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Original article

Evaluation of the effect of *Azotobacter Crococum* and *Pseudomonas putida* on photosynthetic pigments and wheat yield under cadmium stress

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Extended abstract

Introduction

Heavy metal pollution is one of the major problems of human societies in the production of agricultural products and is considered as a major threat to human health. Wheat is a strategic crop in human nutrition and the growing global population requires more agricultural production. To achieve high yields of crops, especially in developing countries, chemical fertilizers and pesticides are widely used, which causes excessive accumulation of heavy metals in agricultural soils and has detrimental effects on human health and other living organisms. Increasing the concentration of heavy metals in the soil on the other hand affects the toxicity and growth and yield of crops. Considering the mentioned cases and the importance of wheat as a major human food, the present study aimed to investigate the effect of plant growth-promoting rhizobacteria (GPR) in reducing the effects of cadmium in two wheat cultivars and their effect on plant pigments. It was done by examining the change in the content of plant pigments.

Materials and methods

The experiment was carried out as a factorial experiment in a completely randomized design with three replications in Research Greenhouse of Agricultural Faculty of Mohaghegh Ardabili University in 2018. Experimental treatments included cadmium chloride stress ($CdCl_2H_2O$ 0, 75, 150 and 300 μM) and the effect of growth promoting bacteria (*Azotobacter* and *Pseudomonas*). The studied traits included content of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio), adjuvant pigments (carotenoids, flavonoids), quantum yield, SPAD, stem dry weight, yield, seed weight and number of seeds.

Results and discussions

The results of analysis of variance of the main photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll) at 24, 48 and 72 hours after cadmium treatment and shoot showed that the interactions of the cultivar in bacteria at 72 hours on chlorophyll a (5% level) and was significant on chlorophyll b and total chlorophyll (1% level) . Comparison of means showed that the highest amount of chlorophyll b (without change compared to control) and total chlorophyll (4.63% compared to

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control) of Karim and chlorophyll a (95.3% compared to control) in Gonbad cultivar in the range 72 hours were observed. The highest amount of chlorophyll a was 6.31 mg / g and total chlorophyll (7 mg / g) was observed from Gonbad cultivar and inoculation with Azotobacter, which was not significantly different from the control. In the case of chlorophyll b, the lowest amount (0.57 mg / g) was obtained from the use of Pseudomonas in Gonbad cultivar and bacterial inoculation in Karim cultivar had no significant effect. The superiority of Karim cultivar over Gonbad cultivar is probably due to the genetically precocious of Karim cultivar and faster maturation process. In addition, different wheat cultivars have significant differences in cadmium accumulation in their organs because plants absorb more cadmium in stress conditions by secreting siderophore to compensate for nutrient deficiencies. Gonbad cultivar is probably more sensitive due to the absorption of heavy metals, which in turn produces more reactive oxygen, and consequently oxidative stress and degradation of photosynthetic proteins and a decrease in chlorophyll content. Among bacteria, genus pseudomonas increases plant phosphorus uptake due to its high ability to dissolve insoluble mineral phosphate. Phosphorus, as an energy carrier, increases the uptake and transport of nitrogen to the leaves, as a result in the production of higher amounts of chlorophyll. Pseudomonas may have produced more chlorophyll by increasing the uptake of trace elements by stimulating the activity of the ATPase protein pump and converting insoluble phosphate into a plant-usable form. The interaction of cultivar in bacteria was significant for carotenoids at 24 hours, flavonoids at 24, 72 and shoot stage at 1% level and for carotenoids at 72 hours and quantum yield at 48 hours at 5% level showed significance. Comparison of the mean interaction of cultivar in bacteria also showed that the highest amount of carotenoids (0.67 mg / g) was obtained in 24 hours from Gonbad cultivar and inoculation with Azotobacter, while in 72 hours of control treatment in Karim cultivar the highest carotenoids (0.61 mg / g) and did not differ significantly from Pseudomonas treatment. The decrease in carotenoids is probably due to their role in the non-photochemical suppression of excited chlorophylls, which disrupts the structure of these pigments and ultimately reduces the amount of these pigments. Cadmium appears to act as a degradation agent for pigments and other macromolecules by increasing ROS accumulation and degrading photosystem II. Non-photochemical suppression of excited chlorophylls can be another cause of degradation and reduction of auxiliary pigments. Among the main stressful effects on stem dry weight, yield, seed weight and number of seeds, the effect of cadmium on these traits (except number of seeds) was significant at the level of one percent. The interaction effect of cultivar on bacteria on stem dry weight (at 1% level) number of seeds (at 5% level) was significant and in other cases no significant. Regarding stem dry weight, Azotobacter was more effective than Pseudomonas on Karim cultivar and the number of seeds of Karim cultivar increased more in the presence of bacteria than the control. This is probably due to the higher resistance of Karim cultivar to cadmium toxicity and also due to the higher efficiency of carbon cycle enzymes in this cultivar.

Conclusions

The results showed that cadmium decreased photosynthetic pigments, increased auxiliary pigments (such as carotenoids) and decreased dry weight, yield, grain weight and number of grains in wheat plants studied. The presence of plant growth stimulating bacteria improved the photosynthetic system, dry weight, seed weight, number of seeds and yield. Application of Azotobacter growth promoting bacterium had the best results, so that in most of the studied traits, it improved the stress effects of cadmium; Therefore, Azotobacter can be used as a bacterium that reduces the stress effects of cadmium in Karim wheat.

***Keywords*:** Heavy metal, Growth-promoting rhizobacteria, Pigment, Wheat

Table 1- Analysis of variance of photosynthetic main pigments under cadmium chloride stress and growth-promoting bacteria in two bread wheat cultivars

S.O.V	df	Chlorophyll a 24h	Chlorophyll a 48h	Chlorophyll a 72h	Chlorophyll a Stem Stage	Chlorophyll b 24h	Chlorophyll b 48h	Chlorophyll b 72h	Chlorophyll b Stem Stage
Genotype(G)	1	0.129^{ns}	0.758*	1.751**	0.010^{ns}	0.0001^{ns}	0.332*	0.021*	0.004^{ns}
Bacteria(B)	2	0.003^{ns}	0.070^{ns}	0.072*	0.011^{ns}	0.073^{ns}	0.212^{ns}	0.023**	0.081^{ns}
Cadmium(C)	3	1.33**	4.73**	1.842**	0.652**	0.465**	1.96**	0.125**	0.294**
G×B	2	0.103^{ns}	0.246^{ns}	0.190*	0.048^{ns}	0.008^{ns}	0.03^{ns}	0.031**	0.015^{ns}
G×C	3	0.030^{ns}	0.125^{ns}	0.172^{ns}	0.020^{ns}	0.009^{ns}	0.105^{ns}	0.003^{ns}	0.021^{ns}
B×C	6	0.20**	0.121^{ns}	0.225**	0.078^{ns}	0.019^{ns}	0.047^{ns}	0.004^{ns}	0.021^{ns}
G×B×C	6	0.022^{ns}	0.086^{ns}	0.032^{ns}	0.032^{ns}	0.005^{ns}	0.027^{ns}	0.003^{ns}	0.017^{ns}
Error	48	0.051	0.142	0.042	0.066	0.019	0.088	0.003	0.029
CV (%)	-	*11.3*	*14.16*	*9.01*	*11.12*	*15.7*	*20.40*	*7.56*	*15.79*

Table 1. Continued

S.O.V	df	Chlorophyll total 24h	Chlorophyll total 48h	Chlorophyll total 72h	Chlorophyll total Stem Stage
Genotype(G)	1	0.086^{ns}	1.125*	1.38**	0.002^{ns}
Bacteria(B)	2	0.012^{ns}	0.066^{ns}	0.088*	0.011^{ns}
Cadmium(C)	3	1.75**	6.59**	1.879**	0.927**
G×B	2	0.092^{ns}	0.193^{ns}	0.210**	0.037^{ns}
G×C	3	0.027^{ns}	0.045^{ns}	0.124^{ns}	0.029^{ns}
B×C	6	0.188^{ns}	0.152^{ns}	0.195*	0.076^{ns}
G×B×C	6	0.014^{ns}	0.078^{ns}	0.028^{ns}	0.038^{ns}
Error	48	0.038	0.191	0.036	0.062
CV (%)	-	*8.9*	*14.37*	*7.82*	*9.74*

ns, * and ** Significant at 5% and 1% probability level

Table 2. Comparison of the average amount of photosynthetic pigments under the influence of cadmium stress, cultivar and growth-promoting bacteria

Treatments		Chlorophyll a (mgg^{-1}) 24h	Chlorophyll a (mgg^{-1}) 48h	Chlorophyll a (mgg^{-1}) 72h	Chlorophyll a (mgg^{-1}) Stem Stage	Chlorophyll b (mgg^{-1}) 24h	Chlorophyll b (mgg^{-1}) 48h	Chlorophyll b (mgg^{-1}) 72h	Chlorophyll b (mgg^{-1}) Stem Stage
Bacteria	**Control**	4.23^{a}	7.49^{a}	5.61^{a}	5.43^{a}	0.73^{a}	2.61^{a}	0.76^{a}	1.35^{a}
	Azotobacter	4.09^{a}	7.22^{a}	5.50^{ab}	5.56^{a}	0.85^{a}	2.25^{ab}	0.70^{b}	1.17^{ab}
	Pseudomonas	4.17^{a}	7.51^{a}	4.99^{b}	5.38^{a}	0.87^{a}	2.03^{b}	0.66^{b}	1.14^{b}
Genotype	**Karim**	3.98^{a}	7.88^{a}	4.71^{b}	5.40^{a}	0.83^{a}	2.55^{a}	0.73^{a}	1.25^{a}
	Gonbad	4.34^{a}	6.94^{b}	6.02^{a}	5.51^{a}	0.81^{a}	2.04^{b}	0.68^{b}	1.19^{a}
Cadmium (μM)	**0**	5.47^{a}	10.61^{a}	6.68^{a}	6.55^{a}	1.17^{a}	3.52^{a}	0.89^{a}	1.53^{a}
	75	4.49^{b}	8.27^{b}	6.09^{a}	5.77^{b}	0.88^{b}	2.61^{b}	0.73^{b}	1.36^{a}
	150	3.76^{c}	6.33^{c}	5.08^{b}	4.96^{c}	0.72^{b}	1.81^{c}	0.63^{c}	1.11^{b}
	300	2.95^{d}	4.42^{d}	3.61^{c}	4.54^{c}	0.49^{c}	1.24^{c}	0.56^{c}	0.89^{b}

Table 2. Continued

Treatments		Total chlorophyll (mgg^{-1})			
		24h	**48h**	**72h**	**Stem Stage**
Bacteria	**Control**	4.96^{a}	10.10^{a}	6.37^{a}	6.78^{a}
	Azotobacter	4.94^{a}	9.47^{a}	6.20^{a}	6.73^{a}
	Pseudomonas	5.03^{a}	9.55^{a}	5.56^{b}	6.52^{a}
Genotype	**Karim**	4.81 ^{a}	10.43 ^{a}	5.44 ^{b}	6.65 ^{a}
	Gonbad	5.15 ^{a}	8.98 ^{b}	6.70 ^{a}	6.71 ^{a}
Cadmium (µM)	**0**	6.64^{a}	14.14^{a}	7.57^{a}	8.08^{a}
	75	5.37^{b}	10.88^{b}	6.82^{b}	7.12^{b}
	150	4.46^{c}	8.14^{c}	5.72^{c}	6.07^{c}
	300	3.44^{d}	5.66^{d}	4.17^{d}	5.43^{c}

The presence of different letters in each column indicates a significant difference by the Duncan test at the 5% level

Table 3. Comparison of mean bacterial × genotype interactions on major pigments over 72 hours

Genotype	Bacteria	Chlorophyll a	Chlorophyll b	Total chlorophyll
		mgg^{-1}		
Karim	**Control**	4.64 ^{b}	0.74 ^{a}	5.38 ^{b}
	Azotobacter	4.69 ^{b}	0.70 ^{a}	5.39 ^{b}
	Pseudomonas	4.77 ^{b}	0.74 ^{a}	5.52 ^{b}
Gonbad	**Control**	6.56 ^{a}	0.77^{a}	7.34 ^{a}
	Azotobacter	6.30 ^{a}	0. 69 ^{a}	6.99 ^{a}
	Pseudomonas	5.19 ^{b}	0.56 ^{b}	5.76 ^{b}

The presence of different letters in each column indicates a significant difference by the Duncan test at the 5% level

Table 4. Comparison of the mean effect of the bacterium× Cadmium on chlorophyll a (24 and 72 h) and total chlorophyll (72 h)

Bacteria	Cadmium	(24h) Chlorophyll a	(72h) Chlorophyll a	(72h) Total chlorophyll
	µM	µgg^{-1}		
Control	**0**	5.67 ^{a}	7.09 ^{a}	8.04 ^{a}
	75	5.53 ^{a}	7.19 ^{a}	7.98 ^{ab}
	150	3.58^{c}	5.47^{c-e}	6.18^{d-f}
	300	2.15 ^{d}	2.66 ^{h}	3.25^{i}
***Azotobacte*r**	**0**	5.66 ^{a}	6.74^{ab}	7.69^{a-c}
	75	3.78 ^{c}	5.92^{b-d}	6.64^{c-e}
	150	3.63 ^{c}	5.11^{d-f}	5.69^{e-g}
	300	3.28 ^{c}	4.24^{fg}	4.77^{gh}
Pseudomonas	**0**	5.09 ^{ab}	6.19 $^{a-c}$	6.97^{b-d}
	75	4.15^{bc}	5.15^{c-f}	5.84 ^{ef}
	150	4.00 ^{c}	4.66^{e-g}	5.28^{f-h}
	300	3.42 ^{c}	3.92^{g}	4.48^{h}

The presence of different letters in each column indicates a significant difference by the Duncan test at the 5% level

Table 5. Analysis of variance of auxiliary pigments under cadmium chloride stress and plant growth-promoting bacteria in two bread wheat cultivars

S.O.V	df	Carotenoids				Flavonoids			
		24h	48h	72h	Stem Stage	24h	48h	72h	Stem Stage
Genotype (G)	1	0.021^{ns}	0.118^{ns}	0.0004^{ns}	0.009^{ns}	0.028^{ns}	0.636^{ns}	0.644**	1.00**
Bacteria (B)	2	0.036**	0.006^{ns}	0.004^{ns}	0.008^{ns}	2.422**	2.538**	0.630**	0.322**
Cadmium (C)	3	0.123**	0.273**	0.077**	0.070**	3.224**	4.377**	2.174**	1.439**
G×B	2	0.046**	0.176^{ns}	0.013*	0.002^{ns}	1.435**	0.392^{ns}	1.209**	1.596**
G×C	3	0.014^{ns}	0.040^{ns}	0.001^{ns}	0.002^{ns}	0.034^{ns}	0.081^{ns}	0.062^{ns}	0.114*
B×C	6	0.004^{ns}	0.009^{ns}	0.0005^{ns}	0.001^{ns}	0.024^{ns}	0.113^{ns}	0.062^{ns}	0.018^{ns}
G×B×C	6	0.001^{ns}	0.010^{ns}	0.003^{ns}	0.005^{ns}	0.031^{ns}	0.146^{ns}	0.042^{ns}	0.022^{ns}
Error	48	0.006	0.028	0.003	0.004	0.089	0.298	0.048	0.058
CV (%)	-	*11.44*	*17.73*	*7.22*	*7.97*	*9.75*	*16.30*	*5.72*	*6.71*

ns, * and ** Significant at 5% and 1% probability levels

Table 6. Analysis of variance of quantum yield and SPAD under cadmium chloride stress and growth-promoting bacteria in two bread wheat cultivars.

S.O.V	df	Quantum yield				SPAD			
		24h	48h	72h	Stem Stage	24h	48h	72h	Stem Stage
Genotype (G)	1	0.029^{ns}	0.021**	0.0009^{ns}	0.014**	0.015^{ns}	0.071^{ns}	0.012^{ns}	0. 032^{ns}
Bacteria (B)	2	0.050**	0.043**	0.038**	0.008**	0.571**	0.348**	0.348**	0.623**
Cadmium (C)	3	0.106**	0.127**	0.170**	0.112**	1.159**	0.187**	0.482**	0.509**
G×B	2	0.0007^{ns}	0.007*	0.0004^{ns}	0.001^{ns}	0.100^{ns}	0.055^{ns}	0.019^{ns}	0.024^{ns}
G×C	3	0.0038^{ns}	0.005*	0.0009^{ns}	0.0006^{ns}	0.043^{ns}	0.076^{ns}	0.098*	0.006^{ns}
B×C	6	0.006^{ns}	0.009**	0.008**	0.002^{ns}	0.135**	0.103**	0.136**	0.028^{ns}
G×B×C	6	0.002^{ns}	0.003^{ns}	0.001^{ns}	0.001^{ns}	0.054^{ns}	0.049^{ns}	0.074*	0.033^{ns}
Error	48	0.003	0.001	0.0008	0.001	0.028	0.030	0.031	0.041
CV (%)		*6.62*	*4.83*	*3.73*	*4.74*	*7.54*	*7.45*	*8.52*	*11.14*

ns, * and ** Significant at 5% and 1% probability levels,

Table 7. Comparison of mean main effects of cultivar, bacterium and cadmium on auxiliary pigments, quantum yield and SPAD.

Treatments		Carotenoids (mgg^{-1})				Flavonoids (µgg^{-1})			
		24h	48h	72h	Stem Stage	24h	48h	72h	Stem Stage
Bacteria	Control	0.56^{a}	0.98^{a}	0.62^{a}	0.72^{a}	7.70^{b}	9.30^{b}	13.65^{b}	12.47^{b}
	Azotobacter	0.46^{b}	0.93^{a}	0.63^{a}	0.66^{a}	10.90^{a}	13.21^{a}	15.27^{a}	13.54^{a}
	Pseudomonas	0.48^{b}	0.95^{a}	0.58^{a}	0.67^{a}	10.71^{a}	12.63^{a}	15.79^{a}	13.72^{a}
Genotype	Karim	0.48^{a}	1.04^{a}	0.61^{a}	0.70^{a}	9.60^{a}	12.51^{a}	15.63^{a}	14.07^{a}
	Gonbad	0.52^{a}	0.87^{b}	0.61^{a}	0.66^{a}	9.94^{a}	10.92^{b}	14.18^{b}	12.42^{b}
Cadmium (µM)	0	0.39^{c}	0.73^{c}	0.50^{d}	0.65^{b}	13.15^{a}	16.03^{a}	17.75^{a}	15.86^{a}
	75	0.65^{a}	1/26^{a}	0.73^{a}	0.79^{a}	10.31^{b}	12.35^{b}	16.15^{b}	13.79^{b}
	150	0.52^{b}	1.03^{b}	0.65^{b}	0.73^{a}	8.76^{c}	10.62^{b}	13.99^{c}	12.32^{c}
	300	0.43^{c}	0.80^{c}	0.56^{c}	0.56^{c}	6.86^{d}	7.85^{c}	11.72^{d}	11.02^{d}

Table 7. Continued

Treatments		Quantum yield				SPAD			
		24h	48h	72h	Stem Stage	24h	48h	72h	Stem Stage
Bacteria	*Control*	0.67c	0.62b	0.58b	0.55b	4.46b	5.00b	3.89b	2.79b
	Azotobacter	0.75b	0.73a	0.68a	0.58ab	5.38a	6.01a	4.60a	3.79a
	Pseudomonas	0.82a	0.73a	0.68a	0.60a	5.57a	5.70a	4.65a	3.76a
Genotype	Karim	0.78a	0.72a	0.65a	0.60 a	5.25a	5.68a	4.37a	3.38 a
	Gonbad	0.71b	0.66b	0.64a	0.55b	5.02a	5.46b	4.39a	3.51 a
Cadmium (μM)	0	0.90a	0.86a	0.79a	0.70a	6.22a	5.92a	5.19a	4.06a
	75	0.78b	0.74b	0.72b	0.64b	5.55b	5.79a	4.53b	3.71ab
	150	0.71c	0.63c	0.63c	0.53c	5.09b	5.61a	4.15b	3.34b
	300	0.59d	0.54d	0.44d	0.43d	3.68c	4.96b	3.65c	2/.67c

Table 8.Comparison of the mean interaction of cultivar in bacteria on auxiliary pigments and quantum yield

Treatments		Carotenoids (mgg^{-1})		Flavonoids(μgg^{-1})			Quantum yield
Genotype	Bacteria	24 h	72h	Stem Stage	24 h	72h	48h
Karim	Control	0.61a	0.64ab	9.24 c	16.28a	15.30 a	0.62 d
	Azotobacter	0.40d	0.58bc	9.56c	15.05a	13.61 b	0.79a
	Pseudomonas	0.43cd	0.59bc	9.99 c	15.55a	13.30 b	0.76 a
Gonbad	Control	0.51bc	0.59bc	6.17 d	11.03b	9.64c	0.62cd
	Azotobacter	0.51bc	0.67a	12.24a	15.49a	13.47b	0.67 cb
	Pseudomonas	0.53ab	0.57c	11. 43 ab	16.02a	14.15ab	0.70 b

The presence of different letters in each column indicates a significant difference by the Duncan test at the 5% level

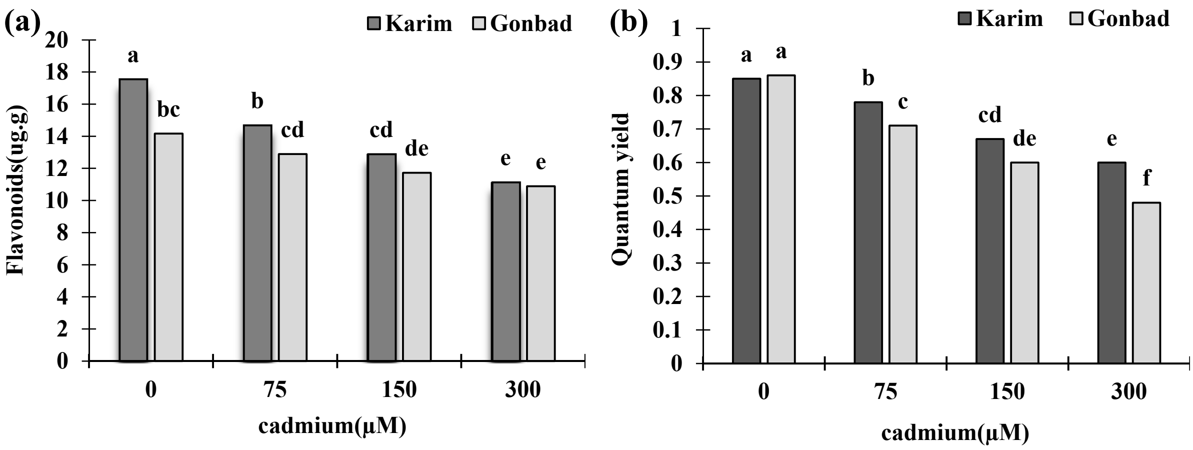

Fig. 1. Comparison of the mean interaction of the number in the medium on flavonoids (a) and quantum yield (b)

Table 9. Differential analysis of dry weight, yield, grain weight and number of grains under stress of cadmium chloride and the effect of stimulating bacteria in two cultivars of wheat bread

S.O.V	df	Dry shoot weight	Yeild	Grain weight	Number of grains
Genotype (G)	1	0.001^{ns}	51.59^{**}	11.84^{**}	0.0861^{ns}
Bacteria (B)	2	0.099^{**}	16.24^{**}	0.516^{*}	0.148^{ns}
Cadmium (C)	3	0.124^{**}	33.45^{**}	1.094^{**}	0.454^{*}
G×B	2	0.089^{**}	2.64^{ns}	0.408^{ns}	0.396^{*}
G×C	3	0.004^{ns}	3.82^{ns}	0.191^{ns}	0.087^{ns}
B×C	6	0.008^{ns}	0.827^{ns}	0.064^{ns}	0.016^{ns}
G×B×C	6	0.002^{ns}	0.684^{ns}	0.082^{ns}	0.004^{ns}
Error	48	0.009	2.16	0.116	0.110
CV (%)	-	8.49	11.35	6.74	12.97

ns, * and ** Significant at 5% and 1% probability levels

Table 10. Comparison of average dry weight, yield, grain weight and number of grains under stress of cadmium chloride and the effect of stimulating bacteria in two cultivars of bread wheat

Treatments		Dry shoot weight	Yeild	Grain weight one hundred grains	Number of grains
		g	mg per plant	mg	
Bacteria	Control	1.19^{a}	148.7^{b}	24.46^{b}	6.28^{a}
	Azotobacter	1.44^{a}	189.6^{a}	26.96^{a}	6.99^{a}
	Pseudomonas	1.21^{b}	179.9^{a}	26.75^{a}	6.79^{a}
Genotype	Karim	1.29^{a}	195.5^{a}	30.22^{a}	6.85^{a}
	Gonbad	1.27^{a}	150.0^{b}	21.88^{b}	6.52^{a}
Cadmium (μM)	0	1.46^{a}	195.0^{a}	28.61^{a}	6.94^{a}
	75	1.36^{ab}	193.4^{a}	26.66^{ab}	7.39^{a}
	150	1.24^{b}	179.5^{a}	26.22^{b}	6.87^{a}
	300	1.05^{c}	123.0^{b}	22.72^{c}	5.55^{b}

The presence of different letters in each column indicates a significant difference by the Duncan test at the 5% level

Table 11. Comparison of the mean interaction of bacteria, genotype on dry stem weight and number of seeds

Genotype	Bacteria	Dry shoot Weight (g)	Number of grains
Karim	Control	1.08^{c}	5.35^{b}
	Azotobacter	1.61^{a}	7.14^{a}
	Pseudomonas	1.20^{bc}	7.10^{a}
Gonbad	Control	1.30^{b}	7.22^{a}
	Azotobacter	1.28^{b}	6.86^{a}
	Pseudomonas	1.21^{bc}	6.48^{ab}

The presence of different letters in each column indicates a significant difference by the Duncan test at the 5% level