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The effect of PGPR (Plant-Growth-Promoting Rhizobacteria) on phytoremediation of cadmiums by canola (*Brassica napus* L.) cultivars of Hyola 401

Nazila Heshmatpure and Mojtaba Yousefi Rad

Department Agronomy, Faculty Agriculture, Islamic Azad University, Saveh Beranch, Saveh, Iran

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ABSTRACT

Cadmium is a heavy metal which induces oxidative stress in plants. This ion is extremely toxic to plants and animals. This study investigates the effects of Cadmium different levels in soil and the species of co-existing micro-organism on the morphological features of canola (Hyola 401 cultivar). The experiment was conducted at Research Farm Greenhouse of Islamic Azad University, Saveh Branch in 2010, as factorial experiment based on completely randomized blocks design with three replication. The studied factors were: Cadmium nitrate (0, 0.1, 1 and 3 mg/kg) and PGPR (without inoculation and inoculation by Pseudomonas fluorescence, strains 11, 4, 169, 11+4, 11+169, 4+169, 11+169+4).The study showed that cadmium exposure led to reduction in fresh and dry weight of root and shoot and stem height, a significant decrease in the number leaves and pods of canola. These effects were intensified by increasing cadmium concentration in soil. Some Plant growth-promoting rhizobacteria strains enhanced plant growth as compared with non-inoculated plants (control). In addition, strains 4, 11 exhibited a higher capacity in coexistence with plant. On the other hand, the results showed that in the presence of different value of cadmium soil, strains 4 and 11 significantly increased plant growth and the cadmium extraction efficiency from soil.

Keywords: Plant Growth Promoting Bacteria, Concentration of Cadmium, Content of Cadmium, Canola (*Brassica napus* L).

INTRODUCTION

Heavy metal pollution by industrial, agricultural and municipal wastewater is seriously threatening the environment of coastal regions, ponds and rivers. Aquatic animals accumulate these contaminants through the food web and release them into the ecosystem, which consequently affects the quality of organic products (8). Considering the ongoing growth of the human population and the level of organic and mineral pollution, it seems vital to find a safe and rather quick method for eliminating these pollutions with the least cost and side effects on the environment. Phytoremediation is one of the best methods for this purpose (1). Effects of heavy metals on plants result in growth inhibition, structure damage, a decline of physiological and biochemical activities as well as of the function of plants. The effects and bioavailability of heavy metals depend on many factors, such as environmental conditions, pH, species of element, organic substances of the media and fertilization, plant species (18). But, there are also studies on plant resistance mechanisms to protect plants against the toxic effects of heavy metals such as combining heavy metals by proteins and expressing of detoxifying enzyme and nucleic acid, these mechanisms are integrated to protect the plants against injury by heavy metals. There are two aspects on the interaction of plants and heavy metals. On one hand, heavy metals show negative effects on plants. On the other hand, plants have their own resistance mechanisms against toxic effects and for detoxifying heavy metal pollution (18). Cadmium is a toxic element which can be spread in nature. Industries, municipal sewage, fuels and chemical fertilizers, especially phosphate ones are among the major sources of this contaminant (2, 14). This metal can be easily absorbed by plants' root and enter the wood tissues via apoplastic and symplastic pathways (13). According to studies, Cadmium causes chlorosis and necrosis of the leaf (9, 3) and damages cell division and growing , affects cell division in the meristematic regions and harms plant's overall growth and health. (17). Plant-Growth-Promoting Rhizobacteria are a group of useful rhizosphere bacteria which can enhance plant growth by direct mechanisms (fixation of nitrogen, production of growth-regulating agents, increasing absorbability of nutrients for the plant, producing growth stimulating hormones and vitamins such as Oxine , Cytokinin and Gibberllin) and indirect mechanisms (production of antibiotics, discharging rhizosphere from iron, competing with root occupying species, generating systemic resistance in the plant, and increasing plant resistance against stresses caused by non-living factors (4). Canola is the most important species in the genus Brassica. Special qualities of this plant, such as capability to be grown in different regions, high oil content, ideal quality of the oil, and its press cake's usage in feeding livestock (11) has made its cultivation attractive for supplying the country's need of raw oil and getting rid of dependency (10) . As an oil seed with more than 40% oil content, Canola is considered as one of the most important plants in development process of oilseeds cultivation and production of vegetable oils in Iran (11). Moreover, Hyola 401 is a cultivar of spring that it does not need verbalization. This cultivar's yield is 2.3-2.8 tons per hectare, with 43-45% oil content, and is considered as an option for sequence cultivation with grains (7, 11). This research is studies ability of absorbing of Cadmium and cleaning up contaminated soil by Canola in presence Plant-Growth-Promoting Rhizobacteria.

MATERIALS AND METHODS

This research was performed in 2010 at Agriculture college research greenhouse of Islamic Azad University, Saveh Branch. Experiment was carried out as factorial based on completely randomized blocks design with three replication. The first factor included inoculation of plants by Plant Growth Promoting Rhizobacteria of *Pseudomonas fluorescence* strains 11, 4, 169 and strains combination 11+4, 11+169, 4+169, 11+169+4 and without inoculation (Control plant). The secondary factor included three levels of cadmium nitrate (0.1, 1, 3) mg/kg and control (no apply salt). Rapeseed (*Brassic*a *napus* L.Hyola 401) is oil seed plant that is not required to verbalization (7). Experiment was conducted as pot and the investigated soil properties are given in Table 1.

Table 1. Some physical and chemical properties of soil

FC	EC ds/m	PH (soil/water)	T.N.V %	O.C. %	Total N %	P (ava) p.p.m	K (ava) p.p.m	Clay %	Silt %	Sand %	Soil texture
15/8	2/9	7/7	13/7	1/3	0/1	65/5	315	14	16	70	Sandy loam

EC= Electrical Conductivity, O.C= Organic Carbon %, T.N.V= Total Neutralizing Value, FC=Field Capacity

Afterward, we consumed 0.8, 8, and 24 mg. of Cadmium in an 8 Kg. pot for obtaining different levels of 0.1, 1, and 3 mg. per Kilogram of soil. The method was first to solve the salt into the water, and then to add the solution to the soil to achieve the field capacity. Next, we put the mixture in plastic bags and stored for 1 month to let the salt to stabilize well into the soil. Then, we filled the experiment pots equally with non-sterile soil and placed them in the greenhouse. Canola seeds mixed with 20 ml of 20% sugar solution, for inoculate of seeds with micro-organism. Then amount of 20gr inoculum was added to the sticky seeds and $CdNO_2$ solution was added to the pots. Seeds were planted in depth of 2 cm each pot, plants were harvested by cutting the shoots from the soil surface. Plant shoots and roots were dried at oven-dried 75^0C for 48 hours. In this experiment studied properties as stem height, root and shoot dry and wet weight, leaf number, pod number, phosphorus concentration and content of roots and shoots. Samples of Cd^2, P extracts were analyzed by atomic absorption and spectrophotometer, respectively. The variance analyses were carried out by SAS software, the mean was compared by Duncan test in 5% level, and the diagrams were drawn by Excel.

RESULTS AND DISCUSSION

The results of variance analysis showed that the effect of Cadmium level on the length and fresh/dry weight of roots was significant ($P<0.01$), and it was significant on wet weight of shoots ($P<0.05$). This factor had no effect on properties such as shoot dry weight, the number of pods (Table 2). The effect of bacterial inoculation on height and pod number was significant ($P<0.01$), and it was significant on wet weight shoots ($P<0.05$), but it had no effect on other properties such as wet and dry root weight, dry shoots weight. Interaction two factors were not significant on studied traits (Table2).

The result of mean comparison shows that Cd_4 has the lowest dry and fresh weight of shoots and roots, and lowest pod number, height, nevertheless, another cadmium levels had no significant on studied traits (Table 3). On the other hand, $Cd_{1, 2, 3}$ had the highest fresh weight (Table3).

Table2. Variance analysis of effect of studied factors on morphological characteristics canola

source	df	Root dry weight	Shoot dry weight	Root wet weight	Shoot wet weight	Number of pods	Height
block	2	29/73**	0/55ns	2/039ns	3/63ns	208/53ns	45/627ns
bacteria	7	0/44ns	1/70ns	4/058ns	3/70*	840/99**	113/50**
Cadmium	3	5/43**	2/74ns	9/215**	5/060*	593/375ns	159/002**
Bacteria*Cd	21	1/45ns	0/488ns	1/766ns	1/266ns	333/517ns	33/89ns
Error	62	1/32	1/35	2/658	1/993	232/090	33/57

Significant in statistical level 5%* * Significant in statistical level 1%* *n.s: Not significant*

Table3: Mean comparison of effect of cadmium on morphological characteristics of Canola

treatment	Shoot dry weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Root fresh weight (g)	Height	Number of pods
Cd1	4/313ab	3/405ab	6/066a	7/612a	32/145a	20/125a
Cd2	4/353a	3/615ab	6/717a	6/938a	29/791a	23/875a
Cd3	4/615a	3/929a	6/505a	7/201a	26/041b	14/625b
Cd4	3/806b	2/8b	5/7b	5/919b	24/541c	13/125b

(Cd1=Control, Cd2= Cadmium 0/1 mg/kg, Cd3= Cadmium 1mg/kg, Cd4= Cadmium 3mg/kg)

In Table 3 is seen $Cd_{1,2}$ has the lowest effect on plant's height and also $Cd_{1,2}$ has the lowest effect on the number of pods, while the effect of $Cd_{3,4}$ is the more on pod number. The result of variance analysis also showed that the effect of bacterial inoculation and cadmium on cadmium concentration and content of roots and shoots was significant ($P<0.05$).

The interaction of both factors on cadmium concentration and content of roots and shoots was not significant. On the other hand, the effect of cadmium on the roots phosphorus content was significant ($P<0.01$), while it showed no meaningful effect on other properties. Likewise, the inoculation factors show that effect on root phosphorus concentration and content of roots and shoots is significant ($P<0.05$) but is not significant on shoot phosphorus concentration of rapeseed (Tables4, 5).

Table 4: Variance analysis of effect of studied factors on concentration and content of Cadmium, Phosphate canola organs.

Source	df	Shoot Cadmium concentration	Root Cadmium concentration	Shoot cadmium content	Root cadmium content
Block	2	0/003ns	0/072*	0/019ns	0/897*
Bacteria	7	0/004*	0/020*	0/156*	0/423*
Cadmium	3	0/001*	0/026*	0/029*	0/616*
Bacteria*Cd	21	0/001ns	0/028ns	0/038ns	0/286ns
Error	62	0/003	0/020	0/102	0/288

Significant in statistical level 5%* * Significant in statistical level 1%* *n.s: Not significant*

Table 5: Variance analysis of effect of studied factors on concentration and content of Cadmium, Phosphate canola organs.

Source	df	Shoot phosphate concentration	Root phosphate concentration	Shoot Phosphate content	Root Phosphate content
Block	2	112/93**	144/23**	1358/39*	2646/88**
bacteria	7	8/34ns	8/99*	323/82*	203/94*
Cadmium	3	8/92ns	29/025ns	685/64ns	1250/94**
Bacteria*Cd	21	5/68ns	2/400ns	154/20	176/44ns
Error	62	8/544	11/45	278/17	234/45

Significant in statistical level 5%* * Significant in statistical level 1%* *n.s: Not significant*

As in figures 9, 10, 11 and 12 is observed the highest cadmium content and concentration in shoots and roots is in Cd_4. Therefore Cd concentrations in plant different organs increased with increasing cadmium concentration in soil. Comparing of average effect of soil Cadmium on Phosphorus concentration of shoots in figures 13 and 14 shows phosphorus concentration of shoots don't react to use of Cd whiles only Cd4 decreased root phosphate concentration. Results of this research suggest that the presence of Cadmium in soil had no effect on Phosphate concentration in different organs Canola.

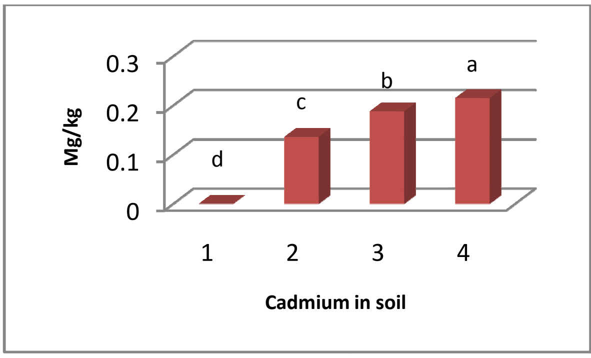

Figure 9: Effect of cadmium on shoot cadmium Concentration.

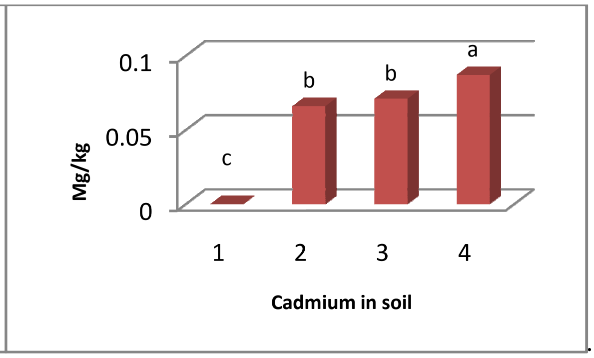

Figure10: Effect of cadmium on root cadmium Concentration.

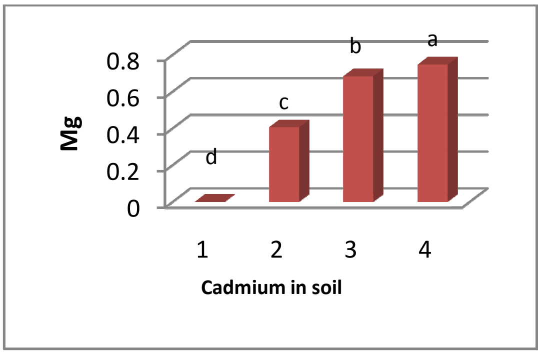

Figure11: Effect of Cadmium on shoot cadmium content.

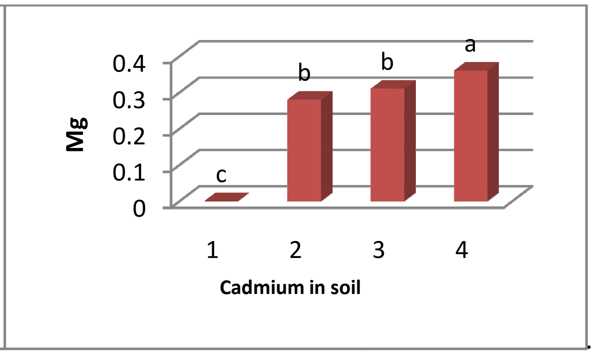

Figure 12: Effect of cadmium on root cadmium content.

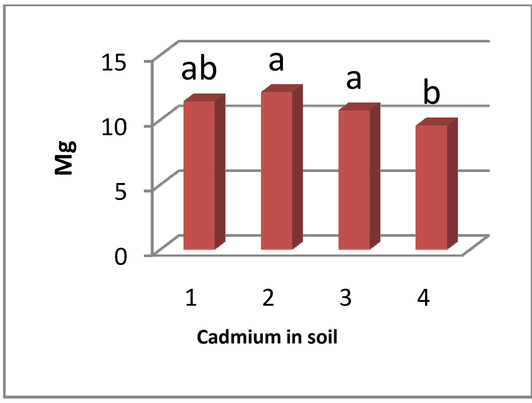

Figure13: Effect of Cadmium on shoot concentration Phosphate.

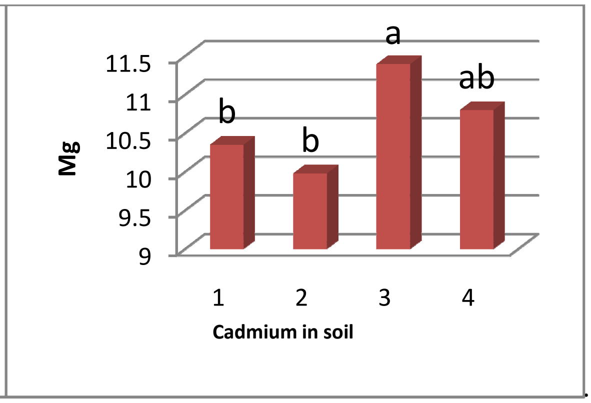

Figure14: Effect of Cadmium on root concentration Phosphate.

Cadmium causes a delay in plant growth process by reducing the mitosis division rate (12). Mayak et al. (2004) points out that under stressful conditions and in times of food poisoning, the production rate of Ethylene increases compared to normal conditions (19, 20). Some researchers believe that the increase in ethylene production rate under stress conditions causes a decrease in growth rate and yield of a plant (6).

Glick et al. (2003) notifies that Ethylene hormone acts as a restriction factor deterring root growth and decelerates the plant growth process(5). Nonetheless, the inoculation of Canola by *Pseudomonas fluorescent* bacteria results an increase in plant resistance against cadmium concentration in soil; which shows the positive effect of these strains on reducing the level of Ethylene in the plant under stress. Mayak et al (2004) in a study on tomatoes indicated that Growth Promoting Rhizobacteria can decrease the negative effect of salt stress and heavy metal contamination of soil and increase the dry/fresh weight of the plant, compared to control plant(19,20). They believe that PGPR make it happen by producing ACC deaminase and reducing Ethylene production. In this research observed that in the presence of cadmium, the strains of 4 and 11 can intensify the morphological properties, such as fresh and dry weight of the roots and shoots, length of the stem, and the number of leaves and pods. The Pseudomonas bacteria increase the immunity of the plants against negative effects of heavy metals like Cd, Zn, Cu, etc (15). The evidences demonstrated that most of the combinations of *Pseudomonas fluorescent* bacteria did not have a positive effect on the growth rate of the plant; probably due to the competition amongst themselves. Only the combination of 169+4+11 and 169+4 strains remarkably contributed in absorption and concentration attributes; as a result of their synergetic effect on each other and on the absorption properties of the plant and coexisting better with canola (Tables 6, 7, 8).

Table6: Effect of Bacteria*Cadmium on morphological characteristics Canola

Number	Cd	B	Root Fresh weight	Shoot Fresh weight	Number	Cd	B	Shoot Fresh weight	Root Fresh weight
1	1	1	7/71a	4/81e	17	1	5	4/886de	4/99cd
2	2	1	6/14c	7/59a	18	2	5	5/376d	6/90bc
3	3	1	6/73bc	6/31c	19	3	5	4/893de	7/38ab
4	4	1	4/88cd	5/44d	20	4	5	4/990de	4/40d
5	1	2	5/82c	6/14cd	21	1	6	6/703bc	5/76c
6	2	2	8/006a	7/083ab	22	2	6	5/990a	6/77bc
7	3	2	6/66bc	6/45bc	23	3	6	6/896ab	8/003a
8	4	2	7/40ab	6/32a	24	4	6	4/856de	5/93c
9	1	3	7/24bc	5/97cd	25	1	7	5/306de	5/14cd
10	2	3	7/57a	7/403a	26	2	7	7/203ab	6/09c
11	3	3	8/29a	7/443a	27	3	7	5/943a	6/81bc
12	4	3	6/42bc	6/07cd	28	4	7	5/996cd	4/91cd
13	1	4	6/60bc	7/326a	29	1	8	7/376a	5/74cd
14	2	4	7/07bc	6/626bc	30	2	8	6/470c	6/93bc
15	3	4	7/45ab	7/280ab	31	3	8	6/823bc	6/27c
16	4	4	7/32ab	5/673d	32	4	8	6/240c	6/08c

(B=bacteria, 1=use of bacteria, 2=not application of bacteria, Cd=Cadmium)

Table7: Effect of Bacteria*Cadmium on morphological characteristics Canola

Number	Cd	B	Height	Number of pods	Number	Cd	B	Height	Number of pods
1	1	1	20/50e	5/66gh	17	1	5	14/66f	3h
2	2	1	31/66ab	29c	18	2	5	29/33bc	13/66f
3	3	1	28/66bc	14/33ef	19	3	5	24/33d	6/66gh
4	4	1	24d	20de	20	4	5	20/66e	3/66h
5	1	2	24d	17e	21	1	6	26abc	24/66d
6	2	2	34a	12/66bc	22	2	6	27/66dc	16/66e
7	3	2	30/33b	2/33h	23	3	6	26/33cd	12/33f
8	4	2	28/66b	9/66g	24	4	6	19/33e	10/66g
9	1	3	30/33b	23/66d	25	1	7	23/33d	7/66gh
10	2	3	33/33a	17e	26	2	7	26/66dc	31c
11	3	3	23/33d	17/3e	27	3	7	24/66d	23/33d
12	4	3	25/33d	15/66e	28	4	7	24d	7gh
13	1	4	34/33a	66a	29	1	8	20e	13/33f
14	2	4	32/33ab	41/33b	30	2	8	23/33d	29/66c
15	3	4	26/33cd	16/66e	31	3	8	24/33d	24d
16	4	4	30/66b	15e	32	4	8	23/66d	123/33d

(B=bacteria ,1=use of bacteria , 2=not application of bacteria, Cd=Cadmium)

Table8: Effect of Bacteria*Cadmium on morphological characteristics Canola

Number	Cd	B	Root dry weight	Shoot dry weight	Number	Cd	B	Root dry weight	Shoot dry weight
1	1	1	5/15a	4/27bc	17	1	5	2/73cd	3/82c
2	2	1	2/69cd	5/14ab	18	2	5	4/42ab	3/19cd
3	3	1	3/97b	4/50b	19	3	5	3/17c	3/80c
4	4	1	1/59e	3/84c	20	4	5	2/17d	2/97d
5	1	2	2/74cd	4/61b	21	1	6	3/40bc	4/31bc
6	2	2	3/65bc	4/05c	22	2	6	3/54bc	4/26bc
7	3	2	4/71bc	5/07ab	23	3	6	4/04b	4/49b
8	4	2	3/24c	4/22bc	24	4	6	3/10c	3/69cd
9	1	3	3/35c	4/29cb	25	1	7	2/76cd	3/62cd
10	2	3	3/60bc	4/72b	26	2	7	3/68bc	4/68b
11	3	3	5/04a	4/86ab	27	3	7	4/06b	4/10bc
12	4	3	2/73cd	4/39bc	28	4	7	2/58bcd	3/84c
13	1	4	3/66bc	4/79ab	29	1	8	3/42bc	4/77ab
14	2	4	3/44bc	3/99c	30	2	8	3/88bc	4/76ab
15	3	4	3/90b	5/63a	31	3	8	3/51bc	4/44b
16	4	4	3/40bc	3/76c	32	4	8	3/57bc	3/71cd

(B=bacteria ,1=use of bacteria , 2=not application of bacteria, Cd=Cadmium)

In figures 15,16 is show that, Mean comparison of the effects of bacterial inoculation on shoot and root cadmium concentration show that Inoculation with different strains was ineffective and strains of 4 ,11,169 has lower Cadmium concentration but strains of 11+169 , 169 +4, 169+11+4 have most cadmium concentration.

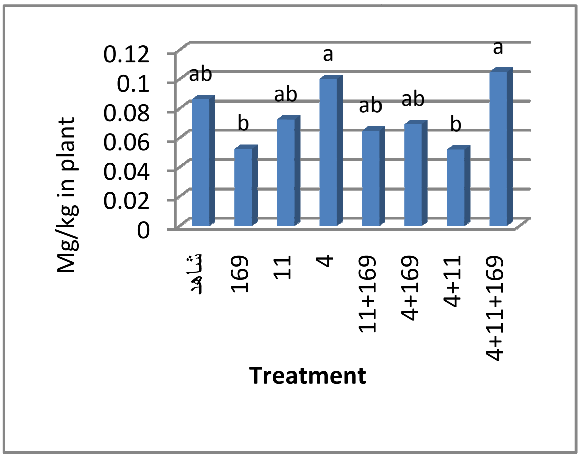

Figure15: Effect of bacteria on shoot Cadmium Concentration.

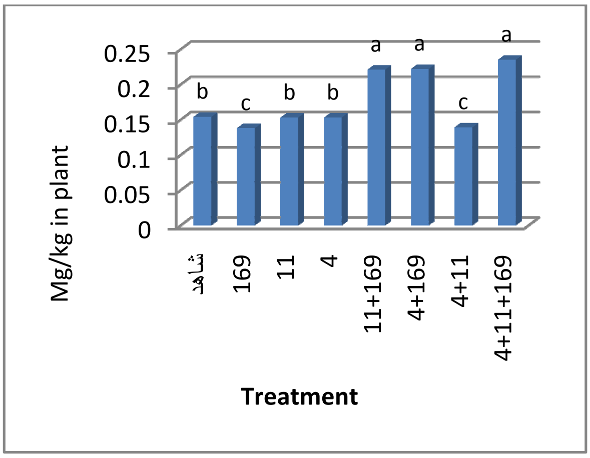

Figure16: Effect of bacteria on root Cadmium Concentration.

Pseudomonas fluorescence Strain 4,11,169 has successful and important role on improvement growth of rapeseed and morphological characteristic On the other hand , the combination of this bacteria same as combinations *Pseudomonas fluorescence* strains (169+4, 169+11+4) have an important role in phosphate absorption and phytoremediation (16). Auxin-producing bacteria and nitrogen stabilizer increased root length, plant growth and nutrient uptake in the presence of toxic concentrations of Cadmium with Sedentary to cadmium (15).

CONCLUSION

We can also infer that bacteria strains 4 and 11 are able to resist against high levels of Cadmium and modify the adverse effect of this metal. The PGPR can also promote the efficiency of Phytoremediation in presence of cadmium and increase plant resistance against Cadmium toxicity. In conclusion, our study shows that Plant-Growth-Promoting Rhizobacteria (PGPR) can deactivate Cadmium and increase the plant growth, the total growth of canola, and intensify the absorption of nutrients, such as Phosphorus and Calcium.

REFERENCES

[1] AA Erakhrumen; Phytoremediation: *Journal of Educational Research and Review*, **2007**, 2, 7, pp. 151-156.
[2] A Baryla; P Carrier; F Franck; C Coulomb; C Sahut; M Havaux; *Journal of stress physiology and Biochemistry*, **2001**, 212, 7, pp. 696-709.
[3] AN Hernandez; M Heydrich; *Tropicales Journal*, **1995**, 6, PP. 5-8.
[4] BR Glick; CL Patten; G Holguin; DM Penrose. *Journal of organic pollutants*, **1999**, 24, pp. 86-179.
[5] BR Glick; *Biotechnology Advances*, **2003**, 21, 5, pp. 383–393.
[6] BR Glick; *Journal of Plant physiol*, **2004**, 251, pp. 1-7.
[7] ES Oplinger; LL Hardman; ET Gritton; JD Doll; KA Kelling; *Alternative Field Crops Manual Journal*, **1989**, 2, 3 pp.39-50.
[8] FA Abadati; S Smaeili; A Riyahi Bakhtiyari; *Journal of environmental studies*,**2005**, 31, 37.
[9] G Zhang; M Fukami; H Sekimoto; *Field crops researches*, **2002**, 77, pp. 93-98.
[10] H Aliyary; V Shekari; *Oilseed plant. Agriculture and physiology*. **2000**, 7,182.
[11] H Kandel; JJ Knodel; Canola production Field guide, North Dakota Weed Control Guide (NDSU) Extension Services, 1st.ed, **2011**, 1280 , PP.4-14.
[12] J Hart; R M Welch; A Wendell; WA Norvell; L A Sullivan; V Kochian; *Journal of Plant Physiology*, **1998**, 116, pp.1413-1420.
[13] L Sanita di Toppi; R Gabbrielli; *Environ Journal*, **1999**, 41, pp.105-130.
[14] M Mejare; L Bulow. *Trends in Biotechnology Journal*, **2001**, 19, pp.67-72.
[15] M Turan; A Esringü; *Plant Soil Environ Journal*, **2007**, 53,1, pp. 7–15.
[16] P Abbaszadeh dehjy; N Saleh Rastin; H Asadi rahmani; K Khavazi; M.CS Thesis. Introduction of Pseudomonas fluorescent bacteria solver as insoluble inorganic phosphates. Department of Agronomy Tehran University, Iran, **2007**.

[17] P Das; S Samantaray; GR Rout; *Journal of Environmental pollution*, **1997**, 98, pp.29-36.
[18] SHP Cheng; *Journal of Environmental science and pollution research*, **2003**, 10, 4, pp. 256-264.
[19] S Mayak; T Tirosh; BR Glick; *Journal of Plant Sci*, **2004a**, 166, pp. 525–530.
[20] S Mayak; T Tirosh; BR Glick; *Plant physiology Journal*, **2004b**, 42, pp. 565–572.