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Benzyladenine Alleviates the Lead Toxicity in Roselle (*Hibiscus sabdariffa* L.) Plants

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ABSTRACT

Results showed that the plants exposed to Pb exhibited a substantial decline in growth, yield, pigment content, anthocyanin, total carbohydrates, nutrient content and catalase (CAT) of roselle plants. However, pretreatment with benzyladenine (BA) mitigated the stress generated by Pb and markedly improved the aforesaid parameters. The Pb increased proline and Pb content in plant. However, the BA treatment attenuated the adverse effects of Pb on these attributes. Dual application of both Pb and BA caused significant increases in the previously mentioned characters compared with the individual treatment in both seasons. Also, the results showed that pretreatment with BA enhanced the antioxidant defense activities in Pb stressed roselle, thus alleviating Pb induced oxidative damage and enhancing Pb tolerance.

Key words: Benzyladenine (BA), lead (Pb), roselle, growth, yield, chemical constituents.

Introduction

Roselle (*Hibiscus sabdariffa* L.) belongs to the family Malvaceae. It is known commonly as "Karkade" in Egypt and most Arab countries (Mohamed *et al.,* 2007). It is one of the most important medicinal plants used in traditional folk medicine. El-Sheikh *et al.* (1990) found that an aqueous extract of roselle at 50-100mg/l was toxic to cercariae and miracidia of *Schistosoma mansonii*. The part of the flower used by customers is the dried and fleshly calyces which have large quantities of organic acids (that is, oxalic, malic, citric and tartaric acids). The calyces have, also, vitamin C and the properties of therapeutic and diuretic, in addition to two types of anthocyanin, namely: hibiscin (delphinidin) and gossyptin (cyanidin) (Peng-Kong *et al.,* 2002). It is mostly planted in India, Africa, Mexico and tropical zone and has a special position in traditional medicine. Plants have been rich medicinal sources for a very long time and had a special status in health caring from both remedy and prevention aspects. Even today, World Health Organization estimates that more than 80% of the people still reckon on traditional medication (Laila *et al.,* 2002). Its soporific action has a favorable effect on the functions of the stomach possession. It kills various types of bacteria and micro-organisms, and as such, decreases blood pressure and causes relaxation of the rest parts of the body (Aziz *et al.,* 2007). The red beverage is also used in jams, tea pies, deserts and sauces. The flowers of roselle are suitable for use as natural food coloring agents. It is used for controlling blood pressure. Also, it has been reported that it is sexual stimulator, appetizer, restorative, cathartic, cancer-protective, anti-cough and refrigerant (Lin *et al.,* 2007). Also, Hussein *et al*. (1989) recorded that roselle seeds contain fixed oils (17%) which are easily refundable and have good cooking properties.

Lead (Pb) is a major anthropogenic pollutant that has been accumulated in different aquatic and terrestrial ecosystems since the industrial revolution. It is one of the most widely distributed heavy metal that is highly destructive to plants and most difficult to control (Verma and Dubey, 2003 and Salt *et al*., 1998). Naturally, Pb is present in soil, sea water, lakes and rivers. It is also a component of lead batteries, rubber, paints, metal products (steel and brass) and dusts (Nasralla and Ali, 1985). Besides natural sources, exhaust fumes of automobiles, chimneys of factories, mining, effluents from storage battery, smelting of Pb ores, fertilizers, additives in pigments, metal plating and pesticides are also major sources of Pb (Eick *et al*., 1999). Due to low solubility and strong binding capacity with soil colloids, Pb has long residence time in soil, causing a large number of direct and indirect effects on plant growth and metabolism. It induces many biochemical and structural changes in biological systems (Balsberg, 1989 and Minaii *et al*., 2008). Pb in soil not only changes the soil microorganism activities and resulted in soil fertility deterioration, but also directly affects the physiological processes and main symptom of Pb stress includes leaf chlorosis (Majer *et al.,* 2002 and Burton *et al*., 1984). Decrease in the root hair development, water potential, plant hormones and stunted growth in plants are caused by the deposition of Pb (Sarkar and Jana, 1986 and Sharma and Dubey, 2005). Different reactions are shown by plants against Pb stress. Some of them are sensitive and the others are more tolerant (Oliver and Naidu, 2003).

Cytokinins are important plant hormones that regulate various processes of plant growth and development; cytokinins appeared to play an important role in the regulation of cell division, differentiation and

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organogenesis in developing plants, enhancement of leaf expansion, nutrient mobilization an delayed senescence, Skoog and Armstrong, (1970) and Hall, (1973). Shudok (1994), reported that chemical structure of cytokinin active substances has determined two groups of adenine cytokinins and urea cytokinins with similar physiological effects, it has pronounced effect of cotyledon growth and expansion and other processes. The effect of cytokinins especially benzyladenine on the plant growth and chemical constituents of different plants have mentioned by Eraki *et al*. (1993) on salvia plants, Mazrou (1992) on datura, Mazrou *et al.* (1994) on sweet basil, Mansour *et al.* (1994) on soybean plants and Vijakumari (2003) on *Andrographis panculata*. Janowska (2014) on calla lily found that the application of BA at concentrations (100, 350 and 600 mg/dm^3) resulted in increasing 2-3 times the flower yield, staying neutral towards flower quality and showed higher greenness index and protein content.

The objective of this study was to determine the effect of Pb^{2+} ion stress and spraying of BA on morphological and physiological changes in roselle plants.

Materials and Methods

Seeds of roselle (*Hibiscus sabdariffa* L.) were obtained from Medicinal and Aromatic Plant Department, Agriculture Research Center, Ministry of Agriculture, Egypt and were sown in the experimental area, Agricultural Faculty, Fayoum University. Healthy seeds were surface-sterilized in 5% (v/v) NaClO for 5 – 10 min. On 1st April, 2014 and on 4th April, 2015, three sterilized seeds were sown in each of 40 cm-diameter plastic pots filled with acid-washed and rinsed sand. One month later, the seedlings were thinned to one plant pot^{-1}. The pots were arranged in an open greenhouse. Hoagland's nutrient solution (0.5 full-strength; Hoagland and Arnon, 1950) was supplied to all the pots for 7 days before the lead ion (Pb^{2+}) treatments (500 or 1000 µM $PbCl_2$ in Hoagland's nutrient solution) was initiated and applied every 2 – 3 days, throughout the experiments.

Benzyladenine (BA; 100 mg. L^{-1}) was sprayed until run-off. The BA was mixed with 0.1% (v/v) Tween-20 as a surfactant and spreading agent, and was applied three times at monthly intervals after sowing.

Treatments :-

1- Control.
2- 100 mg. L^{-1} benzyladenine .
3- 500 µM $PbCl_2$..
4- 500 µM $PbCl_2$ + 100 mg. L^{-1} benzyladenine.
5- 1000 µM $PbCl_2$.
6- 1000 µM $PbCl_2$ + 100 mg. L^{-1} benzyladenine.
Each experiment included 6 treatments, each with three replicates (each replicate = 5 pots), in a randomized complete blocks design.

Data recorded:-

a) Vegetative growth characters:

1- Morphological characters:-

At the flowering stage (aged 120 days) plants from each replicate were randomly chosen and washed in distilled water and were measured the following characters: plant height (cm), number of branches plant^{-1} and fresh (FW) and dry (DW) weights of herb plant^{-1} (g). (the samples were dried in an electric oven at 70^{o} C ± 2 till constant weight and then ground to determine the chemical constituents).

2- Yield and its components:

At the end of experiment (25th of October), when plants reached suitable maturity, the following data were taken: Number of fruits plant^{-1}, fresh weights of sepals plant^{-1} (g) and dry weight of sepals plant^{-1} (g).

b) **Chemical composition:**

Chlorophylls (a & b) and carotenoids concentrations (mg g^{-1} fresh weight of leaf) were determined using colorimetric method as described by Welburn and Lichtenthaler (1984). Total carbohydrates were colorimetrically determined according to the method described by Herbert *et al.* (1971). Nitrogen% was colorimetrically determined by using the orange G dye according to the method of Hafez and Hikkelsen (1981). Phosphorus % was determined according to method described by A.O.A.C. (1995). Potassium % was determined by flame-Photometer Parkin-Elmer model 52 with acetylene burner according to Page *et al.* (1982). Anthocyanin percentage. anthocyanin content was extracted by using acidified methanol (1% HCl). The absorbance of the clear filtered pigment solution was measured spectrophotometrically at 535 nm using the molar absorption coefficient (29 500 M^{-1} cm^{-1} of cyaniding 3-glycoside) as described by Fuleki and Francis (1968) and developed by Du and Francis (1973). Proline concentrations of roots and shoots of different plant samples was extracted by sulphosalicylic acid (3%) then, determined (mg kg^{-1} DW) colorimetrically using acid ninhydrin reagent as outlined by Bates *et al*. (1973). Catalase (CAT) activity was measured following the method of Aebi (1984). CAT activity was determined by measuring the loss of H_2O_2 by the decline in absorbance at 240 nm. Each reaction was carried out in a final volume of 2 ml containing 1.7 ml of reaction buffer, 0.1 ml of 3 mM EDTA, 0.1 ml of crude enzyme extract, and 0.1 ml of 3 mM H_2O_2. The reaction was incubated at 20°C for 10 min. CAT activity was calculated based on extinction coefficient (ε) for H_2O_2 of 0.036 mM^{-1} cm^{-1}, and expressed in EU mg^{-1} TSP. One EU of CAT activity was the amount needed to degrade 1 µmol of H_2O_2 min^{-1} at 25°C. The ascorbate peroxidase (APX) activity in leaves was determined according to Nakano and Asada (1981). Each 1.0 ml reaction mixture contained 0.6 ml reaction buffer, 0.1 ml 0.5 mM ascorbate, 0.1 ml 0.1 mM H_2O_2, 0.1 ml 0.1 mM EDTA, and 0.1 ml crude leaf extract. APX activity was calculated based on the decrease in absorbance at 290 nm using an extinction coefficient for ascorbate of 2.8 mM^{-1} cm^{-1}, and was expressed in EU mg^{-1} TSP. One EU of APX activity was considered to be the amount needed to decompose 1 µmol of ascorbate min^{-1} at 25 °C.

Statistical analysis:

The results were statistically analyzed using the LSD at probability level 5% for comparisons (Gomez and Gomez**,** 1983).

Results and Discussion

a) Effect of lead and benzyladenine on growth characters:

Plant height significantly reduced under lead stress conditions in both seasons and this effect was more pronounced with increasing the rate of lead stress (Table 1). Plant height was reduced by 12.21% at 500 mM Pb^{2+} and by 19.35% at 1000 mM Pb^{2+} compared to control (plants grown without stress and BA).

Table 1: Effect of benzyladenine on the vegetative growth characters of roselle plants grown under lead stress during seasons of 2014 and 2015.

Treatments	Plant height (cm)	No. of Branches plant^{-1}	Fresh weight of herb plant^{-1} (g)	Dry weight of herb plant^{-1} (g)	Plant height (cm)	No. of branches plant^{-1}	Fresh weight of herb plant^{-1} (g)	Dry weight of herb plant^{-1} (g)
	2014				2015			
Control	110.6	18.4	805.2	159.76	113.9	17.8	839.3	166.53
100 mg. L^{-1}BA	118.3	20.5	815.7	160.57	120.5	18.9	849.7	168.59
500 µM $PbCl_2$	97.1	15.2	764.5	151.75	103.7	13.7	796.5	160.58
500 µM $PbCl_2$ +100 mg. L^{-1}BA	102.4	16.1	783.4	159.37	109. 8	17.8	816.3	161.90
1000 µM $PbCl_2$	89.2	8.6	667.7	135.71	87.1	7.4	665.4	132.02
1000 µM $PbCl_2$ + 100 mg. L^{-1}BA	93.5	13.5	723.4	143.82	97.2	11.2	719.6	145.08
LSD 5%	3.5	2.1	7.3	3.82	5.6	1.3	8.6	2.85

BA= benzyladenine, Pb = lead

Number of branches plant^{-1} were reduced by 17.93% and 53.26% at 500 mM and 1000 mM Pb^{2+}, respectively, compared to control. The FW and DW of herb plant^{-1} also showed significant declines under Pb^{2+} ion stress (5.05% and 3.14% at 500 mM) and (17.08% and 15.05% at 1000 mM Pb^{2+}, respectively in the first season. The same was obtained trend in the second season. Lead reduced the morphological parameters (*Khizar et al*., 2013). Decrease in the root hair development, water potential, plant hormones and stunted growth in plants are caused by the deposition of Pb (Sarkar and Jana, 1986. and Sharma and Dubey, 2005). While, significant increases in plant height, number of branches plant^{-1}and fresh and dry weights of herb were noticed with BA spraying alone in both seasons as compared to control. Ramtin *et al.* (2015) on carnation, found that the spraying benzyladenine at 50 µM increased water content, dry weight, total length, floret length and floret diameter before harvesting. However, significant increases plant height, number of branches plant^{-1}and fresh and dry weights of herb were noticed following the exogenously applied BA under both Pb^{2+} regimes. A 5.46% increase in plant height and a 2.92% increase in number of branches were observed following the application of 100 mg. L^{-1} BA plus 500 mM Pb^{2+} as compared to 500 mM Pb^{2+} alone. Also, a 2.62 and 5.02 % increase in fresh and dry weight, respectively, at the same concentrations of Pb^{2+} and BA. It was evident from the data in the same (Table 1) that application of 100 mg. L^{-1} BA plus 1000 mM Pb^{2+} caused a significantly positive effect on attributes aforesaid as compared to 1000 mM Pb^{2+} alone in the first season. The same trend was true in the second season.

b) Effect of lead and benzyladenine on yield and its components:

Table (2) clearly show that the numbers of fruits $plant^{-1}$ and fresh and dry weight of sepals $plant^{-1}$ decreased by 19.56 % and 28.21 and 31.89 % respectively, under 500 mM Pb^{2+} ion stress, and declined by 24.24% and 47.03 and 52.59 %, respectively, under 1000 mM Pb^{2+} ion stress compared to control (plants grown without stress and BA). However, 100 mg. L^{-1} BA increased these characters by 41.80% and 124.38 and 60.40% compared to 1000 mM Pb^{2+} in the first season. The same trend was obtained in the second season. Pan and Xu (2011) found that a 4.5-fold increase in fruit number and a 3.3-fold increase in final seed yield were observed in inflorescences treated with 160 mg. L^{-1} of BA, which resulted from the greater number of female flowers and the newly induced bisexual flowers in BA-treated inflorescences in *J. curcas* plants.

It is obvious form (Table 2) also that pots received both lead and benzyladenine showed the different significant in attributes aforesaid compared with the single treatments, especially 500 or 1000 mM Pb^{2+} treatment in both seasons. Whereas, the number of fruits $plant^{-1}$ and fresh and dry weights of sepals $plant^{-1}$ decreased by 3.90% and 11.40 and 13.44%, respectively, under 500 mM Pb^{2+} ion stress and declined by 12.41% and 27.50 and 31.09%, respectively, under 1000 mM Pb^{2+} ion stress in the first season as compared to control. While, the decreases were 4.75% and 6.71 and 7.41%, respectively, and declined by 11.87% and 26.92 and 29.74%, respectively, in case of interaction between 500 or 1000 mM Pb and 100 mg. L^{-1} BA in the second season compared with control.

Table 2: Effect of benzyladenine on yield and its components of roselle plants grown under lead stress during seasons of 2014 and 2015.

Treatments	No. of fruits $plant^{-1}$	F.W. of sepals $plant^{-1}$ (g)	D.W. of sepals $plant^{-1}$ (g)	No. of fruits $plant^{-1}$	F.W. of sepals $plant^{-1}$ (g)	D.W. of sepals $plant^{-1}$ (g)
	2014			2015		
Control	47.40	137.84	18.75	48.86	142.70	19.30
100 mg. L^{-1}BA	50.92	163.82	22.45	51.28	165.12	22.64
500 µM $PbCl_2$	38.13	98.96	12.77	38.77	100.96	13.07
500 µM $PbCl_2$+100 mg. L^{-1}BA	45.55	122.12	16.23	46.54	133.12	17.87
1000 µM $PbCl_2$	35.91	73.01	8.89	35.24	71.27	9.64
1000 µM $PbCl_2$+ 100 mg.L^{-1}BA	41.52	99.94	12.92	43.06	104.44	13.56
LSD 5%	1.87	7.41	0.40	1.91	5.91	0.58

BA= benzyladenine, Pb = lead

c) Effect of lead and benzyladenine on chemical constituents:

1- Pigments concentration:

Data recorded in Table (3) show that chlorophylls a and b were significantly decreased by lead treatment alone as compared to the control. The inhibition of resolle plants growth following the application of 500 or 1000 mM Pb^{2+} was associated with a significant reduction in chlorophyll a and b concentrations, particularly at 1000 mM Pb^{2+} in both seasons. While, the same treatments from lead significantly increased carotenoids concentration in the first season. The same trend was noted in the second season. Similar results were observed by Dogan and Colak (2009) on wheat, Somashekaraiah *et al*. (1992) on mungbean and *Khizar et al.* (2013) on wheat. While, exogenous application of 100 mg. L^{-1} BA alleviated these harmful effects of Pb^{2+} ions. Also, 500 or 1000 mM Pb^{2+} ion stress reduced anthocyanin percentage of sepals by 3.30 or 12.09% in the first season and 5.59 or 10.06%, respectively, in the second season as compared to control. Appling 100 mg. L^{-1} BA ameliorated the negative effect of Pb^{2+} ions on anthocyanin percentage. Appling 500 or 1000 mM Pb^{2+} ion and spraying the plants by 100 mg. L^{-1} BA led to increase in chlorophyll a, b, carotenoids and anthocyanin in both seasons as compared with 500 or 1000 mM Pb^{2+} alone.

2- Total carbohydrates content:

Exposure of roselle plants to 500 or 1000 mM Pb^{2+} decreased total carbohydrates concentration (Table 3). The maximum decreases in total carbohydrates concentration was 15.97% with 1000 mM Pb^{2+} treatment as compared to control. However, the exogenous application of 100 mg. L^{-1} BA increased total carbohydrates concentration by 25.12% and 12.33%, in the first and second season, respectively, in roselle plants as compared to control. Also, the exogenous application of 100 mg. L^{-1} BA increased total carbohydrates concentration by

11.76% and 7.71%, in the first and second seasons, respectively, in plants exposed to the concentration 1000 mM of Pb^{2+}.

3- Nutrient contents:

Lead stress significantly led to reduction of nitrogen, phosphorus and potassium % as shown in (Table 3). N % was reduced with the increase of Pb^{2+} stress (32.27% under 500 mM Pb^{2+} and 37.85% under 1000 mM Pb^{2+} in the first season as compared to the control.

However, exogenous application of 100 mg. L^{-1} BA restored the N % in both seasons. The percentages of P and K also decreased to 18.75 and 14.88 %, respectively, at 1000 mM Pb^{2+} in the first season as compared to the control. Application of 100 mg. L^{-1} BA helped plants to restore these elements. The same trend in the second season was obtained.

It is evident from data in (Table 3) that the N, P and K percentages significantly increased as a result of dual application of Pb and BA compared with individual treatment. The maximum mean values of N, P and K (%) (2.56, 0.31 and 2.14) were obtained as a result of 500 µM $PbCl_2$ + 100 mg. L^{-1} BA application compared with the other treatments.

Table 3: Effect of benzyladenine on chlorophylls, carotenoid, anthocyanin and macronutrient of roselle plants grown under lead stress during seasons of 2014 and 2015.

Treatments	Chlorophyll a mg^{-1}	Chlorophyll b mg^{-1}	Carotenoids mg^{-1}	Anthocyanin %	Total carbohydrates mg^{-1}	N %	P %	K %
2014								
Control	1.48	0.92	0.302	1.82	180.30	2.51	0.32	2.15
100 mg. L^{-1}BA	1.51	0.97	0.322	1.91	225.60	2.58	0.40	2.19
500 µM $PbCl_2$	1.41	0.82	0.353	1.76	170.30	1.70	0.29	1.98
500 µM $PbCl_2$ + 100 mg. L^{-1}BA	1.44	0.90	0.366	1.79	178.10	2.56	0. 31	2.14
1000 µM $PbCl_2$	1.03	0.79	0.378	1.60	150.50	1.56	0.26	1.83
1000 µM $PbCl_2$ + 100 mg. L^{-1}BA	1.18	0.80	0.391	1.65	168.20	2.40	0.21	1.77
LSD 5%	0.02	0.01	0.007	0.02	7.62	0.05	0.02	0.05
2015								
Control	1.65	1.05	0.319	1.79	188.67	2.70	0.27	2.00
100 mg. L^{-1}BA	1.69	1.07	0.334	1.81	211.94	2.77	0.29	2.42
500 µM $PbCl_2$	1.59	094	0.359	1.69	173.79	2.19	0.21	1.94
500 µM $PbCl_2$ +100 mg. L^{-1}BA	1.61	0.97	0.384	1.74	185.87	2.74	0.25	2.16
1000 µM $PbCl_2$	1.13	0.86	0.396	1.61	157.18	1.07	0.19	1.87
1000 µM $PbCl_2$ + 100 mg. L^{-1}BA	1.34	0.91	0.413	1.65	169.30	1.54	0.18	2.27
LSD 5%	0.02	0.01	0.011	0.02	6.62	0.05	0.02	0.06

BA= benzyladenine, Pb= lead, N=nitrogen, P=phosphorus, K=potassium

4- Endogenous Pb concentration:

Results in this study (Table 4) showed that exposing roselle plants to 500 or 1000 mM Pb^{2+} increased Pb contents in different organs plant whereas no found in the control. Also, found higher Pb concentration in roots rather than in leaves and stems. While, exposing roselle plants to 500 or 1000 mM Pb^{2+} and spraying by BA decreased Pb content in leaves, stems and roots as compared to plants applied by lead in absence of BA.

5- *Proline concentration*:

Data in (Table 4) show that proline concentrations increased with the increase of Pb^{2+} level in growth medium. The maximum increases in proline concentrations were 95.39 and 105.41% in roots and leaves, respectively, occurred in 1000 mM Pb^{2+} as compared to the control. However, the exogenous application of 100 mg.L^{-1} BA increased proline concentration by 138.21 and 141.22%, respectively, in roselle plants exposed to the same concentration of Pb^{2+}.

6- enzymes activity:

Data in (Table 4) show that CAT activity reduced with the increase of Pb^{2+} level in growth medium. However, exogenous application of 100 mg.L^{-1} BA reduced CAT activity by 44.23% in 1000 mM Pb^{2+} treated roselle plants. APX activity was stimulated by Pb^{2+} stress as compared to the control. Maximum APX activity was observed under 1000 mM Pb^{2+}. 100 $mg.L^{-1}$ BA application reduced the activity of APX in Pb^{2+} treated plants, being more marked (14.29%) under 500 mM Pb.

Table 4: Effect of benzyladenine on lead, proline, catalase and ascorbate peroxidase of roselle plants grown under lead stress (Data average of two seasons).

Treatments	Leaf Pb^{2+} mg kg^{-1} DW	Root Pb^{2+} mg kg^{-1} DW	Sepals Pb^{2+} mg kg^{-1} DW	Leaf proline $mgkg^{-1}$ DW	Root proline mg kg^{-1} DW	CAT $EUmg^{-1}$ protein	APX $EUmg^{-1}$ protein
Control	-	-	-	29.6	36.9	156	6.3
100 mg. L^{-1}BA	-	-	-	42.8	51.2	145	5.8
500μM $PbCl_2$	7.3	16.3	5.9	31.2	37.2	131	6.4
500 μM $PbCl_2$+100 mg. L^{-1}BA	5.1	9.5	4.2	55.6	68.5	122	5.4
1000μM $PbCl_2$	12.5	22.3	9.9	60.8	72.1	109	6.9
1000 μM $PbCl_2$+ 100 mg. L^{-1}BA	9.7	15.4	6.2	71.4	87.9	87	6.5
LSD 5%	-	-	-	4.8	5.7	9	0.3

BA= benzyladenine, Pb= lead, CAT= catalase, APX=ascorbate peroxidase

Discussion

The addition of Pb^{2+} ions in the growth medium, particularly at 1000 mM, resulted in a marked reduction on yield and biomass characteristics like plant height, number of branches (Table 1), the herb and sepales fresh and dry weights of roselle (Table 2) due to changes in metabolism and physiology of plants due to Pb. The inhibited growth was associated with pb^{2+} induced reduction in the concentration of chlorophylls (Table 3). Pb has inhibitory effect on morphological parameters and yield reduction has been well described by many researchers (Bashmakov *et al*., 2005 and Khizur *et al*., 2013). Pb has reduced the photosynthetic pigments (chlorophyll a and b) significantly because Pb prevents the incorporation of Fe (iron) in phytoporphyrin ring of chlorophyll molecule, so cause reduction in chlorophyll contents (Nyitrai *et al*., 2002 and Jaleel *et al*., 2009) and also, may be attributed to increase in activity of chlorophyll-degrading enzyme chlorophyllase under stress conditions (Reddy and Vora, 1986). The increase in carotenoids contents in plants (Table 3) due to heavy metal stress may be a strategy adopted by plants to alleviate the toxic effects of free radicals generated under heavy metal toxicity (Singh *et al*., 2006 and Azooz *et al*., 2011). Also, Pb decreased anthocyanine, total carbohydrates and nutrient levels (Table 3) and catalase activity (Table 4) while, increased Pb contents in leaf, roots and sepals, proline in leaf and roots and APX activity (Table 4). Pb^{2+} application led to a deficiency of macronutrients in roselle plants, as shown in this study, which may cause other changes in plant metabolism, but BA application can alleviate this disturbance.

While application of BA increases all except Pb contents, CAT and APX (Table 4). The mechanisms by which benzyladenine stimulated plant growth may by similar to that of other plant growth regulators such as auxins, cytokinins and gibberellins that affect plant metabolism in a positive manner. This may explain the positive results of the benzyladenine on proline and chlorophyll contents under Pb stress that were then positively reflected in the growth of roselle plants. Benzyladenine leads to highest rates of uptake of elementals N, P and K (Table 3), thus leads to a corresponding increase in chlorophyll which can serve as an indicator of the stress induced by alterations in the balance of endogenous hormones (Marschner, 1995). The increase in tolerance to the Pb stress was manifested in terms of improved growth and photosynthetic pigments (chlorophyll a, b and carotenoids: Table 3) and subsequent sepals yield (Table 2). Level of the benzyladenine significantly increase the sepales yield of roselle plants due to the increase fresh and dry weights of herb (Table 1), nutrient status of plant (Table 3) as compared to Pb treatment. These results confirmed that Pb toxicity is linked with the production of free radical which hinders membrane stability and also increases its permeability to the outside environment. But, the exogenous application of BA mitigated the negative effect of Pb^{2+} on growth of roselle.

Conclusion:

Pb exposure alone depressed plant growth, reflected by the inhibition of chlorophyll synthesis, increase of the inhibitory effect on uptake of some nutrient elements and increase of lead concentration in plant. Exogenously applied 100 mg. L^{-1} benzyladenine decrease Pb toxicity in roselle plants exposed to Pb stress, which probably includes not only the regulation of the chloroplast and antioxidant system, but also the reduction of Pb uptake and the improvement of mineral nutrient absorption. Furthermore, Pb induced stress in BA-treated plants may be related to lower Pb levels in cell organelle due to the inhibition of the cell wall that reduce Pb toxicity. This suggests that an appropriate concentration of BA could be used as a potential growth regulator to improve plant growth under Pb stress.

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