RESEARCH PAPER

Alleviating of Lead toxicity by Salicylic acid in common bean (*Phaseolus vulgaris* L.) plants

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ABSTRACT:

This study was conducted in the greenhouse of Biology Department in the College of Science- Salahaddin University- Erbil, which consisted of interaction application of Salicylic acid (SA) concentrations (0,100,200,400 ppm) and Lead (Pb) concentrations (0, 5, 10 ppm) on vegetative growth, yield and chemical components of common bean. It was found that SA significantly decreased the harmful effects of Pb on the vegetative growth characteristics such as plant height, number of leaves/plant¹, number of branches/plant¹, the dry weight of the shoot system, and yield components such as number of seeds/pod¹, dry weight of 100 seeds. The adverse effects of Pb on chemical components such as chlorophyll a and total chlorophyll contents, total protein content of leaves, and total sodium by the influence of salicylic acid.

KEY WORDS: Lead; toxicity; Salicylic acid; common bean.
DOI: http://dx.doi.org/10.21271/ZJPAS.33.s1.9
ZJPAS (2021), 33(s1):86-92.

1. INTRODUCTION:

The Common bean (*Phaseolus vulgaris* L.) is a herbaceous annual plant species, belongs to fabaceae family domesticated independently in ancient Mesoamerica and now grown worldwide both for dry and green bean. Among major food Legumes the Common bean is the third most important worldwide, superseded only by Soybean [*Glycine max* (L.) Merr.], and Peanut (*Arachis hypogaea* L.) (Zeka, 2007). The common bean grown for their tender and green pods, or seeds as an important source of protein (20–25%) and complex carbohydrates (50–60%) (Martinez et al., 2011), dietary fiber, minerals such as iron, zinc, calcium, and phosphorus and vitamins (Carvalho et al., 2012). Salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. SA has been found to play a vital role in the regulation of plant growth, development, and interaction with other factors and in the responses to environmental stresses. Furthermore, it’s role is evident in seed germination, plant growth, fruit yield, glycolysis, flowering, ion uptake and transport, photosynthetic rate, stomatal conductance and transpiration …etc (Sadeghipour and Aghaei, 2012 A, B). It is implicated in hardening response to a biotic stressors and mediates some positive acclimation response to a biotic stress, such as heavy metals, herbicides, low temperature, salinity (Mohsenzadeh et al., 2011), and osmotic stress. Along with stresses, toxic heavy metal stress is an emerging and more effective stress for major crops (Bhardwaj et al., 2009).

Lead (Pb), is one of the heaviest non-essential metals released into the natural environment from a range of anthropogenic activities (Ekmekçi et al., 2009). Upon release, it gets accumulated in the soil and causes toxicity to plants and animals (Kaur et al., 2010). Significant increases in the Pb...
content of cultivated soils have been observed near urban and industrial areas where it tends to accumulate in the surface ground layer (Hussain et al., 2006). Pb contamination in soils not only aroused the changes of soil microorganism and it’s activities and resulted in soil fertility deterioration, but also directly affected the change of physiological indices and, resulted in yield decline (Majer et al., 2002). Pb is taken up by plants mainly through the root system and partly in minor amounts through the leaves and trichomes. Pb toxicity in plant causes such problems a 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. The Pb contamination in the plant environment is known to affect the seed germination, seedling growth, photosynthesis, respiration, nitrate assimilation and other processes (Singh et al., 2003). Mishra and Choudhuri, (1997) found that SA application at a concentration of 0.1 or 0.2 mM reduced the inhibitory effect of Pb²⁺ on seedling growth of two rice (Oryza sativa L.) cultivars, SA increased the fresh and dry mass of shoots and roots in both cultivars under Pb stress conditions. Tavakoli et al.,(2011) observed that effect of SA (1, 5 and 10 µM) and Pb (0.005, 0.01 and 0.015 M) significantly increased the plant height, number of leaves, leaf area, fresh and dry weight as compared with those treated with Pb only in eggplants.

This study was carried out to investigate the ability of SA to alleviating of Pb toxicity through the interaction effects of different concentrations of each of vegetative growth yield and chemical components on common bean.

2. MATERIALS AND METHODS

This study was conducted in the glasshouse of the biology Department, College of Science, Salahaddin University-Erbil, during March 7 2012 to July 5 2012, to investigate the interaction effects of Salicylic acid (SA) and lead (Pb) on growth and development of common bean. The study involved 40 plastic pots each pot with a diameter of 24 cm in length and 21 cm in depth. Each pot filled with 7kg of dry sandy loam soil of Askikalak area, the soil sieved through 2mm pore size sieves, add in each pot three seed were sown. This experiment consisted of combination treatments of foliar spray with different Salicylic acid (SA) concentrations at doses (0, 50, 100, 200, 400ppm) and soil irrigation by two Pb (PbNO₃) concentrations (5, 10 ppm), and involved ten treatments with four replications. The following measurements have been taken for each pot: plant height (cm), number of leaves/plant⁻¹, number of branches/plant⁻¹, shoot dry weight (g), the water content of shoot system, leaf area (cm²), stem diameter, yield components such a number of pods/plant⁻¹, number of seeds/pod⁻¹, dry weight of 100 seeds(g), and chemical contents such as photosynthetic pigments, proline content of leaves, phenol content of leaves, mineral nutrients of leaves.

Chlorophyll content in leaves (mg.g⁻¹) estimated by taking 0.5g of fresh leaves left in 10 ml of absolute ethanol for 24 hrs. In dark condition, this process repeated three times to complete extraction of chlorophyll the final volume reached 30 ml were spectrophotometrically estimates on two wavelength 649and 665 nm as follows (Wintermans and Demote, 1967):  

\[
\mu g \text{ chlorophyll a/ml solution} = (13.70) (A665nm)-(5.76) (A649nm)
\]

\[
\mu g \text{ chlorophyll b/ml solution} = (25.80) (A649nm)-(7.60) (A665nm)
\]

Total chlorophyll =chlorophyll a + chlorophyll b

A=absorbance

nm =nanometer

Proline was determined according to the method as described by (Bates et al., 1973 and Hassan, 2011). The total phenol content was estimated by Folin-Ciocalteau reagent as methods described by (Sharma et al., 2011). The total Carbohydrates were estimated following the Phenol-sulphuric acid method as described by Dey (1990). The total nitrogen was determined by kjeldahl method as described by A.O.A.C, (2010). The total phosphorus was determined, using spectrophotometer method as described by Ryan et al., (2001). The total potassium and sodium are determined by the flame photometer methods as described by Allen, (1974). Total calcium, magnesium, zinc, manganese and iron and lead contents were determined by using atomic absorption method as described by Allen, (1974).

Statistical analysis

The data of this study designed according to Factorial Completely Randomized Designs (Factorial C.R.D) with four replications and ten treatments. Duncan Multiple Range Test used for the comparison of treatment means at 5% for greenhouse parameters and 1% levels for laboratory parameters. The statistical analysis was done by using Statistical Package for Social
Sciences (SPSS version 16 software). For drawing charts, Excel 2007 software used.

3. RESULTS AND DISCUSSION

3.1. Vegetative growth characteristics

Table (1) shows that SA decreased the negative effect of Pb on plant height at different growth stages, which significantly (p≤0.05) increased by Pb$_5$SA$_{200}$, Pb$_5$SA$_{400}$, Pb$_{10}$SA$_{400}$ as compared with controls Pb$_5$SA$_{200}$ and Pb$_{10}$SA$_{400}$ after 15 days from application. And at Pb$_5$SA$_{400}$ as compared with Pb$_5$SA$_0$ after 30 days from application, and at Pb$_5$SA$_{200}$, Pb$_5$SA$_{400}$ as compared with control Pb$_5$SA$_0$, Pb$_{10}$SA$_{100}$, Pb$_{10}$SA$_{200}$, Pb$_{10}$SA$_{400}$ as compared with controls Pb$_5$SA$_0$, Pb$_{10}$SA$_{400}$ after 45 days from application. After 60 days from application at Pb$_5$SA$_{100}$, Pb$_5$SA$_{200}$, Pb$_5$SA$_{400}$, Pb$_{10}$SA$_{50}$, Pb$_{10}$SA$_{100}$, Pb$_{10}$SA$_{200}$, Pb$_{10}$SA$_{400}$ as compared with Pb$_5$SA$_0$, Pb$_{10}$SA$_0$. However, there were significant differences between treatments. The increases in plant height with increasing concentrations of SA in the present investigation are in agreement partially with those finding reported by (Sadeghipour and Aghaei, 2012A). SA treatments showed synergetic effect with endogenous phytohormones auxins, gibberellins and cytokinines, which are causing cell elongation leading to increase of plant height (Mady, 2009).

Table 1: Interaction effects of SA and Pb on plant height at different stages of growth

| Interaction treatments | Plant height (cm) after (days) from application |
|------------------------|-----------------------------------------------|
|                        | 15 days | 30 days | 45 days | 60 days |
| Pb mg.Kg$^{-1}$        |         |         |         |         |
| 5                      |         |         |         |         |
| 0                      | 12.62$^b$ | 15.40$^b$ | 18.32$^c$ | 20.62$^e$ |
| 50                     | 13.22$^b$ | 15.58$^{ab}$ | 19.20$^{bc}$ | 21.90$^{de}$ |
| 100                    | 14.30$^{ab}$ | 16.14$^{ab}$ | 20.17$^{bc}$ | 22.64$^{cd}$ |
| 200                    | 15.17$^a$  | 17.35$^{ab}$ | 22.52$^a$  | 25.23$^{ab}$ |
| 400                    | 15.57$^a$  | 17.92$^a$  | 21.25$^{ab}$ | 24.70$^{ab}$ |
| 10                      |         |         |         |         |
| 0                      | 12.65$^b$ | 15.30$^b$ | 18.70$^c$ | 21.50$^{cd}$ |
| 50                     | 14.02$^{ab}$ | 15.72$^{ab}$ | 20.85$^{abc}$ | 23.97$^c$ |
| 100                    | 13.70$^{ab}$ | 16.25$^{ab}$ | 21.14$^{ab}$ | 24.03$^{bc}$ |
| 200                    | 13.20$^{b}$ | 17.35$^{ab}$ | 22.45$^a$  | 25.87$^a$  |
| 400                    | 15.62$^a$  | 16.87$^{ab}$ | 22.15$^a$  | 24.92$^{ab}$ |

*Means within the same column, with the same letters are not significantly different from each other according to Duncan’s multiple ranges test at 5% level.

Table (2) shows that SA decreased the negative effect of Pb on number of leaves at different growth stages, which significantly (p≤0.05) increased number of leaves by the treatment Pb$_5$SA$_{50}$, Pb$_5$SA$_{400}$, Pb$_{10}$SA$_{200}$ as compared with Pb$_5$SA$_0$, Pb$_{10}$SA$_0$ after 15 days from application and at Pb$_5$SA$_{200}$, Pb$_5$SA$_{400}$, Pb$_{10}$SA$_{200}$, Pb$_{10}$SA$_{400}$ as compared with controls Pb$_5$SA$_0$, Pb$_{10}$SA$_0$ after 30 days from application. And at Pb$_5$SA$_{200}$, Pb$_5$SA$_{400}$ as compared with controls Pb$_5$SA$_0$, Pb$_{10}$SA$_0$ after 45 days from application and after 60 days from application at Pb$_5$SA$_{200}$, Pb$_5$SA$_{200}$, Pb$_5$SA$_{400}$, Pb$_{10}$SA$_{400}$ as compared with Pb$_5$SA$_0$, Pb$_{10}$SA$_0$ and there were significant differences between treatments.). These results partially agreed with those obtained from basil and marjoram plants (Gharib, 2007), common bean plants (Hegazi and El-Shairy, 2007) and pea plants (El-shairy and Hegazi, 2009). SA at 400ppm was the most effective treatments in increasing number of leaves.
According to the results presented in Table (3), SA alleviated the effect of Pb stress on number of branches at different growth stages, which significantly \( (p \leq 0.05) \) increased by \( \text{Pb}_5 \text{SA}_{400}, \) \( \text{Pb}_{10} \text{SA}_{400} \) as compared with controls \( \text{Pb}_5 \text{SA}_0, \text{Pb}_{10} \text{SA}_0 \) after 15, 30, 45 days from application and at \( \text{Pb}_5 \text{SA}_{200}, \text{Pb}_{10} \text{SA}_{400} \) as compared with controls \( \text{Pb}_5 \text{SA}_0, \text{Pb}_{10} \text{SA}_0 \) after 60days from application and there were significant differences between treatments. These results agreed partially with those obtained by Devi et al., (2011) and Ali and Mahmoud, (2013), who pointed out that SA treatments increased the number of branches. The increase in the number of branches could be due to the suppression of apical dominance, thereby diverting the polar transport of auxins towards the basal nodes leading to increased branching (Naz, 2006).

### Table 2: Interaction effects of SA and Pb on number of leaves at different stages of growth

| Interaction treatments | Pb mg.Kg\(^{-1}\) | SA ppm | Number of leaves after (days) from application |
|------------------------|------------------|--------|-----------------------------------------------|
|                        |                  | 15 days | 30days | 45days | 60days |
| 5                      | 0                | 9.00 \(^b\) | 11.75 \(^c\) | 13.75 \(^c\) | 16.00 \(^b\) |
|                        | 50               | 9.75 \(^{ab}\) | 12.00 \(^{bc}\) | 15.25 \(^{bc}\) | 17.50 \(^{ab}\) |
|                        | 100              | 11.00 \(^a\) | 12.75 \(^{abc}\) | 15.00 \(^{bc}\) | 18.50 \(^a\) |
|                        | 200              | 10.50 \(^{ab}\) | 13.50 \(^{ab}\) | 16.25 \(^a\) | 18.25 \(^a\) |
|                        | 400              | 11.25 \(^{a}\) | 14.00 \(^a\) | 16.00 \(^a\) | 19.50 \(^{a}\) |

*Means within the same column, with the same letters are not significantly different from each other according to Duncan’s multiple ranges test at 5% level.

### Table 3: Interaction effects of SA and Pb on number of branches at different stages of growth

| Interaction treatments | Pb mg.Kg\(^{-1}\) | SA ppm | Number of branches after (days) from application |
|------------------------|------------------|--------|-----------------------------------------------|
|                        |                  | 15 days | 30days | 45days | 60days |
| 5                      | 0                | 3.25 \(^b\) | 5.00 \(^b\) | 6.00 \(^c\) | 7.50 \(^c\) |
|                        | 50               | 3.25 \(^b\) | 5.25 \(^{ab}\) | 6.25 \(^{bc}\) | 8.00 \(^c\) |
|                        | 100              | 3.50 \(^b\) | 5.25 \(^{ab}\) | 6.25 \(^{bc}\) | 8.25 \(^c\) |
|                        | 200              | 3.75 \(^{ab}\) | 5.75 \(^b\) | 6.75 \(^{bc}\) | 8.75 \(^{ab}\) |
|                        | 400              | 4.50 \(^{a}\) | 6.00 \(^{a}\) | 7.50 \(^{a}\) | 9.00 \(^{abc}\) |

*Means within the same column, with the same letters are not significantly different from each other according to Duncan’s multiple ranges test at 5% level.
3.2 Yield characteristics

Table (4) shows the interaction effects of SA and Pb on yield characteristics. It is observed that there were no significant differences between treatments.

3.3 Chemical components of seeds and leaves

Table (5) indicated that SA decreased the negative effect of Pb on chlorophyll contents of fresh leaves, which significantly \((p\leq0.01)\) increased chlorophyll \(a\) by \(\text{Pb}_{5}\text{SA}_{100}\), as compared with \(\text{Pb}_{5}\text{SA}_{0}\), and significantly increased by \(\text{Pb}_{10}\text{SA}_{50}\), \(\text{Pb}_{10}\text{SA}_{400}\) as compared with \(\text{Pb}_{10}\text{SA}_{0}\), and there significant differences between 100ppm and 400ppm in total chlorophyll content. These results partially agreed with those obtained by Turkyilmaz \textit{et al.} (2005), who suggested that foliar spray with SA increased chlorophyll \(a\), \(b\), and other photosynthetic pigments in bean under normal field conditions. The stimulation effect of SA on chlorophyll concentration was confirmed by Azooz \textit{et al.} (2011) on broad bean, Fahd and Bano (2012) on maize plants. Table (6) SA treatments decreased the negative effect of Pb on total protein content of leaves, which significantly \((p\leq0.01)\) increased by \(\text{Pb}_{10}\text{SA}_{100}\), \(\text{Pb}_{10}\text{SA}_{400}\) as compared with \(\text{Pb}_{10}\text{SA}_{0}\), SA treatments significantly \((p\leq0.01)\) decreased the content of proline under Pb stress by \(\text{Pb}_{10}\text{SA}_{400}\) as compared with \(\text{Pb}_{10}\text{SA}_{0}\). Data in Table (7) indicated that SA decreased the negative effect of Pb on nitrogen content of leaves. It was observed that there were significant differences between 50ppm with 100 and 400ppm. It is observed that there were significant differences between \(\text{Pb}_{10}\text{SA}_{50}\) with \(\text{Pb}_{10}\text{SA}_{100}\) \(\text{Pb}_{10}\text{SA}_{200}\) and \(\text{Pb}_{10}\text{SA}_{400}\) in potassium content of leaves. The interaction effects of SA and Pb on potassium content in leaves, that there were significant differences between \(\text{Pb}_{5}\text{SA}_{50}\) with \(\text{Pb}_{5}\text{SA}_{400}\). There were no significant differences between treatments in total zinc, iron, and lead content of leaves. SA treatments increased the contents of K, while decreased Na contents in mung bean plant (Khan \textit{et al.}, 2010). SA caused significant increases in the uptake of elements in tomato plant (Amin \textit{et al.}, 2007). These increases in some mineral content might be connected with the increase in photosynthetic pigments which in turn affect the rate of organic compound assimilation (Abou El-Yazeid, 2011).

4. CONCLUSIONS

In conclusion the adverse effects of Pb toxicity alleviated by foliar application of SA in common bean plants on vegetative growth such plant height, number of leaves, number of branches, as well as on yield characteristics like number of seeds per pods and dry weight of 100 seeds, further more on and chemical components such as photosynthetic pigments also chemical contents protein, proline, total phenol, mineral nutrients nitrogen, potassium, sodium, and manganese.
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