Effect of salicylic acid on some plant growth parameters under lead stress in *Brassica napus* var. Okapi

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Abstract

Heavy metals pollution is a big problem for the large parts of the world. Lead is a heavy metal with high toxicity that inhibition of germination and retardation of plant growth are commonly observed due to its toxicity. The very important role of salicylic acid (SA) in response to different stresses, namely, modifications and decreasing the negative effects of stress has been established in different studies. The objective of this research was to evaluate the effect of different concentrations of salicylic acid to enhance the characteristics of *Brassica napus* L. under lead stress. Factorial experimental design was arranged in a completely randomized block design with three replications. Treatments were combination of 7 levels application of lead (0, 0.25, 0.5, 0.75, 1, 1.5 & 2 mM) and 2 concentrations of salicylic acid (5 & 10 µM). The results revealed that increasing lead concentration reduced root and shoot length, leaf area, and root and shoot dry weight, root and shoot fresh weight, specific leaf area (SLA) and leaf weight ratio (LWR). Application of salicylic acid on the other hand, significantly increased the studied traits, although specific leaf weight (SLW) and leaf water content area (LWCA) were significantly increased with an increase in the concentration of $Pb(NO_3)_2$ ($P<0.01$). Generally salicylic acid applications improved plant growth parameters of *Brassica napus* L.

Keywords: salicylic acid; growth parameters; *Brassica napus* L.; lead

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Introduction

Lead contamination is one of the major environmental hazards in contaminated areas because it is among the heavy metals introduced as a major environmental contaminant (Yell Yang, 2000). It is also a non-essential component for plants that has no known biological function (Kim et al., 2002). Lead toxicity due to many aspects of its behavior mimics calcium metabolism and inhibits the activities of many enzymes (Ruley et al., 2004). Existence of polluted material with lead in soil causes the major reduction of agricultural products. Lead is taken up by plants mainly through the root system and partly in minor amounts through the leaves and thrichomes

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(Pallavi and Rama, 2005). Lead toxicity effects in plants usually appear in concentrations higher than 30 micrograms per gram of leaf and leads to reduction in growth and decrease of chlorophyll synthesis (Ruley et al., 2004). It causes such problems as reduction in growth and production levels, yellowing of young leaves, reduction in absorption of essential elements such as iron and reduction in the rate of photosynthesis (Pallavi and Rama, 2005). Toxicity of lead caused inhibition of root growth and high lead concentration in root decreased the root length and had a harmful effect on growth and metabolism in plants (Yell Yang, 2000). Decreasing root length affected unbalanced microtubules (Yell Yang, 2000). The weight of plant organs gradually decreased with increasing Pb concentrations. Kabata - Pendias and Pendias (2001) reported that inhibitory effects of Pb may be due to interaction with other elements and environmental factors. *Brassica napus* L. Which is a herbaceous plant belonging to Brassicaceae with potential of heavy metals absorption and is able to tolerate high concentrations of heavy metals (Ghahreman, 1993) is selected for this research study. Salicylic acid belongs to a group of phenolic compounds that widely exists in plants and now a days is considered as a hormone-like substance. This acid also plays an important role in plant growth and development (Mazaheri Tirani and Manochehri-Kalantari, 2007). SA induces resistance to water deficit and ameliorates the damaging effects of heavy metals, like lead and mercury (Berukova *et al*., 2001). The objective of this work was to investigate whether salicylic acid could be a protectant to ameliorate the influence of lead stress on colza and thereby increasing its lead tolerance. In other words, the study aims at investigating if this plant has the potential to absorb heavy metals and tolerate high concentrations of lead.

Materials and Methods

Plant seeds of canola cultivar Okapi from Institute of Agriculture, Lorestan, Iran was collected and cultivated. The seeds were sterilized with 20% sodium hypochlorite solution. When two leaves grew, seedlings were moved to a plastic container with 650 mL of Hoagland's nutrient solution. After 24 h, treatments were started. The seedlings treated at different concentrations of $Pb(NO_3)_2$ (0, 0.25, 0.5, 0.75, 1, 1.5 and 2 mM) and with salicylic acid at just two

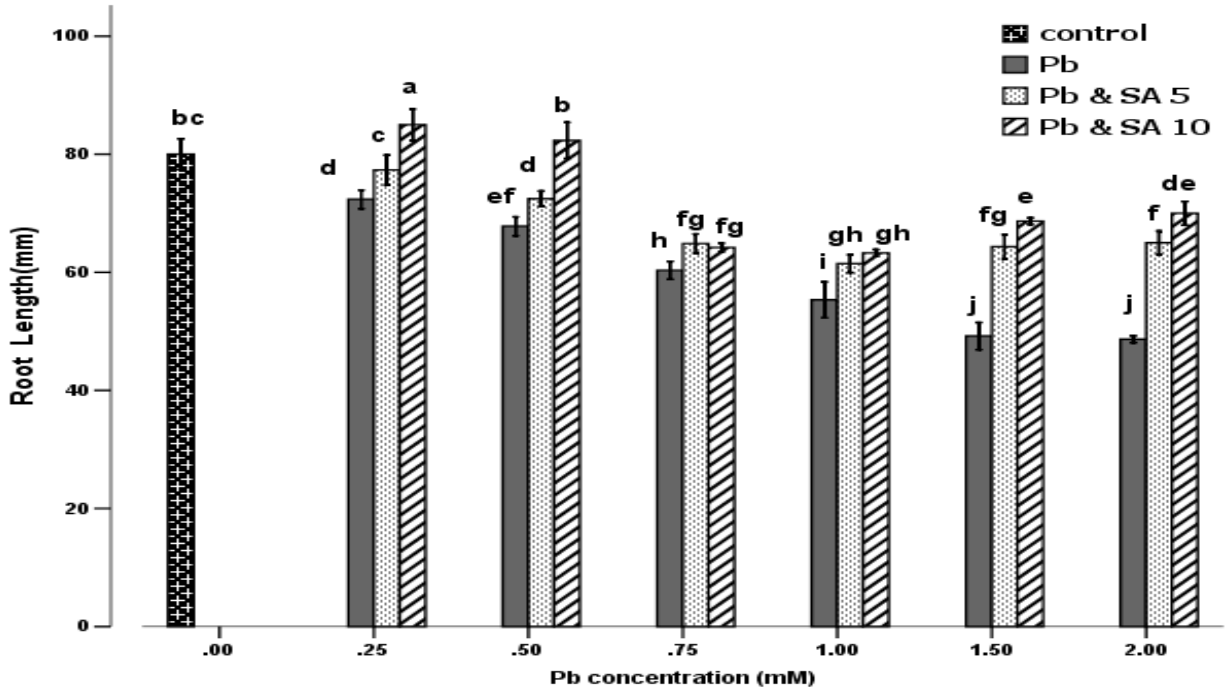

Fig. I. Effect of salicylic acid and lead on root length after 10 days treatment in Hoagland nutrient solution

different concentrations, 5 and 10 µM in three replicates. The treated plants were transferred to a germinator under 20 °C for 10 days. Light and dark period were 16 and 8 hours. 10 days after treatment, plants were removed from the Hoagland nutrient solution and the roots were washed with distilled water and separated from the aerial organ. Then root and shoot lengths were measured using the graph paper in millimetres (mm). Leaf area was also measured in square millimetres (mm^2). Dry and fresh weight of roots and shoots were then measured with a digital scale in grams (g). Specific leaf area (SLA), specific leaf weight (SLW), leaf water content area (LWCA) and leaf weight ratio (LWR) were measured in cm^2. g^{-1}, g /cm^2, g(H_2O)/cm^2 and g . g^{-1} respectively. Various experiments in completely randomized factorial design with three replicates were conducted and the presented data included means of three separate experiments± SE. In order to analyze the data, SPSS software and ANOVA and Duncan test were used and all graphs were done by SPSS software and the results were presented as comparison graphs.

Results

Effect of lead on root growth

Analysis of data on root growth showed that lead poisoning caused significant reduction in root length, so that when lead concentration was increased, the amount of root length was significantly declined (P<0.01). Under lead and salicylic acid treatments, root length significantly increased compared to lead treatment (P<0.01) (Fig.I).

Effect of lead on shoot growth

When lead concentration was increased in cultivar Okapi 10-day treatment, the stem length was significantly decreased compared to control group (P<0.01). Application of salicylic acid 5 and 10 µM concentrations, significantly increased stem length compared to lead treatments (P<0.01) and 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 as compared with control (Fig. II).

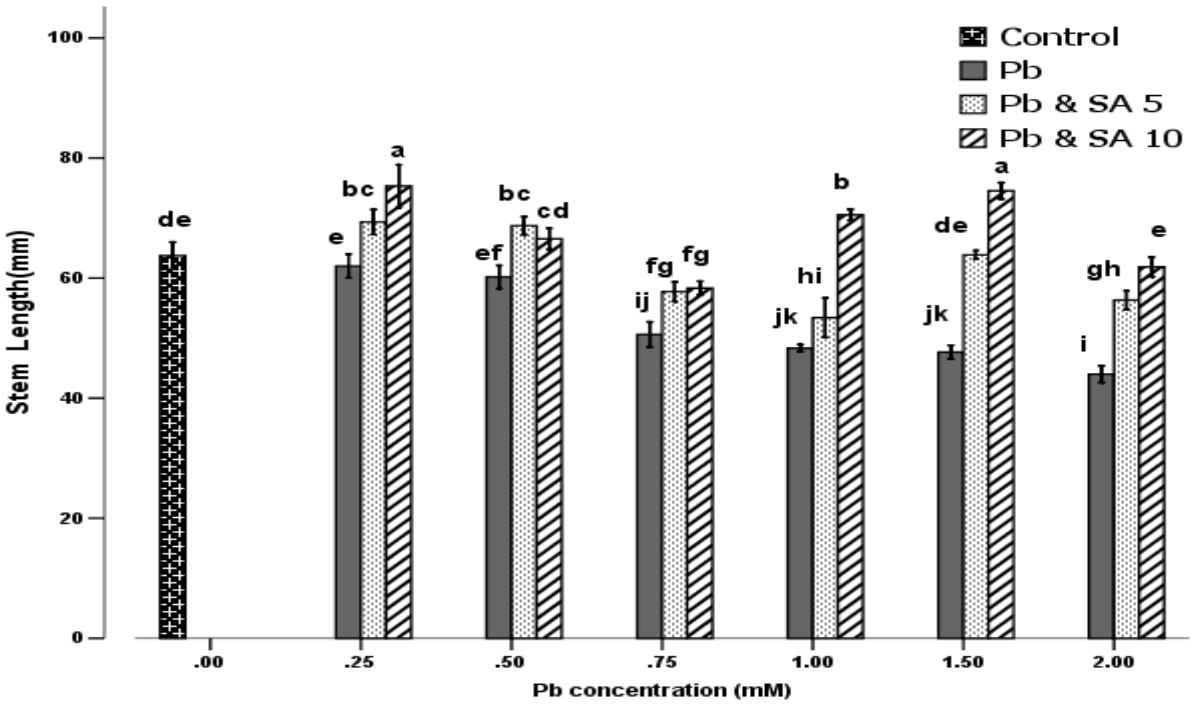

Fig. II. Effect of salicylic acid and lead on stem length after 10 days treatment in Hoagland nutrient solution

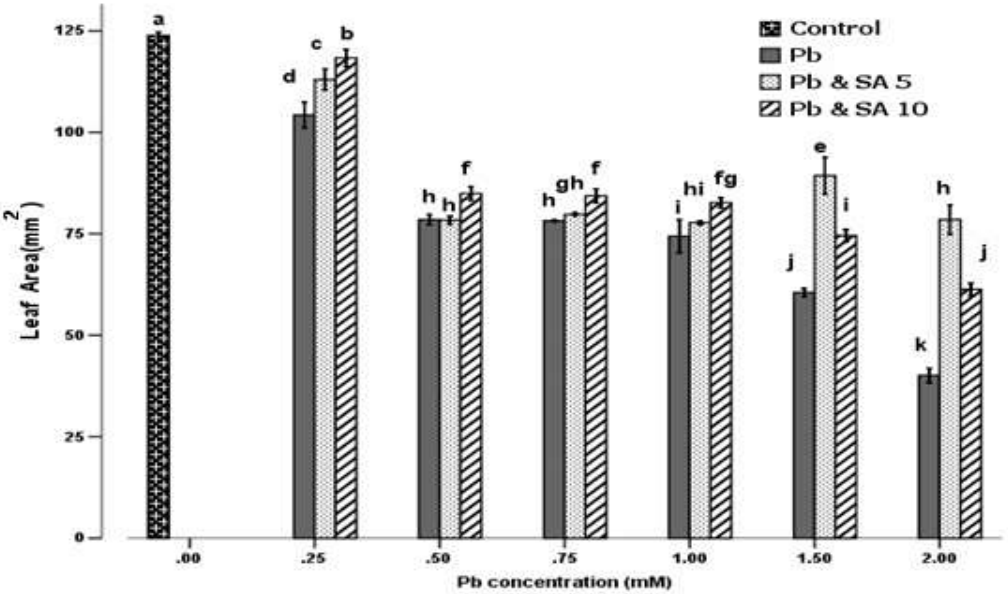

Fig. III. Effect of salicylic acid and lead on leaf area after 10 days treatment in Hoagland nutrient solution

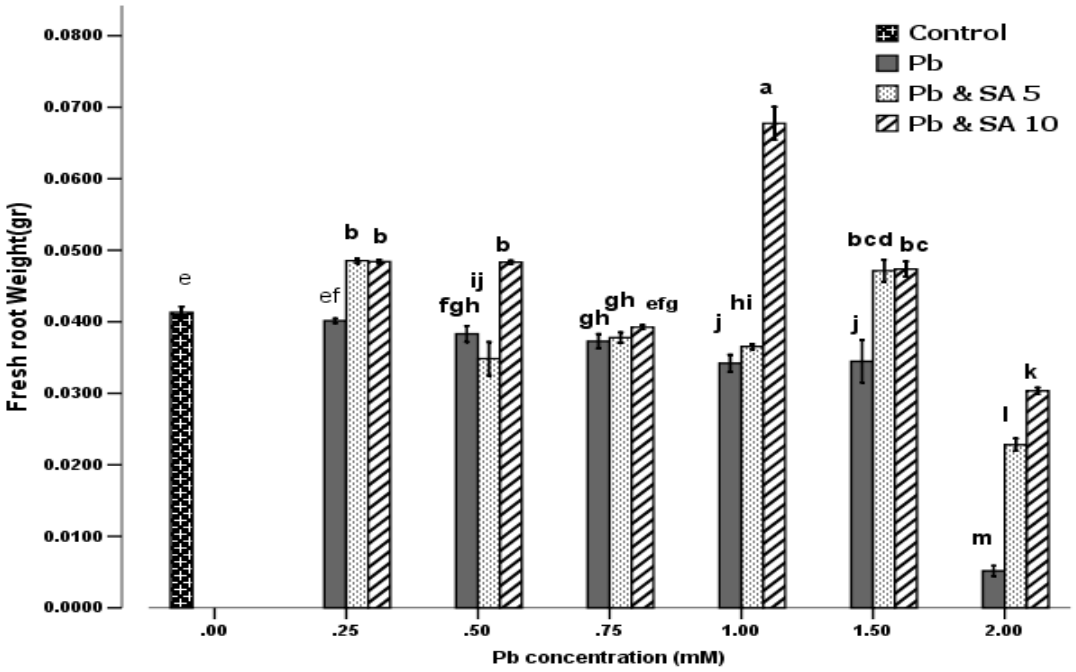

Fig. IV. Effect of salicylic acid and lead on fresh root weight after 10 days treatment in Hoagland nutrient solution

Effect of lead on the leaf area

With increasing concentration of lead, leaf area was decreased significantly (P <0.01) compared with the control group. Two concentrations of salicylic acid with 5 and 10 µM significantly increased (P <0.01) surface of leaf area as compared with lead treatments. The result showed significant difference (P <0.05) between two concentrations of salicylic acid (5 and 10 µM) (Fig. III).

Effect of lead on root and shoot fresh weight

Average fresh weight of roots and shoots under different lead treatments were significantly

decreased (P <0.01). Under Lead treatment with salicylic acid, the fresh weight of root and shoot were significantly increased as compared with lead treatments (P<0.01), so that in lead concentrations of 0.25, 0.5, 1 and 1.5 mM, salicylic acid 10 µM caused an increase in the root fresh weight which was higher than the control group (Fig. IV & V).

Effect of lead on root and shoot dry weight

Increasing concentrations of lead in medium of Hoagland, significantly (P <0.01) decreased dry weight of root and shoot. Application of salicylic acid significantly increased dry weight of root and shoot compared to lead treatments (P<0.01). Two concentrations of

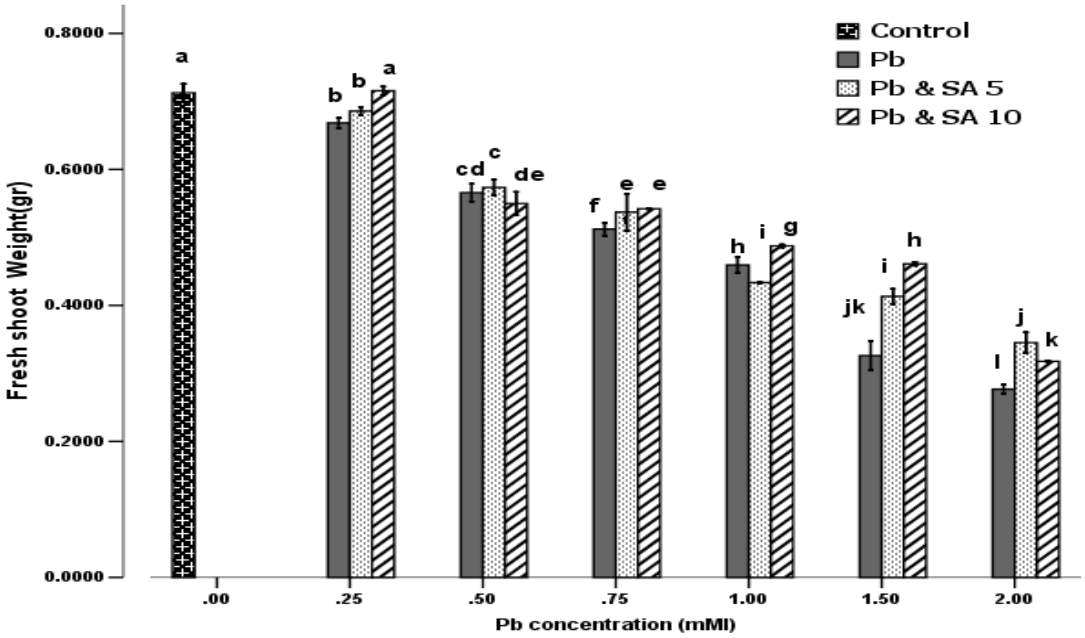

Fig. V. Effect of salicylic acid and lead on fresh shoot weight after 10 days treatment in Hoagland nutrient solution

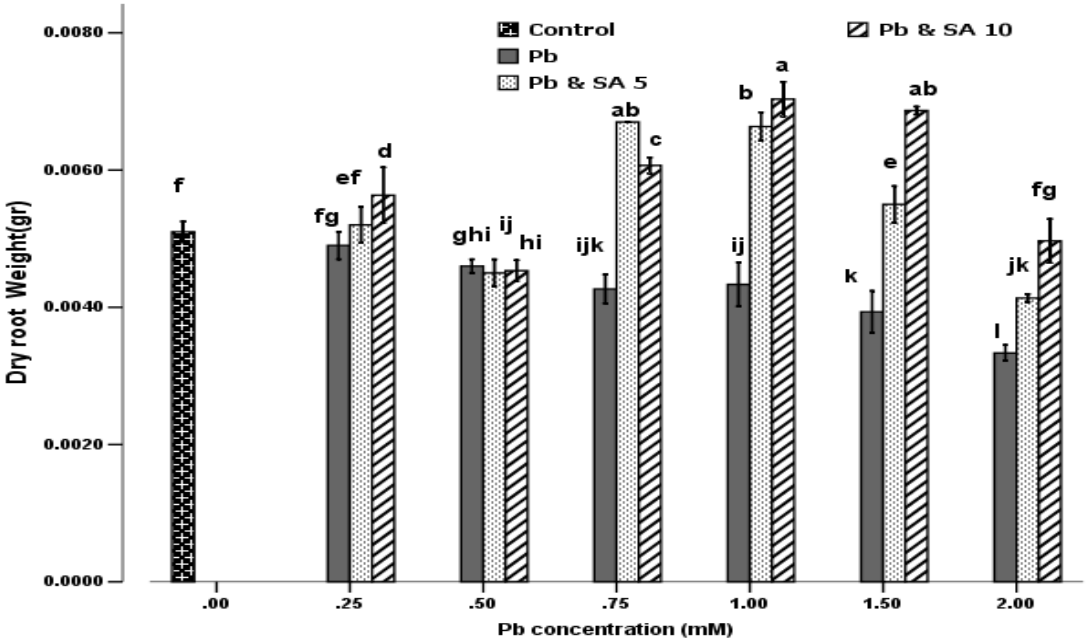

Fig. VI. Effect of salicylic acid and lead on dry root weight after 10 days treatment in Hoagland nutrient solution

salicylic acid had significantly different effects on root dry weight (P <0.01) while they had similar effects on shoot dry weight (P <0.05) (Figs. VI & VII).

Effect of lead on specific leaf area (SLA) and specific leaf weight (SLW)

Increasing lead concentration reduced the average specific leaf area (SLA), while the

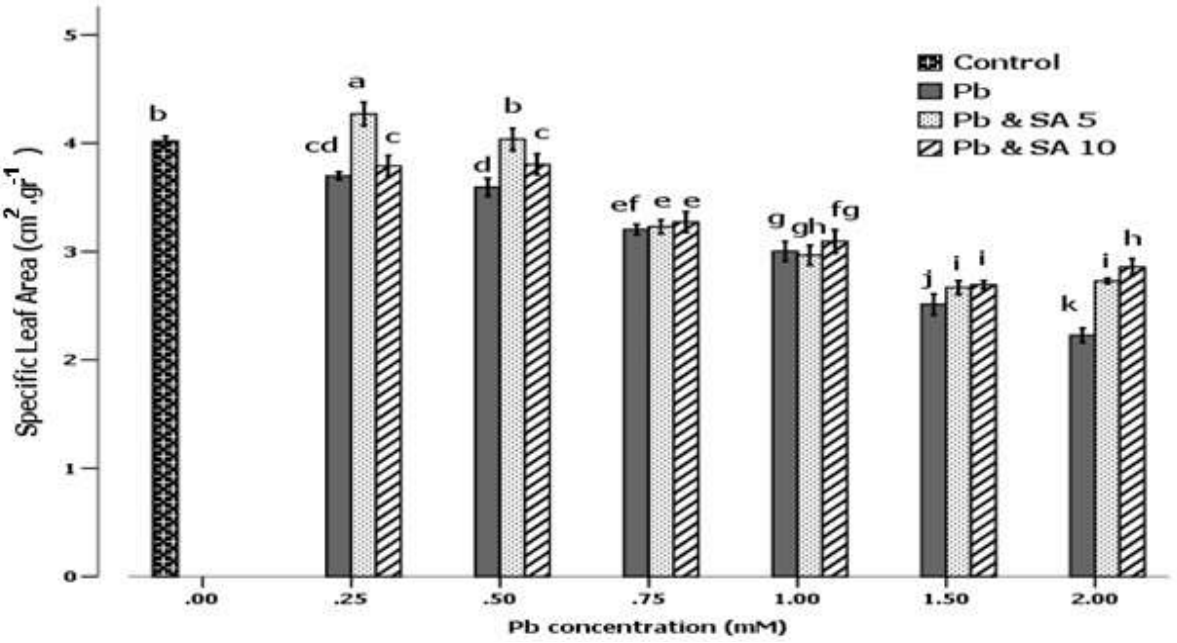

Fig. VII. Effect of salicylic acid and lead on dry shoot weight after 10 days treatment in Hoagland nutrient solution

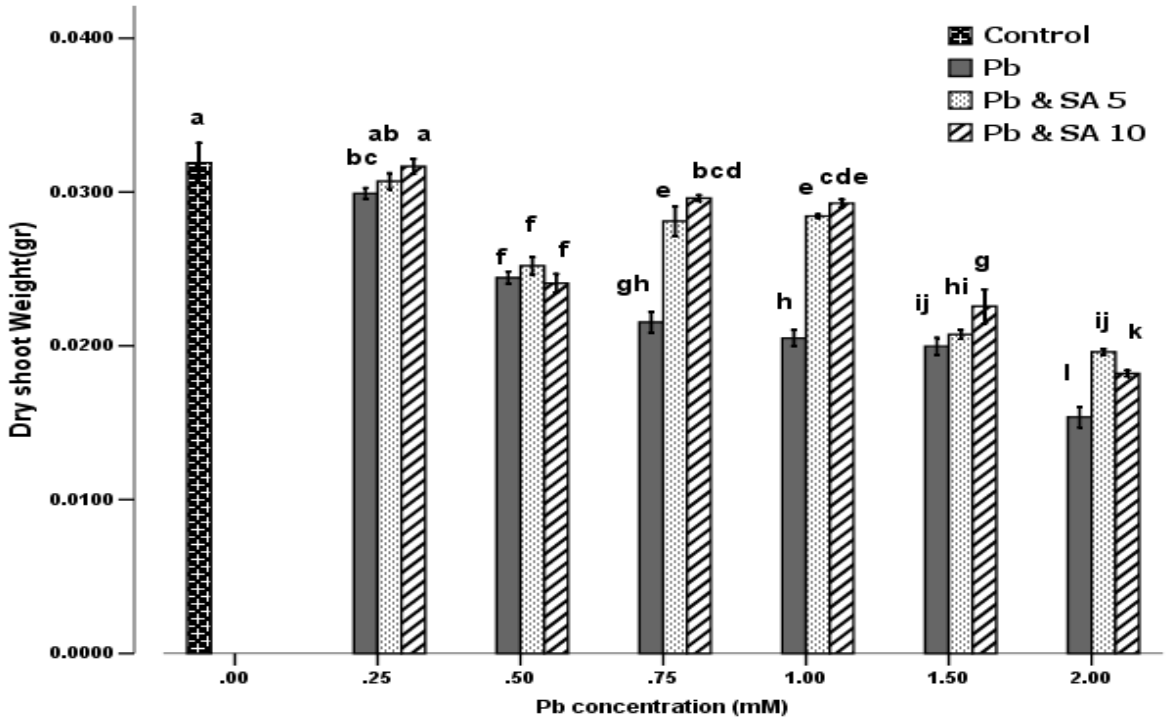

Fig. VIII. Effect of salicylic acid and lead on SLA after 10 days treatment in Hoagland nutrient solution

average of specific leaf weight (SLW) was significantly increased (P<0.01). Combined lead and salicylic acid treatments increased average SLA and significantly decreased average SLW (P <0.01) (Fig. VIII & IX).

Effect of lead on LWR and LWCA

Average LWR with increasing lead concentration decreased while there was a significant increase (P <0.01) in the average LWCA. Treated lead and salicylic acid mean LWR increased and the mean LWCA was significantly

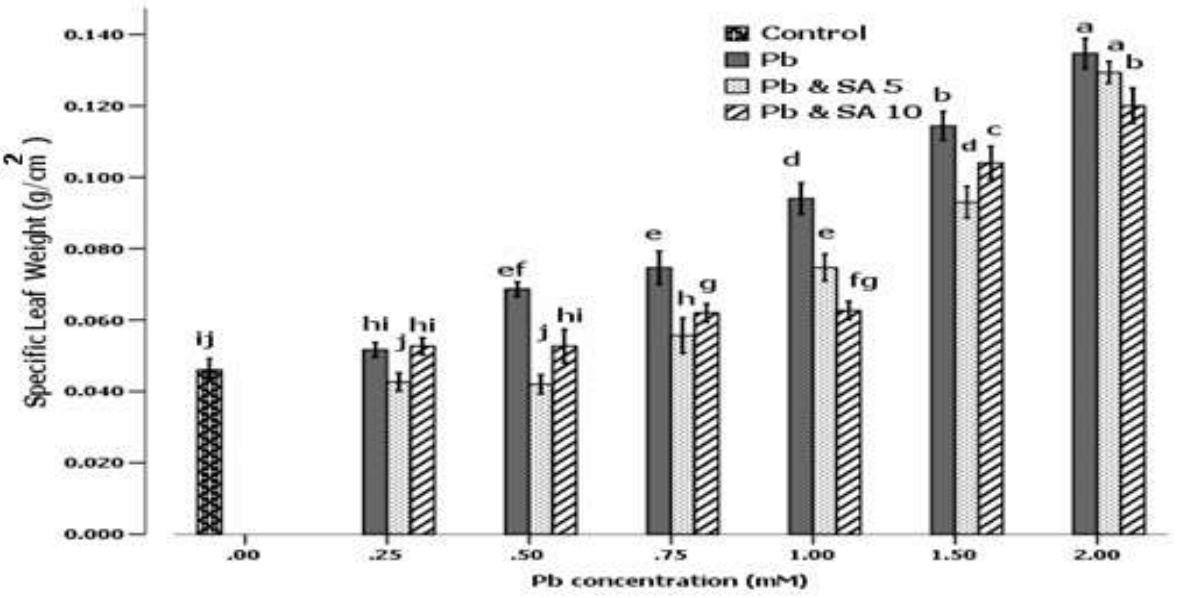

Fig. IX. Effect of salicylic acid and lead on SLW after 10 days treatment in Hoagland nutrient solution

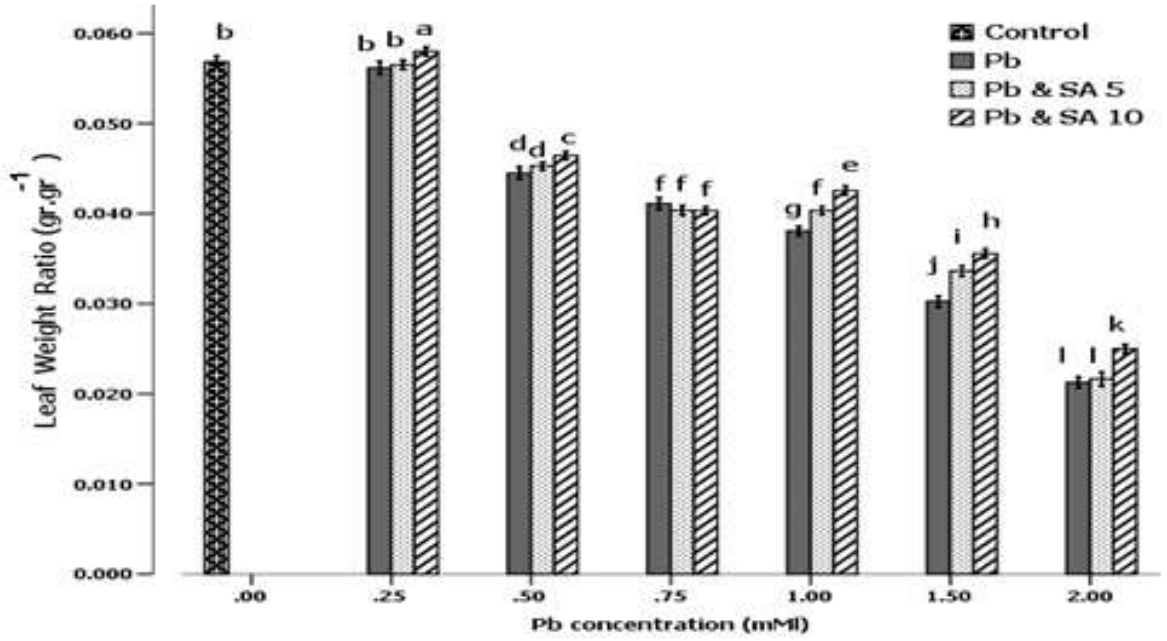

Fig. X. Effect of salicylic acid and lead on LWR after 10 days treatment in Hoagland nutrient solution

decreased (P <0.01) (Figs. X & XI).

Discussion

Changes in growth parameters

According to the results under different treatments of lead in the study, length of root and stem, leaf area, dry and fresh weight of roots and shoots, specific leaf area and leaf weight ratio decreased as compared to the control. On the other hand, specific leaf weight and leaf water content area were significantly increased ($P < 0.01$). Increasing lead accumulation in plant tissues, especially in roots with increasing concentration in the treatments was seen in similar studies (Kosobrukhov and Knyazeva, 2004). Heavy metals by inhibiting cell division or decreased cell expansion in the elongation zone or both of them reduce root length (Nalini and Chandra, 2002). With increase in concentration of lead, the stem length also decreased significantly ($P < 0.01$). Such phenomena, in stem, especially in the meristem area can be observed and in addition to reducing the power division, the elastic properties of cells and their membranes also decreases (Mohanty et al., 1989). On the other hand, treatment of lead caused the slow growth retardation and reduction in leaf area, therefore reduced surface transpiration. It thus limited transport of compounds from stem to other aerial organs and caused the inhibition of aerial organ growth (Pallavi and Rama ,2005). Analysis of data showed that the fresh and dry weight of roots was significantly decreased as plants received Pb in the nutrient solution. Reduction in the root biomass has also been reported in other plants. Similar results about the inhibitory effects of heavy metals such as copper in the plant *Brassica pekinesis* Rupr have been reported (Chao et al., 2006). Salicylic acid with two concentrations 5 and 10 µM significantly increased ($P < 0.01$) the root and stem length, leaf area, fresh weight of roots and shoots, dry weight of roots and shoots, SLA and LWR but significantly ($P < 0.01$) decreased LWCA and SLW. There are numerous reports about the role of salicylic acid on the growth of plants, including that application of salicylic acid, improves growth and yield plant responses to environmental stress (Pal et al., 2002). Also treated with 0.5 mM salicylic acid, cell division of apical meristem in wheat seedlings was increased and plant growth improved (Shakirova et al., 2003).

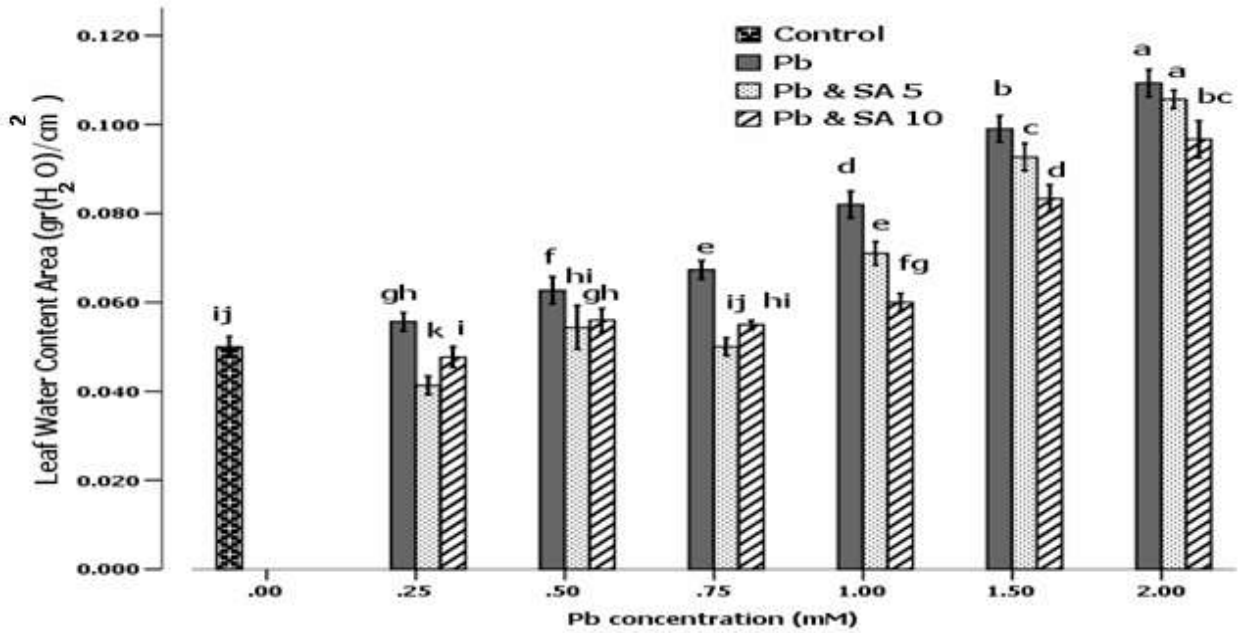

Fig. XI. Effect of salicylic acid and lead on LWCA after 10 days treatment in Hoagland nutrient solution

Conclusions

Heavy metals, particularly lead, are a toxic factor limiting crop production. As such, modification of heavy metal stresses is important. In this study, the antioxidant properties and role of salicylic acid-like hormone was examined and the modifying role of this compound in lead stress was shown as it could inhibit the damaging effects of lead.

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