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The Biphasic Role of Cupper and Counteraction with *Azospirillum brasilense* Application on Growth, Metabolities, Osmotic Pressure and Mineral of Wheat Plant

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Abstract

The interactive effect of different Cu^{++} 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^{++} treatments, while shoot organ response positively to this treatment, root response negatively. The positive effect of Cu^{++} 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^{++} 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. *Azospirillum brasilense* inoculation increased the accumulation of soluble sugar and soluble protein which reflected an increase in the production of fresh, dry matter and water content with increasing values of osmotic pressure of the tested plants under Cu^{++} treatment. Finally, wheat plants response differentially to Cu^{++} treatment according to its organ and *Azospirillum brasilense* treatment improved wheat plant efficiency to tolerate the effect of Cu^{++} stress.

Keywords

Cu^{++} Treatment, *Azospirillum brasilense*, Wheat

1. Introduction

Cu^{++} is required within the plant cell, in at least six locations: cytoplasm, the en-

doplasmic reticulum (ER), mitochondrial inner membrane, stroma chloroplast, apoplast and lumen thylakoid [1]. Cu^{+} was contributed in many biochemical and physiological activities because it is able to exit in multiple oxidation states *in vivo*; cupper can exit as Cu^{++} and Cu^{+}; the cation Cu^{++} is bound with nitrogen in histidine side chains, whereas Cu^{+} prefers the interaction with the sculpture in cystein or methionine. Cu^{++} was considered as the structural element in metall proteins, which involved in chloroplasts electron transport and mitochondria and in oxidative stress response. Cu^{+} has the ability to bind small molecules such as O_2 as ligands, thus, Cu acts as the cofactor of a large number of oxidase [2]. Soil Cu^{++} defficiency or in excess amount can cause the undesirable effect in plant growth and survival by adversely affecting physiological activities in plants which later effect on agriculture and human healthy. Thus, for healthy growth of plant and survival, Cu^{++} must be absorbed from soil, transported, distributed in different plant cells regulated carefully. Soil fertility is lowered periodically related to soil erosions, the diminished of nutrients, and increasing of toxic ions, water logging and undistributed nutrient compounds. Recently, using biofertilizers has a promising component of nutrient supply in agriculture. Biofertilizers useful for crops are *Azospirillum*, *Azotobacter*, P-solubilizing bacteria, blue green algae and mycorrhizae [3] [4]. *Azospirillum*s species are among facultative endophytic diazotrophic group which found on the surface and interior of roots [5]. *Azospirillum* has been reported as plant growth promotion in field and nursery plants, resulting in significant effects in plants [6].

Romero *et al.* (2003) and Sangeeth *et al.* (2008) [7] [8] were studied *Azospirillum* spp inoculation effects of indigenous selectively isolated from various black pepper in enhancing growth as total dry weight and increasing nutrient uptake as nitrogen, phosphorus and potassium in treated plants.

Thus, this work was carried out to study the more important features concerning Cu^{++} stimulant or toxicity and try to increase wheat plant tolerance by *Azospirillum* inoculation treatments on growth, metabolic constituents and osmotic pressure of wheat plants grown for 21 days.

2. Materials and Methods

2.1. Experimental Sites and Cupper Treatments

Grain of cv. Giza 168 was obtained from Beni Suief, Seds Center, Egypt, Agricultural pharmacies in bags, already a factory prepared for research. Wheat plant was considered as one of the most important crop plants in Egypt because of its contribution as main nutrient foods for people. From investigation was carried out by Hamdia and Shaddad (2016) showed that the salt tolerance (0.0, 20 mM, 50 mM, 100 mM, 150 mM, 200 mM and 300 mM NaCl levels) of the four wheat cultivars, during vegetative growth and crop yield stages ranked as the following: cv. Sakha 94 > Gimiza11 > cv. Gimiza 10 > cv. Giza 168. This means that cv. Sakha 94 was the superior and cv. Giza 168 was the interior. Select cv. Giza 168 because it was most salt sensitive. Wheat grains 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. Wheat (cv. Giza 168) plants were grown in vermiculate for one week and then transplanted in plastic pots fill with clay soil without Cu^{++} treatment (control) and under different Cu^{++} concentration as $CuSO_4$ salt, 5 mM, 10 mM, 20 mM and 25 mM were added to the soil in such a way that the soil solution acquired the assigned Cu^{++} concentrations at field capacity in growth chamber (Forma Scientific, Marietta, Ohio, U.S.A.) at 30/25˚C, 12 h day/night cycles and 60 Wm^2). 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). Soil particle of clay is <0.002 invisible to naked eye. Considerations of working in controlled environments were followed by Tibbitts & Langhans (1993) [9].

2.2. Cupper Treatments with *Azospirillum* Inoculation

Treatments of plants with different concentration began when seedlings transplanted in the plastic pot. The previous treatment group was repeated for *Azospirillum brailense* inoculation, inoculum was prepared by El-Komy (1992) [10] at bacteriology laboratory in Minia University, Faculty of Science, Botany and Microbiology Department.

2.3. Laboratory Analysis for Metabolities

A week after the plants was used for analysis after 21-days. Dry matter was determined after drying plants in an aerated oven at 70˚C to constant mass. Soluble sugar was determined by the anthrone-sulfuric acids method [11]. Soluble protein contents were measured according to Lowry *et al.* (1951) [12]. The osmotic pressure of tissue sap was measured by advanced wide-range Osmometer 3W2. Na^+ and K^+ were determined Flamphotometeric by Williams and Twin (1960) [13]. Ca^{++} and Mg^{++} were determined by Schwarzenbach and Biedermann (1948) [14].

3. Statistical Analysis

The experimental data were subjected to the one way analysis of variances (ANOVA test) using the SPSS version 11.0 to quantify and evaluate the source of variation and the means were separated by the least significant differences, L.S.D. at P level of 0.05% [15]. The percentage presented in the following tables was calculated by the data of fresh, dry matter, water content, length, soluble sugar, soluble protein, minerals and osmotic pressure of shoot and root at reference control plants, with different Cu^{++} concentrations 5 mM, 10 mM, 20 mM and 25 mM and with Cu^{++} concentrations plus *Azospirllum* inoculation of wheat cv. Giza 168. The data was compared by plants grow at control (untreated), the other different Cu^{++} concentration and with Cu^{++} under *Azospirillum* inoculation. The fresh, dry matter, length and water content were determined as g $plant^{-1}$, chemical constituents (soluble sugar, soluble protein and mineral con-

tent) were determined as mg·g^{-1} d.m. Osmotic pressure was determined as (m Osmo/kg H_2O).

4. Results

The date in Table 1 reveals that fresh and dry matter of shoot and root response differentially to different Cu^{++} concentrations treatments. The low Cu^{++} concentration 5 mM significantly increase the production of fresh and dry matter of both shoot and root of wheat plants. The percent of increase at that level was 97.1%, 65%, 23.5% and 16.7% of fresh and dry matter of shoot and root, respectively. The moderate and high in Cu^{++} concentrations (from 10 mM to 25 mM) were significantly increasing the fresh and dry matter of shoot while significantly decreases these parameters in root organ compared with uncupper plant treatment. The percent of increase in fresh and dry matter in shoot and root at 25 mM Cu^{++} concentration was 32.7% and 25%, while the percent of reduction in root was 20% and 46.7% as compared with control plants.

The length of shoot and root was markedly increased with increasing Cu^{++} concentration up to 5 mM, above which a reduction was detected until reach a low levels at 25 mM Cu^{++} (Table 2). The percent of reduction at that level of Cu^{++} was 18.2% and 21.9% for shoot and root of wheat plants. Water content significantly increases with increasing Cu^{++} concentration in both shoot and root; the high values were produced at 5 mM for both organs (Table 2). This activation was more observed at low and moderate level of Cu^{++} treatment. Soluble sugar was significantly increased at 5 mM Cu^{++} concentration in shoot, after that, it become more or less unchanged compared with control plant (Table 3). While, Cu^{++} treatment significantly decreased this content in root

Table 1. The effect of different Cu^{++} concentrations and *Azospirillum* inoculation on fresh and dry matter g plant^{-1} in shoot and root of wheat plants grown for 21-days.

Treat.	Shoot				Root			
mM	F. m. g·plant^{-1}	%	D. m. g·plant^{-1}	%	F. m. g·plant^{-1}	%	D. m. g·plant^{-1}	%
Control	0.104	100	0.02	100	0.051	100	0.03	100
5 mM	0.205**	197.1	0.033**	165	0.063**	123.5	0.035**	116.7
10 mM	0.155	149.0	0.027**	135	0.039**	76.4	0.015**	50.0
20 mM	0.194*	186.5	0.028**	140	0.042	82.4	0.016**	53.3
25 mM	0.138	132.7	0.025**	125	0.041	80	0.016**	53.3
Cont. + *Az.*	0.227**	218.3	0.039**	195.0	0.057	111.8	0.035**	116.7
5 mM + *Az.*	0.262**	251.9	0.035**	175.0	0.064**	125.5	0.028*	93.3
10 mM + *Az.*	0.215**	206.8	0.034**	170.0	0.064**	125.5	0.028*	93.3
20 mM + *Az.*	0.174	163.5	0.029**	145.0	0.052	101.9	0.023**	76.8
25 mM + *Az.*	0.156	150.0	0.029**	145.0	0.055	107.8	0.024**	80.0
L. S. D. 5%	0.1		0.003		0.01		0.002	

**Highly significant > 0.05.

Table 2. The effect of different Cu^{++} concentrations and *Azospirillum* inoculation in Length (Cm), water content ($g \cdot plant^{-1}$) of shoot and root of wheat plants grown for 21 days.

Treatments	Length (Cm)				Water content ($g \cdot plant^{-1}$)			
mM	Shoot	%	Root	%	Shoot	%	Root	%
Control	22	100	7.3	100	0.084	100	0.021	100
5 mM	24.3**	110.5	8.2*	112.3	0.172**	204.8	0.028	133.3
10 mM	20.3**	92.3	7.7	105	0.128	152.3	0.024	114.3
20 mM	20**	90.9	6.2**	84.9	0.166**	197.6	0.026	123.8
25 mm	18**	81.8	5.7**	78.1	0.113	134.5	0.025	119.0
Control + Az.	19.5	88.6	8.0	109.6	0.188**	223.8	0.022	104.8
5 mM + *Az.*	26.2**	119.0	8.0	109.6	0.227**	270.2	0.036**	174.4
10 mM + *Az.*	21**	95.5	8.0	109.6	0.181**	215.5	0.036**	171.4
20 mM + *Az.*	21**	95.5	7.5	102.7	0.141**	167.8	0.029**	138.1
25 mM + *Az.*	19**	86.4	6.5	89.0	0.127	151.2	0.031*	147.6
L. S. D. 5%	0.6		0.9		0.05		0.01	

**Highly significant > 0.05.

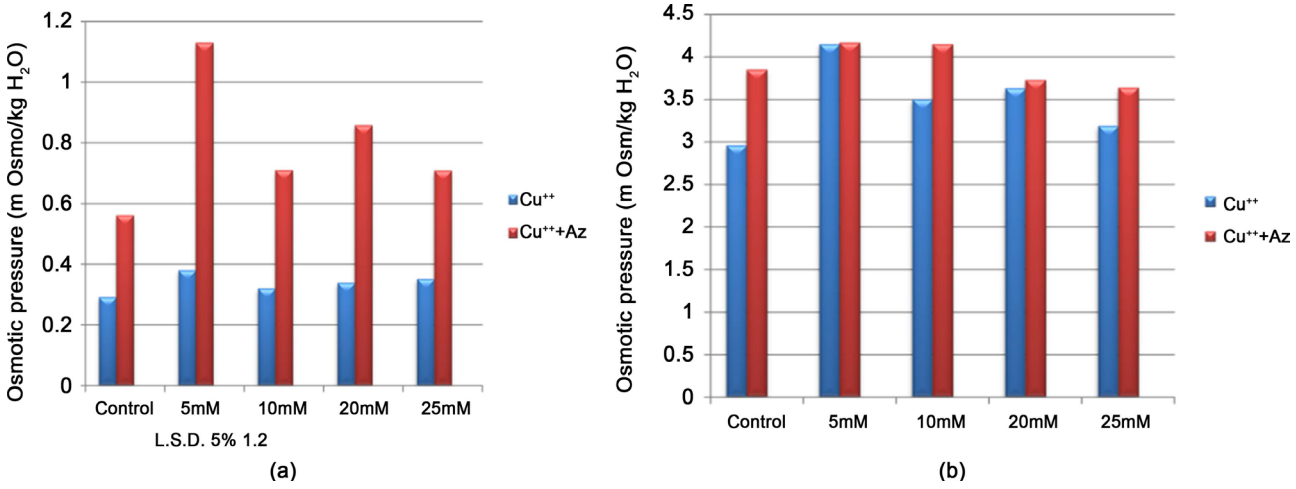

Figure 1. Counteraction of Cu^{++} and treatment with *Azospirillum brasilense* on osmotic pressure (m Osmo/kg H_2O) of shoot (a) and root (b) of wheat plants grown for 21 days.

especially at higher Cu^{++} concentration. Soluble protein was markedly accumulated in both shoot and root of wheat plant (Table 3). This accumulation was higher in shoot than in root organ and at 5 mM Cu^{++} concentration in case of root. Osmotic pressure of wheat plants was markedly increased with increasing Cu^{++}concentration in shoot and root (Figure 1(a), Figure 1(b)). This activation effects was more in root than in shoot organ of wheat plant. It is worthy to note that osmotic pressure (OP) represent a higher value at 5 mM Cu^{++} concentration in shoot and root organ compared with untreated plants. This runs parallel with fresh, dry matter, length and water content. Mineral content of wheat plants showed a variable response to Cu^{++} treatments. Sodium content was tended to

increase in shoot and significantly accumulated in root with increasing Cu^{++} treatment (Figure 2(a), Figure 2(b)). This accumulation was pronounced at 25 mM Cu^{++} concentration, the percent of increase was 266.6% and 233.3% of shoot and root respectively compared with untreated plants. K^{+} in content was markedly increased in both shoot and root with increasing Cu^{++} treatment, it decreased in root as compared with control plants (Figure 3(a), Figure 3(b)). It is worthy to note that the high value in K^{+} accumulation in shoot was detected at 5 mM and 10 mM Cu^{++} concentration. Ca^{++} content was significantly increased at 5 mM Cu^{++} concentration in shoot while at 10 mM Cu^{++} produced the same value of control, above that a reduction was recorded (Figure 4(a), Figure 4(b)). In root

Table 3. The effect of different Cu concentrations and *Azospirillum brasilense* inoculation on soluble sugar (mg·g^{-1} d. m.) and soluble protein (mg·g^{-1} d. m.) in shoot and root of wheat plants grown for 21-days.

	Soluble sugar				Soluble protein			
	Shoot	%	Root	%	Shoot	%	Root	%
Control	32.1	100	63.3	100	32.7	100	34.4	100
5 mM	45.1**	169.5	56.3**	88.9	31.5	96.3	43.8**	127.7
10 mM	33.5**	104.4	54.9**	86.8	34.8**	106.4	31.9**	92.7
20 mM	30.4**	91.7	48.3***	76.3	43.8**	133.9	35.8**	104.1
25 mm	33.4**	104.0	33.5**	52.9	41.9**	128.1	36.1**	104.9
Control + *Az.*	53.3**	166.0	150.9**	238.4	32.4	99.1	30.2**	87.8
5 mM + *Az.*	46.3**	144.2	71.9**	113.6	34.3**	104.9	33.9	98.5
10 mM + *Az.*	47.7**	148.6	98.6**	155.8	40.1**	122.6	38.9**	113.1
20 mM + *Az.*	35.0**	109.0	79.9**	123.2	43.7**	133.6	41.9**	133.4
25 mM + *Az.*	36.9**	114.9	45.4**	71.7	51.4**	157.2	43.2**	125.6
L. S. D. 5%	1.1		1.2		1.3		0.8	

**Highly significant > 0.05.

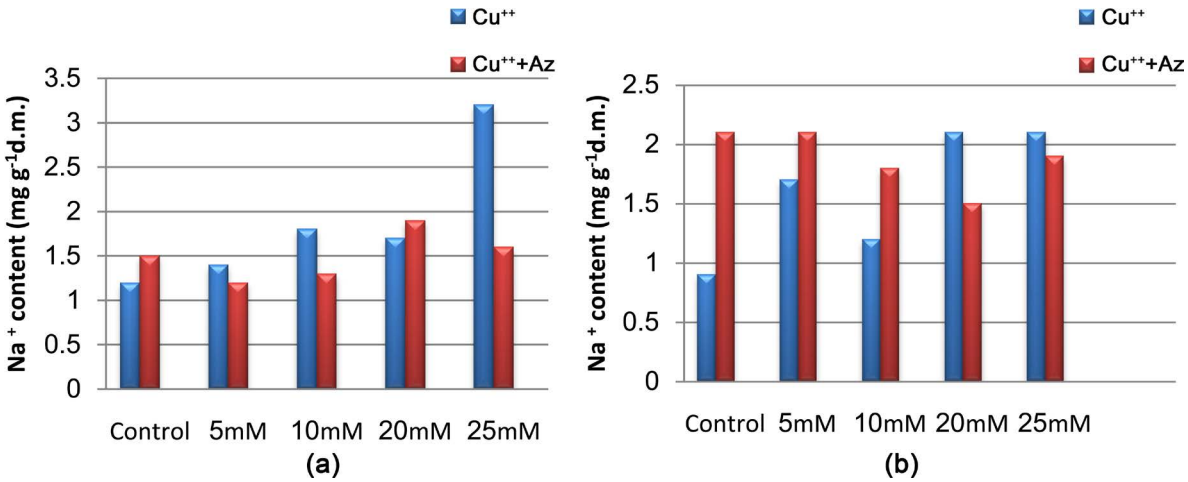

Figure 2. Effect of Cu^{++} treatment with *Az.* inoculation on Na^{+} content (mg·g^{-1}d.m.) in shoot (a) and root (b) wheat plants grown for 21 days. (a) L.S.D. 5% 0.011; (b) L.S.D. 5% 0.013.

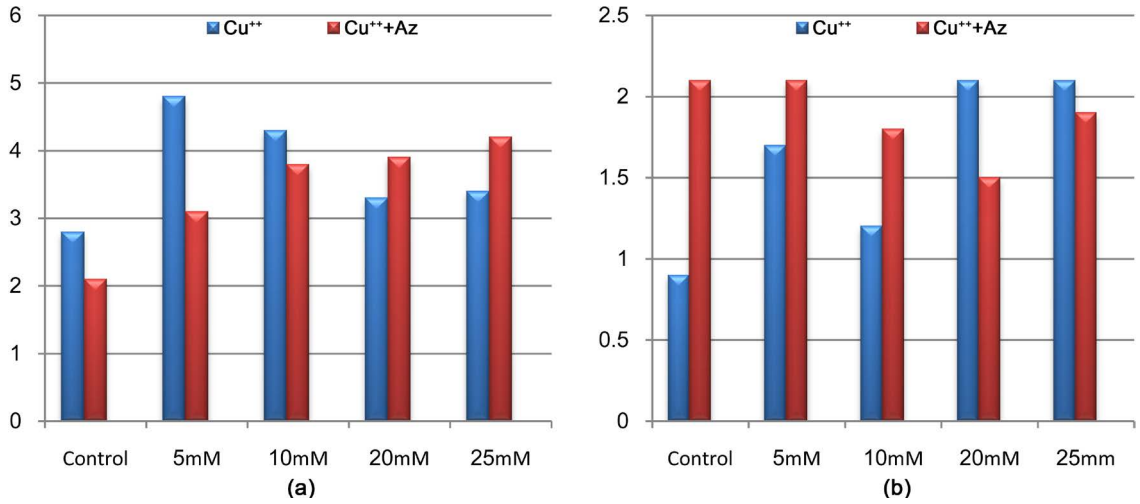

Figure 3. Effect of Cu^{++} treatment with *Az.* inoculation on K^{+} content ($mg \cdot g^{-1}$ d.m.) in shoot (a) and root (b) of wheat plants grown for 21 days. (a) L.S.D. 5% 0.014; (b) L.S.D. 5% 0.015.

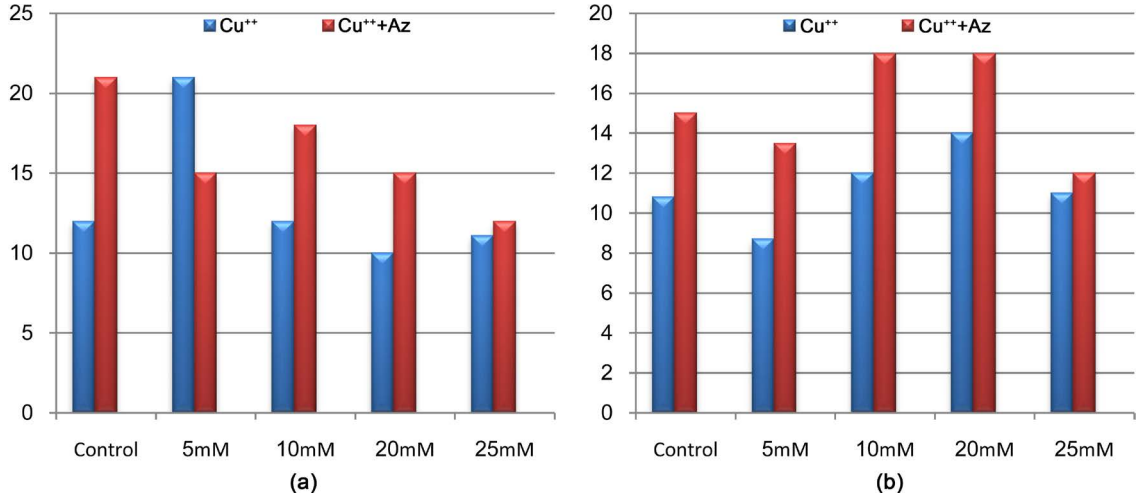

Figure 4. Effect of Cu^{++} treatment with *Az.* inoculation on Ca^{++} content ($mg \cdot g^{-1}$ d.m.) in shoot (a) and root (b) of wheat plants grown for 21 days. (a) L.S.D. 5% 0.014; (b) L.S.D. 5% 0.015.

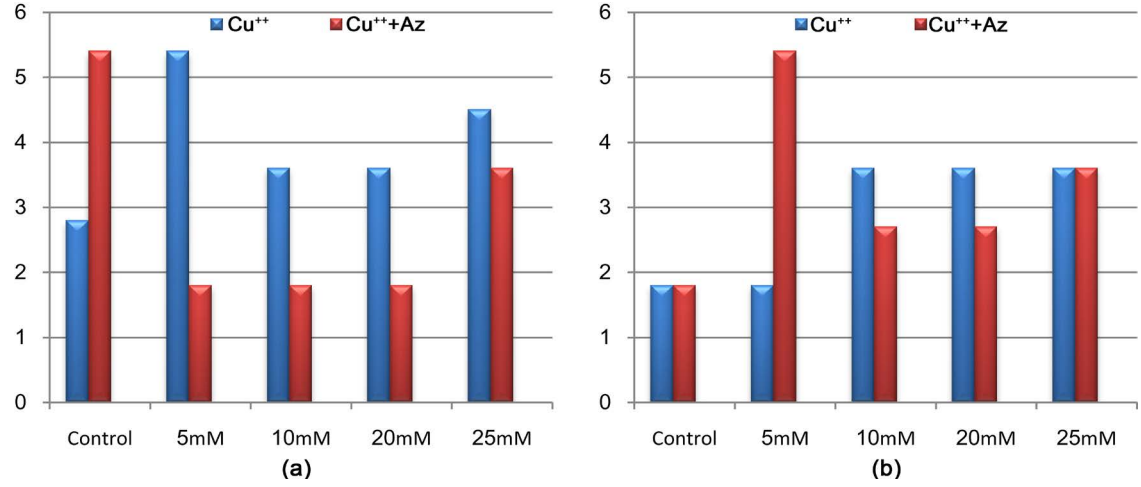

Figure 5. Effect of Cu^{++} treatment with *Az.* inoculation on Mg^{++} content ($mg \cdot g^{-1}$ d.m.) in shoot (a) and root (b) of wheat plants grown for 21 days. (a) L.S.D. 5% 0.014; (b) L.S.D. 5% 0.015.

Ca^{++} was significantly increased with elevated Cu^{++} application. Also, Increasing Cu^{++} concentration elevated Mg^{++} accumulation in both shoot and root, the high values was observed at 5 mM Cu^{++} level in shoot while in root the high value was recorded at 25 mM Cu^{++} compared with uncupper treatment (Figure 5(a), Figure 5(b)). *Azospirillum* inoculation significantly enhanced the production of fresh and dry matter in shoot and root of wheat plants when compared with control plant (Table 1). This activation was more prominent at low Cu^{++} concentrations (5 mM and 10 mM). The percent of increase in fresh and dry matter of shoot at 5 mM Cu^{++} was 151.9% and 75% for shoot and 25.5% for root fresh matter respectively. The percent of increase at 25 mM was 50% and 45% in fresh and dry matter of shoot, while in root no significant effect was observed. *Azospirillum* application stimulates the length in shoot and root of wheat plant as compared with both untreated plant and the corresponding Cu^{++} concentration (Table 2). Also, water content of shoot and root was progressively increased with increasing Cu^{++} concentration. This increase was reaching 2-folds especially at lower and moderate (5 mM and 10 mM) Cu^{++} treatment and in shoot than in root organ (Table 2). The percent of activation in water content at 5 mM Cu^{++} concentration was 170.2% and 71.4 % of shoot and root at 5 mM Cu^{++} level and 151.5 and 71.4 at 10 mM Cu^{++} level over the control value 100%. *Azospirillum* application significantly elevates the accumulation of soluble sugar and soluble protein in both shoot and root compared with control plants (Table 3). This elevation was prominent at lower and moderate Cu^{++} concentration in soluble sugar content and in soluble protein at the higher Cu^{++} concentration in both shoot and root of wheat plant. The percent of increase in case of soluble sugar at 10 mM with *Azospirillum* inoculation was 48.6% and 55.8% over the control value 100% in shoot and root, in case of soluble protein at 25 mM with *Azospirillum* treatment this percent was 57.2% and 33.4% over the control plant. *Azospirillum* application significantly enhanced the values of OP in shoot with increasing Cu^{++} concentration. The percent of activation was reached 3-folds at 5 mM Cu^{++} concentration than the control or the corresponding Cu^{++} concentration plant. In root, there is a slight increase of OP with increasing Cu^{++} concentration, the high values was recorded at 5 mM Cu^{++} concentration. *Azospirillum* inoculation significantly decreases the Na^{+} accumulation with increasing Cu^{++} concentration in shoot and in root at higher Cu^{++} value of wheat plant (Figure 2(a) & Figure 2(b)). Potassium and calcium content increased with the elevating Cu^{++} concentration in shoot and root of wheat plants (Figure 3(a), Figure 3(b) & Figure 4(a), Figure 4(b)). Mg^{++} remain unchanged in shoot and root with *Azspirillum* treatments at all levels of Cu^{++} concentration, at 5 mM in root a high value was produced than the other Cu^{++} concentration (Figure 5(a) & Figure 5(b)). Data in Table 4 showed that relative nutrient content increased with increasing Cu^{++} content in shoot and root until reached higher values at 25 mM Cu^{++} concentration with *Azospirillum* application, except of this trend relative nutrient content decreased in case of root Mg^{++}. Lai *et al.* (2008) [16] have proposed a new type of data analysis which considers both biomass and nutrient

Table 4. The effect of different Cu^{++} concentrations and *Azospirillum* inoculation on relative nutrient accumulation of shoot and root of wheat plants.

Treat. mM	Sh. Na^{+}	Sh. K^{+}	Sh. Ca^{++}	Sh. Mg^{++}	Ro. Na^{+}	Ro. K^{+}	Ro. Ca^{++}	Ro. Mg^{++}
Cont.	0.925	0.56	0.426	0.284	1.66	0.162	0.339	2.66
5 mM	0.931	0.837	1.15	0.230	1.67	0.164	0.670	4.6
10 mM	2.76	1.74	1.07	0.229	1.88	0.453	0.770	0.768
20 mM	5.51	2.72	2.9	0.580	1.99	1.95	1.59	0.78
25 mM	5.6	4.55	11.5	3.47	3.71	2.65	4.7	0.78

content of plants. This new type of analysis has shown the importance of the mineral content of plant. The relative nutrient accumulation rate can be calculated by the following relationship:

$$\frac{\text{Biomass in inoculated}}{\text{Biomass in non-inoculated}} \times \frac{\text{Mineral content in inoculated}}{\text{Mineral content in non}^{-}\text{inoculated}} \times 100$$

Our data showed that relative nutrient content increased with increasing Cu^{++} content until reached a higher values at 25 mM Cu^{++} concentration with *Azospirillum* application. Inoculation facilitated in the plant yield and mineral content by some selected mechanisms or by a cascade of mechanisms operating simultaneously under suitable conditions.

5. Discussion

The present work was conducting the biphasic role of different Cu^{++} concentrations (5 mM, 10 mM, 15 mM and 25 mM) and treatments with biofertilizers *Azospirillum brasilense* on growth, metabolites, osmotic pressure and mineral content of wheat plants. Shoot and root of wheat plant were differentially response to Cu^{++} treatments, while shoot organ response positively to this treatment, root response negatively. Cu^{++} acts as activator metal in shoot organ while acts as inhibitory metal in root organ. This activation effect in shoot organ was concomitant with the increase in the production of fresh and dry matter which was related with the accumulation of soluble sugar and soluble protein that share in increasing osmotic pressure of the cell sap. This accumulation can be functioning in the increase of water uptake of wheat plants which support the view that Cu^{++} successes in the utilization of carbohydrate and N-metabolism and finally reflected on the increase of growth parameters [17] [18]. Thus, plants required Cu^{++} for normal growth and development, when it was not enough specific symptoms to develop on young leaves and reproductive organs [19]. However, the negative response of root to Cu^{++} treatment was related with the inhibition of fresh and dry matter which produced as the result of the decrease in the accumulation of soluble sugar and soluble protein at lower Cu^{++} concentration and the increase in OP. This means that OP increases the efficiency of root organ to survive under the inhibitory effect of Cu^{++} addition. It is worthy to mention Yuan *et al.* (2013) [20] observed that heavy metal copper (Cu^{++}) is an essential microelement required for normal plant growth and development, but it in-

hibits primary root growth when in excess. On the other hand, redox properties that make Cu^{++} essential element also contributed to its inherent toxicity in shoot of wheat plant. Redox cycling between Cu^{++} and Cu^{+} can catalyze the production of highly toxic hydroxyl radicals, with subsequent damage to cells at level of lipids, membranes, nucleic acids, proteins and other biomolecules [21]. The mechanism underlying how excess Cu^{++} functions in this process remains to be further elucidated. At this position from our result, Cu^{++} acts as chemical fertilizers for shoot wheat plants or the tested soil suffering from Cu^{++} deficiency while caused an inhibition effect in root organ. Wheat plant can tolerated the addition of Cu^{++} from 10 mM to 20 mM. This tolerance was related with the increase in water uptake of shoot and roots which concomitant with the increase of mineral content in both wheat organs as Na^{+}, K^{+}, Mg^{++} and root Ca^{++}. However, a significant reduction was observed at 25 mM Cu^{++} concentration in shoot and root of wheat plant, this reduction was more in root than in shoot. Actually, lower level of Cu^{++} treatment 5 mM, induced an increase in soluble sugar, shoot K^{+}, $Ca^{++}$and Mg^{+} in the cell sap, this resulting an increasing effect in OP which finally activated more water uptake. This also in turn was resulting an increase in the production of growth parameters (fresh and dry matter) and in shoot and root of wheat plants. Also, the increase of mineral content in shoot as compared with root especially K^{+}, Ca^{++} and Mg^{++} indicted that Cu^{++} increase wheat efficiency to translocate these minerals from root to shoot which functioning in their share in osmotic adjustment of the cell sap resulted an increase in water content especially at lower and moderate Cu^{++} concentrations. This reflected also on the tolerant of shoot organ than root to the Cu^{++} toxicity. Genotypical differences in tolerance to copper are well known in certain species and ecotypes of natural vegetation [22] [23].

Azospirillum inoculation exhibited a promotion in the accumulation of soluble sugar and soluble protein, which concomitant with the increase in OP. Finally, this activation reflected on the production of fresh and dry matter of the tested plants under Cu^{++} treatment. It can be observed from the data that shoot response higher than root to the *Azospirillum* inoculation which reflected the interaction effect of the two fertilizers Cu^{++} and *Azospirillum* application. Thus, *Azospirillum* and Cu^{++} counteracted to enhancement wheat plant growth and the higher activated effect was observed at low cupper concentration (5 mM Cu^{++}). The ability to form plant hormones is a property of microorganisms and PGPB, *Azospirillum* species stimulate and facilitate plant growth [24] [25]. *Azospirillum s*pp. has ability to produce plant hormones, polyamines and amino acids in culture media [26]. Among these hormones, indoles, mainly indole-3-acetic acid IAA [27], and gibberellins (GA3) of several kinds [28] may play a significant role. Nitrogen fixation was considered as a major mechanism by which *Azospirillum* enhanced plant growth [28] [29]. Also, a possible strategy for improvement plant is a lowering of metal toxicity in contaminated soils which inhibited plant growth. Although, the bacterium tolerate only moderate concentrations of metals and many toxic compounds [30]. The present data

showed that relative nutrient content increased with increasing Cu^{++} content until reach a higher values at 25 mM Cu^{++} concentration with *Azospirillum* application. Inoculation facilitated in the plant yield and mineral content by some selected mechanisms or by a cascade of mechanisms operating simultaneously under suitable conditions [31] [17] [32]. Finally, wheat plants response differentially to Cu^{++} treatment according to its organ and *Azospirillum brasilense* treatment increased wheat plant efficiency to tolerate the effect of Cu^{++} stress.

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Abbreviations

ER, endoplasmic reticulum;
IAA, indole-3-acetic acid;
GA_3, gibberellins;
OP, osmotic pressure;
Azospirillum, *Az.*