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Impact of salicylic acid on the growth and photosynthetic pigment of canola (*Brassica napus* L.) under lead stress

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Abstract

In order to study the effect of lead and different salicylic acid on the growth, photosynthetic pigment of two canola cultivars (Opera and Okapi cultivars), experiment were conducted with seven levels of $Pb(NO_3)_2$ and three levels of salicylic acid (0, 5 and 10 µM) . The experiments were conducted in randomized complete block design arrangement in split factorial with three replications.Canola seedlings were grown for 12 days in hydroponic culture in presence of $Pb(NO_3)_2$ (0, 0.25, 0.5, 0.75, 1, 1.5 and 2 mM). The results indicated that the increase of lead concentration caused a significant reduction in root and stem length, shoot and root dry weights and leaf area. Also photosynthetic pigments significantly decreased.The extent of toxicity increased with the increasing concentration of lead. Seedlings treated with both lead and salicylic acid showed an increase in most of the parameters compared to plants without salicylic acid treatment.

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Introduction

trace elements

Toxic trace elements are increasing in all compartments of the biosphere; including, air, water and soil, as a result of anthropogenic processes. For example, the metal concentration in river water and sediments increased several thousand fold by effluents from industrial and mining wastes (Siegel, 2002). Heavy metal pollution of the environment which is increasing day by day has exerted deleterious effects on plants and consequently to man and animals through the food chain (Jones *et al*., 1973). Although lead is a non-essential element, plants can absorb it from soil, water and air through their roots and leaves (Zimdahl and Koeppe, 1977).

Effect Lead on plant growth parameters

Lead is a ubiquitous pollutant in the environment widely distributed in soil and waters (Callan, 2011). High lead concentration in soil decreases the germination and has a harmful effect on growth and metabolism in plants (Pang *et al*., 2002,Khan, 2009). Low concentration of lead can inhibit some vital plant processes such as photosynthesis, mitosis, root growth and water absorption (Lin *et al*., 2009: Estrella, 2009). Various abiotic stresses can decrease the chlorophyll content in plants (Ahmad *et al*., 2007). Heavy metal could affect chlorophyll concentration of leaves through inhibition of synthesis of chlorophyll or the acceleration of its degradation (Sharma and Dietz, 2006). Salicylic acid (SA) is a plant phenolic compound that is now considered a hormone – like endogenous regulator and its role in the defense mechanism against biotic and abiotic stresses has been well documented (Horvath *et al*., 2007).

Effect of Salicylic acid on plant growth parameters

Salicylic Acid **(**SA) and related compounds have been reported to induce significant effects on various biological aspects in plants. These compounds influence in a variable manner; inhibiting certain processes and enhancing others (Khodary, 2004). Salicylic acid treatment resulted in interfering with membrane depolarization, stimulating photosynthetic machinery, increasing the content of chlorophyll as well as blocking wound response in soybeans, and an increase in the yield of various crop species (Andrey *et al*., 2012). Thus, the aim of the present study was to investigate lead action on some plant growth characteristics of Brassica napus L. (Opera and Okapi) cultivars and the potential antitoxic effects of salicylic acid that was chosen as antitoxicant to lead.

Materials and methods

Experimental design

The experimental protocol had been installed in the Agricultural Experiment Station of Islamic Azad University, Boruojerd Branch at Iran. Seeds of canola (*Brassica napus* L., cv Okapi and Opera) were germinated on vermiculite and grown hydroponically. The seeds were sterilized with 20% sodium hypochlorite solution. When two leaves grew, seedlings were moved to a plastic container with 650 mL of Hogland's nutient solution (Hoagland and Arnon, 1950). Various experiments in completely randomized factorial desing with three replicates were conducted and the presented data included means of three separate experiments ±SD.

Seedling treatments

After 24 h, treatments were started, the seedlings treated at different concentrations of Pb(No3)2 at (0, 0.25, 0.5, 0.75, 1, 1.5 and 2 mM) and salicylic acid at 3 concentrations (0, 5 and 10 µM) in 3 replicates. The treated plants were transferred to a germinator under 200C, 16/8-h light/dark period, light was enforced with 12 Fluorescent tubes 60W.

Plant analysis

15 days after treatment and humidity of 75%, the plants were removed from the Hogland nutrient solution and roots were washed with distilled water and separated from aerial organ. Then dry weight of roots and shoots were measured with a digital scale in grams(g), root and stem lengths were measured using the graph paper in (mm), leaf area was also measured in (mm2). Chlorophyll a, b and a+b content were measured in (mg /g fw) according to (Arnon *et al*., 1957).

Statistical analysis

All data obtained from the effects of different treatments in the present study were subjected to analysis of variance (ANOVA). SPSS software and Duncan test was used and all graphs were down by Excel software and the results were presented as comparison graphs.

Results and discussion

Effect of Lead and Salicylic acid on growth parameters

Root growth

Analysis of variance data on root growth showed that lead poisoning caused significant reduction in root length of both cultivars, So that when lead concentration increased, the amount of root length was significantly declined ($P < 0.01$).In both cultivars Under lead and salicylic acid treatments, root length significantly increased compare to lead treatment ($P < 0.01$). The results given in fig.1 indicate percentage of reduction of this parameters in opera was higher than the okapi.

Table 1. Chlorophyll a, b and a+b content of canola(*Brassica napus* L. cv. Okapi and Opera) in response of Pb treatments in presence or absence of salicylic acid.

Okapi				Opera		
Pb(mM)/ SA(µM)	Chlorophyll a (mg /g f w)	Chlorophyll b (mg /g f w)	Chlorophyll a+ b (mg /g f w)	Chlorophyll a (mg /g f w)	Chlorophyll b (mg /g f w)	Chlorophyll a+b (mg /g f w)
0/0	$0.0051 \pm 6.42\times10^{-5}$	$0.0028 \pm 1.52\times10^{-5}$	$0.0078 \pm 1.10\times10^{-4}$	$0.0095 \pm 4\times10^{-4}$	$0.0062 \pm 2\times10^{-4}$	$0.0104 \pm 4.50\times10^{-4}$
0.25/0	$0.0041 \pm 9.64\times10^{-5}$	$0.0028 \pm 1.44\times10^{-4}$	$0.0070 \pm 1.04\times10^{-4}$	$0.0089 \pm 3.60\times10^{-4}$	$0.0041 \pm 1.52\times10^{-4}$	$0.0095 \pm 3.05\times10^{-4}$
0.25/5	$0.0050 \pm 9.45\times10^{-5}$	$0.0034 \pm 9.07\times10^{-5}$	$0.0082 \pm 1.10\times10^{-4}$	$0.0091 \pm 2.88\times10^{-4}$	$0.0047 \pm 1.32\times10^{-4}$	$0.0096 \pm 3.21\times10^{-4}$
0.25/10	$0.0051 \pm 9.29\times10^{-5}$	$0.0034 \pm 7\times10^{-5}$	$0.0083 \pm 1.35\times10^{-4}$	$0.0090 \pm 4.04\times10^{-4}$	$0.0057 \pm 2.51\times10^{-4}$	$0.010 \pm 4.50\times10^{-4}$
0.5/0	$0.0037 \pm 5.68\times10^{-5}$	$0.0028 \pm 1.13\times10^{-4}$	$0.0065 \pm 1.36\times10^{-4}$	$0.0054 \pm 3.60\times10^{-4}$	$0.0034 \pm 1.52\times10^{-4}$	$0.0062 \pm 2.64\times10^{-4}$
0.5/5	$0.0049 \pm 8\times10^{-5}$	$0.0031 \pm 7.93\times10^{-5}$	$0.0081 \pm 1.26\times10^{-4}$	$0.0056 \pm 4.04\times10^{-4}$	$0.0036 \pm 1.52\times10^{-4}$	$0.0065 \pm 3.51\times10^{-4}$
0.5/10	$0.0052 \pm 5.5\times10^{-5}$	$0.0026 \pm 8.14\times10^{-5}$	$0.0079 \pm 1.25\times10^{-4}$	$0.0058 \pm 2.08\times10^{-4}$	$0.0046 \pm 2.64\times10^{-4}$	$0.0066 \pm 3.60\times10^{-4}$
0.75/0	$0.0029 \pm 6.65\times10^{-5}$	$0.0018 \pm 1.22\times10^{-4}$	$0.0048 \pm 1.25\times10^{-4}$	$0.0038 \pm 2.51\times10^{-4}$	$0.0024 \pm 2.64\times10^{-4}$	$0.0044 \pm 4.50\times10^{-4}$
0.75/5	$0.0037 \pm 5.50\times10^{-5}$	$0.0022 \pm 4.16\times10^{-5}$	$0.0059 \pm 1.31\times10^{-4}$	$0.0042 \pm 2.64\times10^{-4}$	$0.0026 \pm 2.08\times10^{-4}$	$0.0048 \pm 2.51\times10^{-4}$
0.75/10	$0.0042 \pm 5.85\times10^{-5}$	$0.0021 \pm 1.04\times10^{-4}$	$0.0061 \pm 9.07\times10^{-4}$	$0.0045 \pm 3.51\times10^{-4}$	$0.0029 \pm 1.52\times10^{-4}$	$0.0051 \pm 3.21\times10^{-4}$
1/0	$0.0024 \pm 8.14\times10^{-5}$	$0.0016 \pm 1.07\times10^{-4}$	$0.0029 \pm 1.25\times10^{-4}$	$0.0030 \pm 3.60\times10^{-4}$	$0.0020 \pm 1.52\times10^{-4}$	$0.0031 \pm 4.16\times10^{-4}$
1/5	$0.0033 \pm 6.08\times10^{-5}$	$0.0018 \pm 8.71\times10^{-5}$	$0.0036 \pm 1.04\times10^{-4}$	$0.0030 \pm 2.08\times10^{-4}$	$0.0022 \pm 2.17\times10^{-4}$	$0.0035 \pm 4.72\times10^{-4}$
1/10	$0.0040 \pm 6.11\times10^{-5}$	$0.0019 \pm 7.21\times10^{-5}$	$0.0042 \pm 1.50\times10^{-4}$	$0.0034 \pm 4.50\times10^{-4}$	$0.0025 \pm 2.64\times10^{-4}$	$0.0036 \pm 3.51\times10^{-4}$
1.5/0	$0.0018 \pm 7.23\times10^{-5}$	$0.0012 \pm 1.15\times10^{-4}$	$0.0023 \pm 1.10\times10^{-4}$	$0.0018 \pm 2.51\times10^{-4}$	$0.0011 \pm 2.08\times10^{-4}$	$0.0026 \pm 4\times10^{-4}$
1.5/5	$0.0028 \pm 4.93\times10^{-5}$	$0.0014 \pm 8.71\times10^{-5}$	$0.0031 \pm 1\times10^{-4}$	$0.0021 \pm 3.51\times10^{-4}$	$0.0017 \pm 1.52\times10^{-4}$	$0.0032 \pm 3\times10^{-4}$
1.5/10	$0.0035 \pm 5.29\times10^{-5}$	$0.0018 \pm 9.29\times10^{-5}$	$0.0039 \pm 1.26\times10^{-4}$	$0.0025 \pm 3.60\times10^{-4}$	$0.0021 \pm 1.52\times10^{-4}$	$0.0034 \pm 4.16\times10^{-4}$
2/0	$0.0011 \pm 9.07\times10^{-5}$	$0.0012 \pm 1.06\times10^{-4}$	$0.0019 \pm 1.52\times10^{-4}$	$0.0093 \pm 3.05\times10^{-4}$	$0.0007 \pm 2\times10^{-4}$	$0.0015 \pm 5.03\times10^{-4}$
2/5	$0.0020 \pm 5.13\times10^{-5}$	$0.0015 \pm 9.64\times10^{-5}$	$0.0029 \pm 1.25\times10^{-4}$	$0.0011 \pm 3.78\times10^{-4}$	$0.0012 \pm 1.52\times10^{-4}$	$0.0017 \pm 3.21\times10^{-4}$
2/10	$0.0032 \pm 6.80\times10^{-5}$	$0.0018 \pm 6.42\times10^{-5}$	$0.0036 \pm 1.10\times10^{-4}$	$0.0015 \pm 3.51\times10^{-4}$	$0.0016 \pm 2.51\times10^{-4}$	$0.0024 \pm 4.16\times10^{-4}$

Stem growth

When lead concentrations were increased in both Cultivars, the stem length were significantly decreased compared to control grop ($P < 0.01$). In two cultivars with applications of salicylic acid 5 and 10 µM concentrations, stem length were significantly increased compare to lead treatments ($P < 0.01$). In Okapi cultivar Concentration of 10 µM salicylic acid was more effective so that in lead concentrations of (1, 1.5 and 0.25 mM), stem length increased az compared with control, but in opera cultivar between 5 &10 µM salicylic acid significantly effect was not seen. Percentage of reduction of steam lenght in opera was higher than the okapi. (Fig. 2).

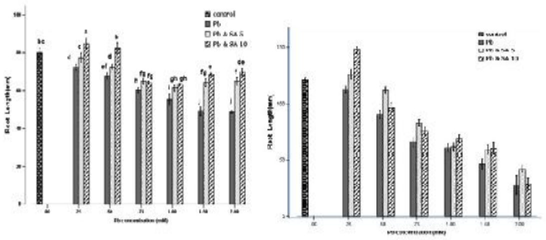

Fig. 1. Response of root length of canola under Pb and salicylic acid treatments. Each value is the mean of three replicates and the bars indicate standard deviation (Left fig. in Okapi and Right fig. in Opera).

Leaf area

With increasing concentrations of lead, leaf area in both cultivars were decreased significantly (P <0.01) than control grop. In both cultivars, Two concentrations of salicylic acid with 5 and 10 µM significantly increased (P <0.01) surface of leaf area comper to treatments lead, between two concentration of salicylic acid in two cultivars, significantly diffrent was seen (P <0.05). Percentage of reduction of leaf area in opera was higher than the okapi (Fig. 3).

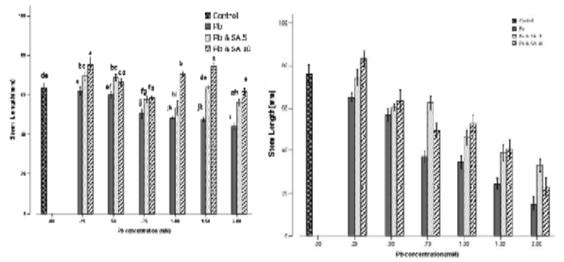

Fig. 2. Response of stem length of canola under Pb and salicylic acid treatments. Each value is the mean of three replicates and the bars indicate standard deviation (Left fig. in Okapi and Right fig. in Opera).

Root and shoot dry weight

Increasing concentrations of lead, significantly(P <0.01) decrease dry weight of root and shoot in both cultivars. Application of salicylic acid, significantly increased dry weight of root and shoot compared to lead treatments (P <0.01). Two concentrations of salicylic acid had significantly different effects on root dry weight of Okapi (P <0.01) While they had similar effects on root and shoot dry weight of Opera and shoot dry weight of Okapi (P <0.05) Percentage of reduction of root and shoot weight in Opera were higher than the okapi (Fig. 4).

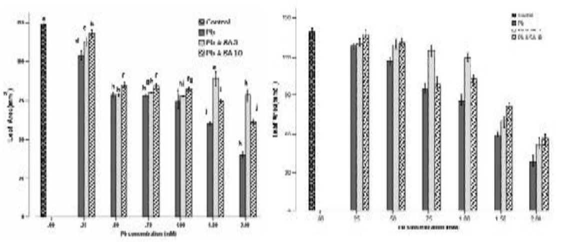

Fig. 3. Response of Leaf area of canola under Pb and salicylic acid treatments. Each value is the mean of three replicates and the bars indicate standard deviation (Left fig. in Okapi and Right fig. in Opera).

Chlorophyll content

Table 1 shows that the pigments (chlorophyll a, b and a+b) content of lead treated canola plants were significantly decreased below that of the controls in both cultivars. In the presence of lead concentrations, decreasing of okapi cultivar pigments (chlorophyll a, b and a+b) contents were higher than the poera. The beneficial effect of SA was seen with pigment content a,b and a+b in okapi, also pigment content b in opera was shown significantly effect, but statistically significant was nat seen in pigment a and a+b in opera (Table1).

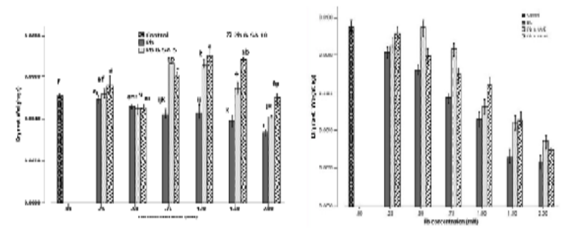

Fig. 4. Response of Dry root weight of canola under Pb and salicylic acid treatments. Each value is the mean of three replicates and the bars indicate standard deviation (Left in Okapi and Right in Opera).

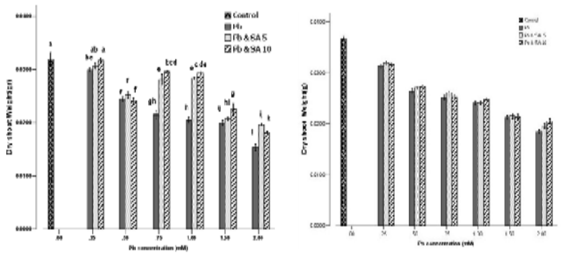

Fig. 5. Response of Dry shoot weight of canola under Pb and salicylic acid treatments. Each value is the mean of three replicates and the bars indicate standard deviation (Left fig. in Okapi and Right fig. in Opera).

Discussion

Heavy metals such as Pb are not essential for plants which are adversely affected by the increasing levels of these metals in the soil environment (John *et al.*, 2009). Application of the test levels of lead (II) nitrate to canola plants adversely influenced their growth parameters (root and shoot lengths, dry weights of shoots and roots and leaf area) as compared with those in the control plants (Fig. 1,2,3,4). Root and stem lengths, leaf area, dry weights of roots and shoots in each cultivar decreased by increasing lead concentration in solutions. Decreasing root length was affected by unbalanced microtubules that was suggested by (Yang *et al.*, 2000). Similar results have been shown by (Saied *et al.*, 2005: Chiraz Chaffei ., 2012). Inhibition of root growth under heavy metal toxicity is a result of heavy metal-induced inhibition of cell devision in root tips and elongation rate of cells that mainly occurs by an irreversible of proton pump responsible for the process (Liu *et al.*, 2004). The weight of plant organs gradually decreased with increasing pb concentrations in both cultivars. This conclusion is confirmed by (Azz, 2005). Pb toxicity commonly reduced the absorption of Co2 due to reduced leaf surface area (Azmat *et al.*, 2009). Reduced leaf area suggest that the persence of excess lead in the nutrient medium reduces water uptake and transport (Azmat *et al.*, 2006), which may effect the photosynthesis system of plant (Haider *et al.*,2006). Exogenous SA applications increased most of these parameters as compared to the control under pb stress. Similar results were reported by (El- Tayeb 2005) on barley, Stevens *et al.*, 2006 on tomato, and (Gunes 2007) on maize. They observed that exogenous SA treatments ameliorated the negative effects of salt stress on fresh and dry weight of plants. Shruti and Singh (2009) showed that salt-induced deleterious effects in maize seedlings were significantly eliminated by the pretreatment of SA. It is concluded that 0.5 mM salicylic acid improves the adaptability of maize plants to Nacl stress. The ability of SA to increase plant growth, decreasing the adverse effects of pb stress on plant growth and productivity, may have significant implications in improving plant growth and overcoming the yield barier arising from pb stress. Khodary(2004) also recorded an increase in fresh and dry weights and leaf area of stressed maize plants with SA treatment (Figers 1, 2, 3, 4). This result was coincided with our results. The photosynthetic pigments (chlorophyll a, b and a+ b) as a chief component of the photosynthetic system governing the fresh matter participation, decreased significantly ($p<0.01$) in pb-treated plants in comparsion to controls (Table I). Several reports show chlorophyll biosynthesis inhibition by metals in higher plants (Ahmad *et al.*, 2007:lanaras *et al.*, 2006: Devi *et al.*, 2007). The decline in chlorophyll content in plants exposed to pb stress is belived to be due to (a): inhibition of important enzymes, such as ∂- aminolevulinic acid dehydratase (ALA-dehydratase) and protochlorophyllide reductase. Associated with chlorophyll biosynthesis, (b): impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls, (c): Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase, (d): the replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecule. Our results, corroborated with the findings of Sharma and Dietz (2006). The results presented by those authors support the findings in this work. Rahimi, 2011 suggested that chlorophyll b is mainly associated with photosystem-II antenns, a decrease in chlorophyll b concentration might lead to structural/ conformational changes in the photosystem-II antenns (Rahimi *et al.*, 2011). SA applications significantly increased the pigment content in leaves of opera and okapi in pb treated plants, except for the chlorophyll a and a+b of opera under lead treatment (Table 1). The increase in chlorophyll content with SA confirmed the reports by (Yildirim *et al*: 2008).

Conclusions

In conclusion, our results indicate that (1) the exposition of Brassica napus L. to different concentrations of pb results in other parameters in both cultivars decrease were observed, (2) the results hint that application of SA improves plant growth and yield in hydroponic culture, (3) finally, the results in our study also support a major role of SA in modulating the plants' response to lead stress.

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