32.09**	52.76	43.66**	87.97	76.12**	65.18
	350	28.36**	46.63	38.81**	78.20	69.03**	59.11
$CuSO_4$ + IAA	0	93.27**	153.35	94.04**	189.49	170.96**	146.38
	100	96.54**	158.73	115.77**	233.28	199.04**	170.42
	200	101.54**	166.95	108.85**	219.33	209.62**	179.48
	350	99.23**	163.15	49.63	210.80	200.77**	171.91
L. S. D. 0.05%		1.32		2.55		2.02	
Cv. Assiut 125	0	32.09	100	28.36	100	78.73	100
$CuSO_4$	100	20.15**	62.79	35.08**	123.69	85.08**	108.06
	200	23.13**	72.09	25.00**	88.16	87.31**	110.90
	350	21.27**	66.28	24.25**	85.53	142.31**	180.75
$CuSO_4$ + IAA	0	80.19**	249.89	106.54**	375.69	190.58**	242.06
	100	77.12**	240.31	110.00**	387.90	172.89**	219.59
	200	74.04**	230.72	82.31**	290.24	151.15**	191.99
	350	73.46**	228.93	75.00**	264.48	158.08**	200.78
L. S. D. 0.05%		1.26		1.79		0.78	

**Highly significance compared with control plants.

Cu^{++} the percent reduction in potassium content in roots, stems and leaves was 29.1%, 45.4% and 16.1% respectively, which indicated that stems were more responding to Cu^{2+} while leaves were the least responding, roots were intermediate. The data reveal that the Cu^{2+} treatments enhanced the calcium accumulation in roots, stems and leaves of cv. Assiut 84 (**Table 5**). This stimulatory effect on the calcium content was more pronounced in roots than in the other two plant organs. At the level of 350 ppm the percent of increase was 60%, 27.8% and 26.7% in roots, stems and leaves respectively. The data reveal that treatment with Cu^{2+} retarded the transport and accumulation of calcium in the different organs of the broad bean cultivar Assiut 125 (**Table 5**). This inhibitory effect was much more pronounced in roots than in stems or leaves, especially at the lower doses of Cu^{2+}. However, the harmful effect of the highest dose of Cu^{2+} on calcium transport seemed to be more or less similar in the three plant organs. At the level of 350 ppm Cu^{++} the calcium content in roots, stems and leaves was 57.14%, 66.67% and 63.16% respectively. In cv. Assiut 84 the data revealed that Cu^{2+} up to the level of 100 ppm induced insignificant changes in magnesium

Table 4. Effect of $CuSO_4$ and $CuSO_4$ plus IAA treatments on potassium content (mg. $g.^{-1}$ d. m.) in the different organs of the broad bean cultivar Assiut 84 and Assiut 125.

Treatment	$CuSO_4$ (ppm)	Root	%	Stem	%	Leaves	%
	0	5.3	100	12.2	100	21	100
	100	5.4	101.89	12.9**	105.74	19.9**	94.76
$CuSO_4$	200	5.6	105.66	13.2**	108.20	18.7**	89.05
	350	5.6	105.66	12.6**	103.28	18.1**	86.19
	0	5.1	96.23	12.1	99.18	20.5	97.62
	100	6.4**	120.76	13.9**	113.93	23.1**	104.79
$CuSO_4$ + IAA	200	7.0**	132.08	17.6**	144.26	22.1**	105.24
	350	6.8**	128.30	15.3**	125.41	21	100
L. S. D. 0.05%		0.32		0.16		0.42	
Cv. Assiut 125	0	6.2	100	13	100	20.6	100
	100	5.8**	93.55	10**	76.92	18.8**	91.26
$CuSO_4$	200	5.1**	82.26	8.9**	68.46	17.8**	86.41
	350	4.4**	70.97	7.1**	54.62	17.3**	83.98
	0	5**	80.65	12.3**	94.62	19**	92.23
	100	5.**1	82.26	13.2*	101.54	22.2**	107.77
$CuSO_4$ + IAA	200	6.4	103.23	14.4**	110.77	19.6**	95.15
	350	5.8**	93.55	12.8*	98.46	17.8**	86.41
L. S. D. 0.05%	0.05	0.21		0.14		0.16	

**Highly significance compared with control plants.

in roots (100% of control), then a slight reduction was obtained (Table 6). This reduction was consistent at the levels from 200 ppm to 350 ppm Cu^{++} (about 15%). In stems, unexpected promotion in magnesium content was recorded. It was 134.04%, 134.04% and 210.64% of the control at the levels of 100 ppm, 200 ppm and 350 ppm Cu^{++} respectively. In leaves, magnesium contents remained mostly around those of control plants even at the highest dose of copper. In cv. Assiut 125 the data exhibited that the level of 100 ppm Cu^{++} induced mostly insignificant changes in magnesium content in the three plant organs, then a gradual reduction was reported by the further increase in Cu^{2+} (Table 6). The percent of this reduction varied among the three tested plant organs. At the level of 350 ppm Cu^{++}, the percent of reduction was 35.3%, 46.3% and 55.2% in roots, stems and leaves respectively.

Interaction of Cu^{2+} with IAA

A remarked and progressive accumulation in dry matter yields was exhibited when the Cu^{++}-treated plants were sprayed by 200 ppm IAA (Table 1). This stimulatory effect was much more pronounced in stems and leaves than in roots of two tested cultivars. The leaf area of cv. Assiut 84 increased from 0 to 350 ppm

Table 5. Effect of $CuSO_4$ and $CuSO_4$ plus IAA treatments on calcium content (mg. g.$^{-1}$ d. m.) in the different organs of the broad bean cultivars Assiut 84 and Assiut 125.

	$CuSO_4$ (ppm)	Root	%	Stem	%	Leaves	%
Cv. Assiut 84	0	3.75	100	4.5	100	11.25	100
	100	7.5**	200	5.25**	116.67	18.75**	166.67
$CuSO_4$	200	6**	160	6.25**	138.89	15.25**	135.56
	350	6**	160	5.75**	127.78	14.25**	126.67
	0	4.9**	130.67	7.1**	157.78	11.25	100
$CuSO_4$ + IAA	100	6**	160	6.75**	150	12.75**	113.33
	200	6.6**	176	6.2**	137.78	15.0**	133.33
	350	6.75**	180	6.5**	144.44	18.0**	160
L. S. D. 0.05%		0.16		0.19		0.24	
Cv. Assiut 125	0	5.25	100	4.5	100	14.25	100
	100	3.75**	71.43	3.75**	83.33	12.0**	84.21
$CuSO_4$	200	3.0**	57.14	3.75**	83.33	12.0**	84.21
	350	3.0**	57.14	3.0**	66.67	9.0**	63.16
	0	4.5**	85.71	4.5	100	13.5**	94.74
$CuSO_4$ + IAA	100	4.5**	85.71	6.75**	150	16.5**	115.79
	200	5.25	100	6**	133.33	12.75**	89.47
	350	4.75**	90.48	4.5	100	12**	84.21
L. S. D. 0.05%		0.17		0.19		0.25	

**Highly significance compared with control plants.

Cu^{++}. Then a slight reduction was exhibited only at the highest dose of Cu^{++} (about 15%). A marked and progressive increase in leaf area was exhibited as a result of IAA treatment in cv. Assiut 84 (**Figure 1**). Also, the slight reduction in leaf area at the level of 350 ppm Cu^{++} was completely eliminated by IAA treatment. Moreover, the leaf area increased by 14.42% as a result of 350 ppm Cu^{++} and IAA treatments. Hormonal treatments completely alleviated the drastic effect of the higher doses of Cu^{++} on the leaf area of cv. Assiut 125. Again, the data of leaf area indicated the superiority of cv. Assiut 84 (**Figure 1**). A marked and progressive additional increase in these values was exhibited as a result of IAA treatments, whatever the level of Cu^{++} used and the fraction of the photosynthetic pigment analyzed in cv. Assiut 84 (**Figure 2(a)**). Interestingly, the contents of chlorophyll a, chlorophyll b and carotenoids was about 2-fold those of control as a result of 350 ppm Cu^{++} plus IAA. A marked and progressive accumulation in the photosynthetically active pigments was obtained when the Cu^{2+}-affected plants were sprayed by IAA in cv. Assiut 125 (**Figure 2(b)**). This promoting effect was more pronounced in carotenoids and chlorophyll a than in chlorophyll b at most Cu^{2+} levels. At the level of 350 ppm Cu^{++} plus IAA the percent of increase in chlorophyll a, chlorophyll b and carotenoids was 46.9%, 0.0% and 66.1%, respectively. In roots and stems of cv. Assiut 84 the

Table 6. Effect of $CuSO_4$ and $CuSO_4$ plus IAA treatments on magnesium content (mg. $g.^{-1}$ d. m.) in the different organs of the broad bean cultivars Assiut 84 and Assiut 125.

	Treatment	Root	%	Stem	%	Leaves	%
Cv. Assiut 84	0	5.4	100	2.35	100	7.2	100
$CuSO_4$	100	5.4	100	3.15**	134.04	8.4**	116.67
	200	4.6**	85.19	3.15**	134.04	7.05	97.92
	350	4.6**	85.19	4.95**	210.64	7.3	101.39
$CuSO_4$ + IAA	0	5.85**	108.33	5.4**	229.79	7.65**	106.25
	100	12.6**	233.33	7.2**	306.38	12.15**	168.75
	200	18.45**	341.67	9**	382.98	10.35**	143.75
	350	17.1**	316.67	9.45**	402.13	9.9**	137.5
L. S. D. 0.05%		0.18		0.20		0.15	
Cv. Assiut125	0	7.65	100	2.7	100	13.05	100
$CuSO_4$	100	9.45**	123.53	2.25**	83.33	13.05	100
	200	6.3**	82.35	2.25**	83.33	8.1**	62.07
	350	4.95**	64.71	1.45**	53.70	5.85**	44.83
$CuSO_4$ + IAA	0	11.7**	152.94	4.5**	166.67	13.55**	103.83
	100	19.35**	252.94	9.9**	366.67	12.75	97.70
	200	9.45**	123.53	5.85**	216.67	12.5**	95.79
	350	8.55**	111.77	5.85**	216.67	11.8**	90.42
L. S. D. 0.05%		0.10		0.14		0.29	

**Highly significance compared with control plants.

soluble carbohydrate fraction seemed to be more or less unchanged with a general tendency to decrease especially at the severe dose of Cu^{++} level as a result of IAA treatment (Table 2). In leaves the soluble carbohydrates increased progressively, elevated at the levels of 100 ppm and 200 ppm Cu^{++} and remained more or less unchanged at the level of 350 ppm Cu^{++} (Table 2). In cv. Assiut 84 considerable production of proteins was recorded in roots, stems and leaves of the Cu^{++}-affected broad bean as a result of phytohormonal treatment, approached 2-fold, especially in roots and stems (Table 3). Phytohoromonal treatment resulted in a marked and progressive accumulation in soluble carbohydrates and soluble proteins content in roots, stems and leaves in cv. Assiut 125 (Table 3). Hormonal treatments considerably accumulated potassium in roots and stems of cv. Assiut 84 (Table 4). At the level of 350 ppm Cu^{++}, the percent of increase in potassium content in roots and stems was 28% and 57% respectively when compared with the absolute control samples. In leaves, IAA completely alleviated the drastic effect of Cu^{2+} on potassium content (Table 4). Hormonal treatments completely alleviated the harmful effects of Cu^{2+} on the absorption and accumulation of potassium, whatever the concentration of Cu^{2+} used in cv. Assiut 125. Interestingly, in cv. Assiut 84 the pattern of changes in calcium content seemed to take place when the Cu^{++}-affected plants were sprayed by IAA (**Table 5**). Moreover, there is some additional activation in the absorption and accumulation of calcium, especially in leaves. IAA improved in cv. Assiut 125 the absorption and accumulation of calcium and the drastic effect of Cu^{2+} on calcium content was completely ameliorated as a result of IAA treatments (**Table 5**). A surprising accumulation of magnesium was exhibited when this cultivar was sprayed by IAA in cv. Assiut 84 (**Table 6**). This was much more pronounced in roots and stems than in leaves. At the level of 350 ppm Cu^{++} the percent of increase in magnesium was 216.67%, 302.13% and 37.5% in roots, stems and leaves respectively. A highly significant increase in magnesium content in roots and stems of cv. Assiut 125 was obtained under IAA treatment (**Table 6**). This stimulatory effect in magnesium content was much more pronounced in stems than in roots. At the level of 200 ppm Cu^{++} the percent of increase was 23.53% and 116.67% in roots and stems respectively. In leaves, the drastic effect of Cu^{2+} on magnesium content was eliminated by IAA treatment, whatever the level of Cu^{2+} used (**Table 6**).

5. Discussion

The data of the dry matter exhibited the biphasic effect of copper on the growth criteria of the two broad bean cultivars. While the lower and moderate doses of Cu^{2+} (100 ppm and 200 ppm) stimulated the growth of the two cultivars, the higher dose revealed the opposite event where the growth dropped in both cultivars. The growth stimulation caused by the lower and moderate doses of Cu^{2+} was more pronounced in cv. Assiut 84 than in cv. Assiut 125 and also at the higher Cu^{2+} concentration the growth dropped slightly in cv. Assiut 84 and highly significantly in cv. Assiut 125. Accordingly, the cv. Assiut 84 was considered as a Cu^{2+}-tolerant cultivar while the cv. Assiut 125 was the Cu^{2+}-sensitive. Such biphasic responses to Cu^{2+} were also revealed by other investigators Fageria (2002); Gao *et al.* (2008) [28] [29] using upland rice and *Jatropha curcas* L. seedlings respectively. The lethal doses of Cu^{2+} were recorded to differ among species and varieties. While Deef (2007) [30] reported that the biomass production of Cu^{2+}-treated *Rosmarinus officinalis* plants increased at lower treatments (100 to 200 ppm) and decreased gradually above 800 ppm Cu^{++}. Lara and Luca (2005) [31] reported that the peach rootstock *Prunus cerasifera* Mr.S. 2/5 plantlets grown *in vitro* on media containing either 10 or 50 μM of $CuSO_4$ did not show any visible signs of copper toxicity. At the higher Cu^{2+} concentration (100 μM of $CuSO_4$), Cu^{2+} toxicity symptoms appeared on the older leaves. In the present work, the differential responses to the different Cu^{2+} concentrations were not only observed between the two broad bean cultivars but also among their plant organs. At the level of 350 ppm Cu^{++}, the percent of reduction in dry weight of roots and stems of the cv. Assiut 84 was 74.13 % and 79.23% of control, respectively, which means that the roots and stems responded almost similarly (26% and 21% reduction, respectively), yet the root is more sensitive. Surprisingly, in leaves of this cultivar some promotion rather than inhibition was recorded (about 4% of control) at the same Cu^{++} level. In cv. Assiut 125 some differences were observed. At the level of 350 ppm, the percent of reduction in roots was 41% and in stems and leaves it was around 30%, which means that: The great inhibition was obtained in roots, stems and leaves responded similarly (30% of control). Thus, the responses to Cu^{2+} treatments differed greatly among the two cultivars and even among their plant organs. If we take into consideration the relation between growth and Cu^{2+} concentration, one can say that in cv. Assiut 84 the toxic ion effect was more or less similar in roots and stems and there is no toxic ion effect in leaves (promotion rather than inhibition), which probably means that the received amount of Cu^{2+} in leaves was much less than in roots or stems. This cultivar restricted the amount of Cu^{2+} transported to the leaves in order to maintain it as a micronutrient rather than inhibitor. There is also evidence that Cu^{2+} should be excluded from the leaves because of its inhibitory function against photosynthesis (Graham, 1981) [32]. Other situation was exhibited in cv. Assiut 125. The Cu^{2+} might be distributed similarly in stems and leaves (as the percent of reduction was more or less similar) while the highest accumulation of Cu^{2+} was in roots. It has been demonstrated that an excess of Cu^{2+} can inhibit the growth of young seedling, root elongation and cause damage to root epidermal cells and root cell membranes [33] [34]. The differential effect of Cu^{2+} on root and shoot growth could be accounted for by the fact that Cu^{2+} is accumulated mainly in roots and to a minor extent in shoots [13] [35]. In our study, an interesting point was also reported, that the role of Cu^{2+} as a fertilizer was more obvious than its toxicity. At the level of 200 ppm Cu^{++} the dry matter yield exceeded by more than 55% in the leaves of cv. Assiut 84 and by more than 31% of control in the leaves of cv. Assiut 125. This indicates the deficiency of Cu^{2+} as a micronutrient in the cultivated soil. Beneficial effect of Cu^{++} on yield of annual crops has been reported by Galrão (1999); Ursuzula (2008) and Diaz *et al.* (2017) [3] [36] [37]. Also, Fageria (2002) [28] reported that Cu^{2+} fertilization increases dry matter yield of upland rice and common bean. The concentrations of the photosynthetic pigments varied among the two broad bean genotypes as well as among the used concentrations of Cu^{2+}. Our data revealed that Cu^{2+} treatment stimulated the synthesis of the photosynthetically active pigments even at the highest dose of Cu^{2+}. This was more pronounced in cv. Assiut 84 than cv. Assiut 125. However, there is some reduction in chlorophyll b content in cv. Assiut 125 at the highest Cu^{2+} dose. Diaz *et al.* (2017) [3] showed that 50 µM and 100 µM of Cu^{2+} concentration induced a significant reduction in chlorophyll a content and exhibited oxidative stress evaluated through an increase in malondialdehyde levels on *C. quitensis* seedlings *in vitro*. Interestingly, the concentrations of photosynthetic pigments, especially in cv. Assiut 84, are not relative to the growth criteria particularly in roots and stems and also to some extent in cv. Assiut 125. However, pigments contents are in direct relationship with the metabolic rate (carbohydrates and proteins) in most cases. Thus, the relationship between growth and photosynthetic pigments as well as the production of metabolites of the two tested broad bean cultivars seemed to be complicated. Fagrasova (2001) [38] who reported that growth was more sensitive to Cu^{2+} than chlorophyll synthesis. Additionally, the considerable improvement of growth criteria of the two broad bean cultivars under IAA treatment was also associated by a marked and progressive increase in chlorophyll a, chlorophyll b and carotenoids, which was more obvious in carotenoids. There is a big difference in the accumulation and distribution of carbohydrates among the two broad bean cultivars and even among the different organs of the same cultivar, as well as the concentrations of Cu^{2+} used. In cv. Assiut 84, the Cu^{2+} treatment stimulated the production of carbohydrates whatever the concentration of Cu^{2+} and the plant organ tested. This accumulation was much higher in roots (about 100% increases in comparison with the absolute control at the highest Cu^{2+} dose) than stems or leaves. In cv. Assiut 125, while leaves and roots maintained carbohydrates contents around the control values, in stems, a marked and progressive accumulation of carbohydrates was recorded (about 50% over the control). In general, cv. Assiut 84 was the carbohydrates accumulator in comparison with cv. Assiut 125 (the Cu^{2+}-sensitive cultivar), whatever the plant organ tested and the level of Cu^{2+} used. Accordingly, the differences in the accumulation of carbohydrates among the two broad bean cultivars and among their plant organs might be used as a suitable selection criterion for Cu^{2+} tolerance of the two broad bean cultivars. Such contrasting results of Cu^{2+} effects on carbohydrates metabolism were also found by Deef (2007) [30] who studied the effect of different concentrations of Cu^{2+} on *Rosmarinus officinalis* and reported that all carbohydrates fractions increased under Cu^{2+} treatments until 200 ppm as compared with control. In cv. Assiut 84 the accumulation of proteins in leaves is opposite to the carbohydrates whose highest accumulation was located in roots. Is there a correlation between the huge accumulation of carbohydrates in roots and the production of energy needed for the active absorption of ions from the soil? Also, is there a correlation between the huge accumulation of proteins in leaves and the defense mechanisms adopted by this cultivar, particularly when taken into consideration the highly significant accumulation of the photosynthetic pigments in the leaves of this cultivar? Diaz *et al.* (2017) [3] evaluated the effect of Cu^{2+} (II) ions (control, 100 and 500 μM) on *C. quitensis* seedlings *in vitro*, determining morpho-physiological and biochemical variables. Cu^{2+} showed a significantly negative effect on the development of new shoots (500 μM) and floral apex appearance (100 μM). The analyzed Cu^{++} concentrations significantly affected leaf and root length and induced a significant increase in guaiacol peroxidase (G POD) enzyme activity. The highest proline accumulation took place in seedlings subjected to 500 μM. This is the first study to demonstrate evidence of Cu^{2+} effects on morphological, physiological and biochemical variables in *C. quitensis*. Vuksanović *et al.* (2017) [39] study five different European black poplar (*Populusnigra* L.) genotypes were studied *in vitro* for their tolerance to Cu^{2+} based on morphological parameters, biomass accumulation and pigment content, as well as Cu^{2+} accumulation in aboveground plant parts. Also, the effect of the pH of the medium and Cu^{2+} concentration was examined in order to optimize the evaluation of Cu^{2+} tolerance *in vitro*. Hamdia *et al.* (2017) [13] showed that response of wheat plants to different osmotic stress levels varied among the different organs root, shoot and spike and the situation of these organs with application of two Cu^{2+} levels 5 mM and 25 mM as $CuSO_4$. Data also showed further stimulatory effect on growth parameters by Cu^{2+} applications with either concentration (7.5 mM or 25 mM). Irrigating the soil with either 7.5 or 25 mM $CuSO_4$ induced a huge accumulation in soluble sugar, soluble protein and nitrate reductase. Hamdia (2017) [40] study interactive effect of different Cu^{2+} concentrations (5 mM, 10 mM, 20 mM and 25 mM) and treatments with biofertilizers *Azospirillum brasilense* on growth, metabolites, minerals and osmotic pressure of wheat plants was investigated. Shoots and roots of wheat plant were differentially response to Cu^{2+} treatments, while shoot organ response positively to this treatment, root response negatively. The positive effect of Cu^{2+} in shoot organ was concomitant with the increase in the production of fresh, dry matter, length and water content and this related with the accumulation of soluble sugar, soluble protein and mineral as a result of increasing osmotic pressure. On the other side, the negative effect of Cu^{2+} on root organ was concomitant with the decrease in production of fresh, dry matter, length and water content that related with the reduction in the accumulation of soluble sugar and mineral with the insignificant change in osmotic pressure. It is worthy to mention that while the cv. Assiut 84 controlled the interconversion between carbon and nitrogen at any concentration of Cu^{2+}, in cv. Assiut 125 some disturbances were recorded, that while the carbohydrates contents increased in some plant organs, the protein contents decreased slightly. This leads us to point out that the heavy metal tolerance was linked with equilibrium in the conversion between carbohydrates and nitrogen metabolism while heavy metal injury leads to the disturbance among the two components [13] [29] [40]. When the plants of cv. Assiut 84 and cv. Assiut 125 were sprayed by IAA the concentration of both carbohydrates and proteins were stimulated considerably parallel to the observed stimulation in the growth criteria of the two tested cultivars as a result of IAA treatment. It can be pointed out that there is a close correlation between the growth criteria and carbohydrates and proteins metabolism, which depends upon the activity of the photosynthetic apparatus as well as the green area and the machinery of water flow (the growth of roots was considerably stimulated by IAA treatment).The differences in the responses to Cu^{2+} among the two selected broad bean cultivars were mirrored by the differences in the absorption, accumulation and compartmentation of potassium, calcium and magnesium in the different organs of the two cultivars. Our data reveal that while Cu^{2+} had a marked stimulatory effect in the absorption and accumulation of calcium in the different organs of cv. Assiut 84, it, on the other hand, significantly inhibited the accumulation of this cation in the different organs of cv. Assiut 125. Moreover, the absolute amount of calcium was markedly higher in cv. Assiut 84 than in cv. Assiut 125, whatever the plant organ tested. Also while Cu^{2+}-stressed cv. Assiut

84 maintained potassium and magnesium levels around the control values and some promotion occurred especially in roots and stems, these cations dropped markedly in cv. Assiut 125 as a result of Cu^{2+} treatments. Also, as in the case of calcium, the highest amount of absorbed potassium and magnesium achieved into the leaves of the two broad bean cultivars. This uphill movement of potassium and magnesium was more pronounced in cv. Assiut 125 than cv. Assiut 84. Similary, Fageria, 2002 [28] working on the effect of various levels of Cu^{2+} on rice and bean plants, reported that while Cu^{2+} decreased the concentration of calcium and magnesium in rice, it, on the other hand, induced insignificant changes in these cations in bean plants. Deef (2007) [30] reported that increasing Cu^{2+} concentration from 50 to 3200 ppm in the medium decreased the nitrogen, phosphorus, potassium, calcium and iron content of *Rosmarinus officinalis* shoot system. The low rate in the absorption of potassium, calcium and magnesium in cv. Assiut 125 when compared to cv. Assiut 84 might be linked with the differences in the biomass content of the roots among the two cultivars. The high absorption zone of cv. Assiut 84 might permit this cultivar to absorb a sufficient amount of macronutrients and also to force up these elements to the leaves which in turn share in the high affinity of the reactive center of the photosynthetic apparatus and consequently the manufacture of carbon and nitrogen and equilibration among the two components. Deef (2007) [30] attributed the reduction in the absorbance of cations to the competition among them. This is not the case in our study. The antagonistic effect theory also seems to be problematical. This is supported by the results obtained by Gareia-Legaz *et al.* (2005) [41] who reported that in rice there was no correlation in potassium and sodium transport and concluded that the genes affecting sodium uptake had not apparently co-segregated with those involved with potassium uptake. This theory was also recommended when the Cu^{2+} stressed plants sprayed with IAA where a lot of these cations were transported in the same trend from the soil solution into the different parts of the two broad bean plants without any competition among these cations. The uptake, translocation and distribution of mineral ions are affected by various growth regulators among others by IAA. These effects can be evoked by changes in the rate of photosynthesis of a new biomass [13]. Massoud *et al.* (2018) [42] stdudy the mehanisms of Cu^{2+} alleviation toxicity in germinating pea seeds by IAA, GA_3, Ca and citric acid. This strategy might be important in heavy metals tolerance mechanisms of crop plants (the sufficient amount of elements and the huge accumulation of them in leaves) which might be evoked up the machinery of water flow from the soil solution into the aerial parts of the plants. Ion regulation is an essential factor of the mechanisms of heavy metals tolerance.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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