Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 6[4] March 2017: 48-54

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

The Alleviating Effect of Salicylic Acid on Early Seedling Growth Of Soybean Under Zinc and Lead Stress

Siddhi Gupta, Manoj Kr. Meena, Soumana Datta
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India 302004
Email: smart.siddhi1989@gmail.com

ABSTRACT
Salicylic acid is an important signal molecule modulating plant response to stress. To assess the effect of the exogenous application of salicylic acid (0.5m M SA) on 7days seedling of soybean (Glycine max (L.) Merr.), pot experiments were conducted. The seeds were subjected to different concentrations of both Zinc (250, 500, 750, 1000 and 1250 mg/l) and Lead (200, 400, 600, 800, 1000 mg/l) as Zinc sulphate and lead acetate with 0.5m M SA as compared to control. The morphological growth parameters such as germination percentage, root length, shoot length fresh weight and dry weight analysis were assessed. Our earlier studies have showed as prior paper of mine showed that Lead concentrations of 1000mg/l significantly decreased the percentage of germination and root length. However, at low levels of zinc (250 and 500 mg/l), showed a significant increase in germination percentage and also increased root shoot length was observed. But at high levels (750–1250mg/l) of zinc detrimental effect on root growth and germination was noted. However, the SA treatment enhanced growth & attenuated the adverse effects of Pb and Zn on these attributes. The results indicated the possible role of SA as a potential inhibitor of heavy metal toxicity by strengthening the internal immunity in soybean.
KEYWORDS: *Soybean, Salicylic acid (SA), Lead (Pb), Zinc (Zn), Seedling growth.*

Received 11.12.2016 Revised 15.01.2017 Accepted 23.02.2017

INTRODUCTION

Soil is of vital importance to plant growth and development, providing all kinds of nutritious elements. However, with the rapid industrial and agricultural development worldwide, agricultural soils are slightly to moderately contaminated with toxic heavy metals that restrict the crop plants to reach their full genetic potential, Significant loss by reducing the crop productivity is also seen. Organic manure can play a great role in improving nutritional status of poor soils (1) The noxious heavy metals in different valence states include zinc (Zn), arsenic (As), chromium (Cr), cadmium (Cd), mercury (Hg), copper (Cu), nickel (Ni) and lead (Pb), Of these Zinc is an essential element for several biochemical processes in plants, such as cytochrome and nucleotide synthesis, auxin metabolism, chlorophyll production, enzyme activation and membrane integrity. Hyper accumulation of Zn in plants, however limits shoot and root growth (2) and also inhibit the uptake of iron that lead to iron deficiency which is characterized by pale yellow to white interveinal chlorosis on the younger leaves, may eventually lead to necrosis of the leaf blades and growing points. Lead on the other hand is not an essential element for growth of plants, nor does it participate in cell metabolism. The primary effect of Pb toxicity in plants is a rapid inhibition of root growth due to the inhibition of cell division in the root tip. High concentration of Pb alters the transcriptional process, denatures the proteins and disturbs photosynthesis (3).

Soybean is an important oilseed crop which ranks second in oilseed after groundnut in India. Soybean contains about 20 percent oil and 40 percent protein (as against 7.0 per cent in rice, 12 per cent in wheat, 10 per cent in maize and 20-25 percent in other pulses) (4) Soybean oil is basically used in cooking as edible oil that containing no cholesterol and almost none of the saturated fat so it is an ideal food for heart patients. (2) Shows that the accumulation of Pb and Zn in soybean seedlings not only seriously affects seedling growth and soybean yield, but also exerts toxic effects on human health by food chain. As a novel endogenous plant hormone, salicylic acid (SA) is considered to have beneficial physiological effects on plants growing under heavy metal stress. The results of numerous researches showed that SA can alleviate the stress effects of many heavy metal elements, in varieties of crops (5). For example, exogenously applied SA attenuated the adverse effects of Pb and Hg on cellular membranes in some rice cultivars (6). Exogenous application of SA can mitigate Cd^{2+} toxicity in pea seedlings. Such alleviating effect of SA was also examined in soybean seedlings grown under Cd^{2+} stress (7). Plant growth promoting bacteria (PGPRs) also help in the alleviation of side effect of heavy metals on plants by solubilisation of metals and by increasing plant growth hormonal activity and availability (8, 9).Owing to considerable evidence of the adverse effects of Pb and Zn on plant growth, it was hypothesized that SA can assuage the injurious effects of both heavy metal on soybean. Thus, the primary objective of this work was to examine whether or not SA could mitigate the Pb and Zn - induced inhibition of plant growth.

MATERIALS AND METHODS

The present investigation was carried out to find the alleviating effect of Salicylic acid on seedling growth on 7days seedling of soybean under heavy metal stress.

Plant material and Chemicals

The certified seeds of soybean (*Glycine max* (L.) Merr.) Produced by Vigor Biotech Pvt. Ltd., variety JS-95-60 was procured from Agriculture station, Kota, Rajasthan. The seeds with uniform size, color and weight were chosen for experimental purpose. Chemicals were used zinc sulphate ($ZnSO_4.7H_2O$) and lead acetate $(CH_3COO)_2$ $Pb.3H_2O$, with various concentrations (250, 500, 750, 1000, 1250 mg/kg and 200, 400, 600, 800, 1000 mg/kg respectively) were used in the experiments.

Soil collection and physiochemical properties

Agricultural soil was collected from the experimental farm (depth 0-15cm) in Rajasthan University campus, Jaipur. The soil was air dried under room temperature and then sieved through 2-mm mesh. Some basic physiochemical properties were measured at Durgapura, Jaipur (2). Four (4) kg soil was weighed and loaded into each pot.

Experimental setup

The experiments were set up in in green house by Botany Department, University of Rajasthan, Jaipur during the month of April, 2015 in natural outdoor conditions where the photoperiod was 12 h and the average temperature was 30^0 C. Four kg of soil was filled in pots 30 cm high and 25 cm in diameter. Five concentrations of lead (200, 400, 600, 800, 1000 mg/kg) and zinc (250, 500, 750, 1000, 1250 mg/kg) were applied as lead acetate and zinc sulphate forms respectively. No other supplement nutrients were applied. Pots without added heavy metals were taken as controls. Soybean seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for two minutes and thoroughly washed with double distilled water. Ten sterilized seeds of soybean were sown equidistantly at 2 cm deep in each pot. Three replicates were used for each concentration. Watering of pots was done on alternate days.

Germination and growth measurements

The number of seeds germinated in each treatment was counted on the seventh day and germination percentage (%G) was calculated by following formula [2]:

%G = (number of germinated seeds / total number of plant seeds) × 100.

The emergence of radical was taken as a criterion for germination. Seedlings from each replica was selected for recording the morphological parameters such as root and shoot length, dry and fresh weight. Seedlings were cut at the root-shoot junction and the length of their root and shoot was measured with a metric scale and expressed in centimetres. The fresh weight of seedling samples was recorded on an analytical balance and expressed in gram per seedling. Later, seedling were dried in an oven at 80^o C for 24 hours to get constant dry weight. After 24 hours the dry weight was recorded.

Stress tolerance index

Stress tolerance index is a useful tool for determining the high yield and stress tolerance potential of genotypes. Stress tolerance indices for different growth parameters were calculated using following formulae [2]:

RLSTI = (Root length of stress plant / Root length of control plant) × 100

SLSTI = (Shoot length of stress plant / Shoot length of control plant) × 100

SFSTI = (Seedling fresh weight of stress plant / Seedling fresh weight of control plant) × 100

SDSTI = (Seedling dry weight of stress plant / Seedling dry weight of control plant) × 100

Statistical analysis

Each treatment was analysed with at least three replicates and the standard error (SE) was calculated. The data were expressed in mean data ± SEM (Standard error of mean).

RESULT AND DISCUSSION

Heavy metal stress adversely affected growth parameters (shoot and root length, shoot and root dry weight [2] in the present study, applications of SA gradually lightened the negative effects of heavy metal stress on growth parameters.

GERMINATION PERCENTAGE:

It has been recorded earlier by us that the presence of lead (Pb) and zinc (Zn) in the soil significantly decreased the values of germination percentage in a concentration dependent manner. At low level of zinc (250 and 500 mg/l) showed a significant increase in germination but growth process beyond these levels (750–1250mg/l) are impacted adversely. However lead exhibited gradual decrease in germination percentage. At a **c**oncentration of 1000mg/l, lead inhibited significantly seed germination by 23.0% (2).In the present study, In control plants, SA (0.5m M) caused significant increase in germination percentage in comparison to non-treated (control) plant. The follow-up treatment of the stressed plants with SA (.5mM) reduced the damaging effect of lead and zinc by increasing the germination percentage. Similar ameliorative role of SA was also observed in *Brassica juncea,* exposed to Mn toxicity (10) and *Cucumis melo* L. exposed to cadmium (11).These findings strengthened our results that the damaging effects caused by lead and zinc were mitigated by exogenous application of SA in the form of enhanced early seedling growth.

ROOT LENGTHS

Performance of soybean was affected adversely compared to the control when plants were subjected to high concentration of lead and zinc. In the present study when applying exogenous SA (0.5mM) in soil, the growth performance of plant improved. Highest value of root length was observed (12.63 cm/seedling) at 500 mg/L zinc and (10.2 cm/seedling) at 200mg/l lead concentrations with .5mM SA respectively as compared to control. Similar ameliorative role of SA was observed in earlier study of *Brassica napus* var. Okapi. exposed to lead stress [12] and in Rice (*Oryza sativa*) under Cadmium Stress (13).

SHOOT LENGTH:

Previous finding shows that the highest decrease in mean shoot length of soybean seedling was found 5.25 cm/seedling and 4.34 cm/seedling at 1250 mg/L zinc and 1000mg/l lead concentrations respectively [2]. This stress is reduced by application of salicylic acid (0.5mM). As we found shoot length (6.55 cm/seedling and 5.15 cm/seedling) of soybean seedling at 1250 mg/L zinc and 1000mg/l lead concentrations respectively with 0.5mM salicylic acid. Highest shoot length (15.09cm/seedling and 11.5 cm/seedling) were observed at 500 mg/L zinc and 200mg/l lead concentrations respectively as compared to control seedlings in the present study. Similar role of SA was observed in earlier study of *Brassica napus* var. Okapi. exposed to lead stress [12], in Rice (*Oryza sativa*) under Cadmium Stress [13] and in Rice under mercury and lead stress [6].

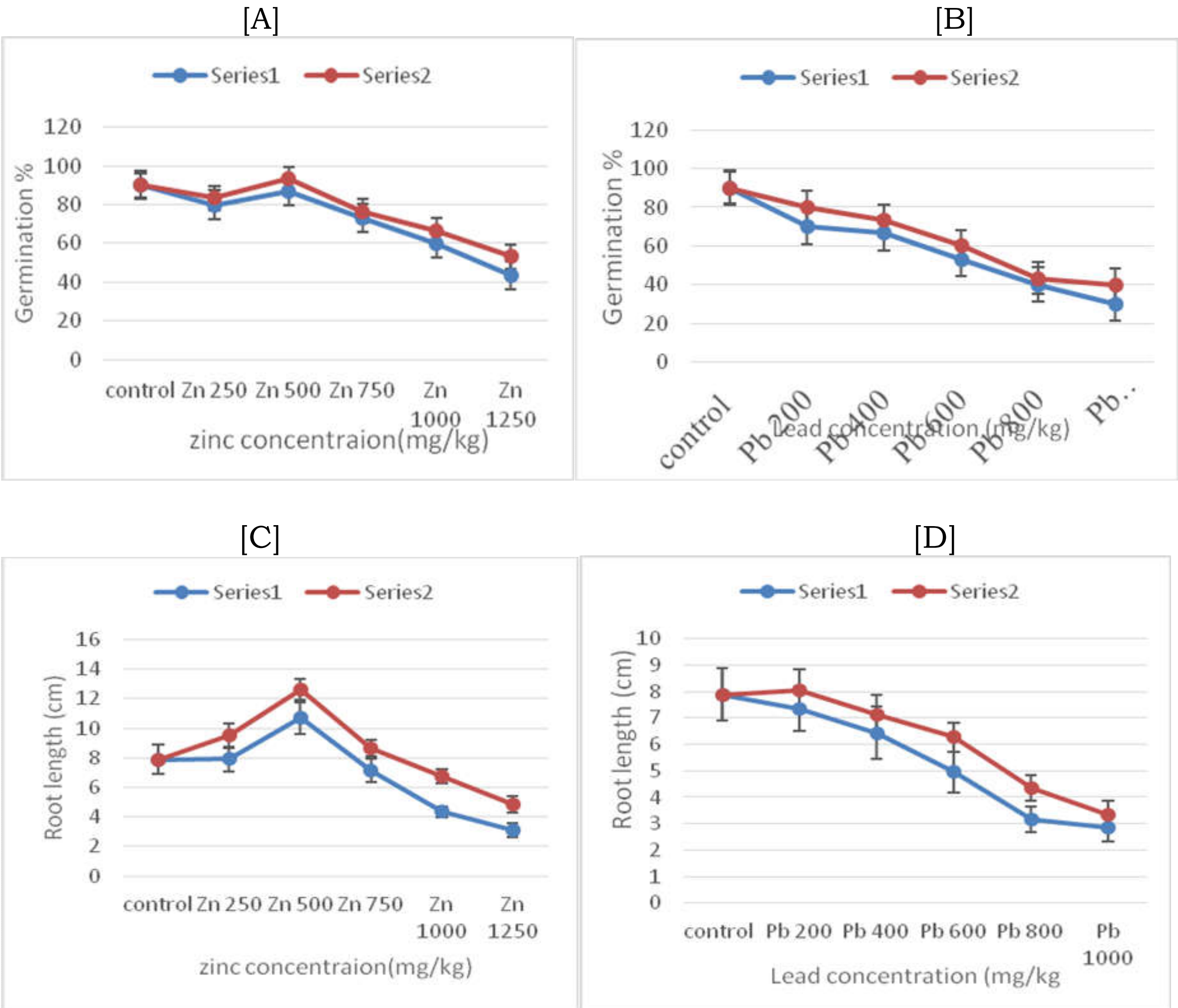
FRESH AND DRY WEIGHT:

Fresh and dry weight of seedling of soybean decreased significantly under the influence of different concentration of both lead and zinc respectively, compared with the control plants [2].Under Lead and zinc treatment with salicylic acid, the fresh weight of root and shoot were significantly increased as compared with lead and zinc treatments alone($P<0.01$). Highest fresh weight (11.85gm/seedling and 8.45gm/seedling) and dry weight (.91gm/seedling and .72gm/seedling) were observed at 500 mg/L zinc and 200mg/l lead concentrations respectively in as compared to control seedlings in the present study. The beneficial effect of SA was seen on all growth parameters in soybean. The same positive effect of SA on growth in the presence of Cd was reported [14] that being exposed to cadmium, reduced root and shoot length and fresh weight in barley seedlings and SA treatment decreased Cd toxicity. These results in response to Cd stress and SA are also in agreement with results in pea plant [15].

Table 1: Stress tolerance index (%) of *Glycine max* (L.) Merr. Growing in Lead and Zinc contaminated soil with 0.5 mM salicylic acid

Groups		RLSTI (%)	SLSTI (%)	SFWSTI (%)	SDWSTI (%)
	Control	100 ± 00	100 ± 00	100 ± 00	100 ± 00
Zinc sulphate (mg/l)) + SA(.5m M)	250	120.53 ± 0.89	114.61 ± 0.98	113.75± 0.82	114.38 ± 0.52
	500	160.07± 1.06	128.67 ± 0.84	142.53± 0.45	162.97 ± 0.74
	750	109.75± 0.78	108.03± 0.65	90.47± 0.77	96.93± 0.66
	1000	85.93± 0.57	89.13± 0.86	70.32± 0.58	86.32 ± 0.45
	1250	61.21± 0.67	57.80± 0.59	56.74± 0.42	59.43 ± 0.8
Lead Acetate (mg/l)) + SA(.5m M)	200	102.02± 1.03	95.89± 0.82	101.25± 0.93	99.52± 0.86
	400	90.62± 0.87	88.13± 0.54	99.73± 0.83	94.52 ± 0.72
	600	79.59± 0.93	72.05± 0.63	84.17 ± 0.46	84.43± 0.56
	800	55± 0.66	62.37± 0.45	70.32± 0.64	64.15± 0.73
	1000	42.33 ± 0.63	47.03± 0.31	48.92 ± 0.49	52.59± 0.43

Number of experiments (N) = 3; Sample size (n) = 30; Values were expressed as mean ± SEM; **RLSTI:** Root length stress tolerance index; **SLSTI:** Soot length stress tolerance index; **SFSTI:** Seedling fresh weight, stress tolerance index; **SDWSTI:** Seedling dry weight stress tolerance index**.**



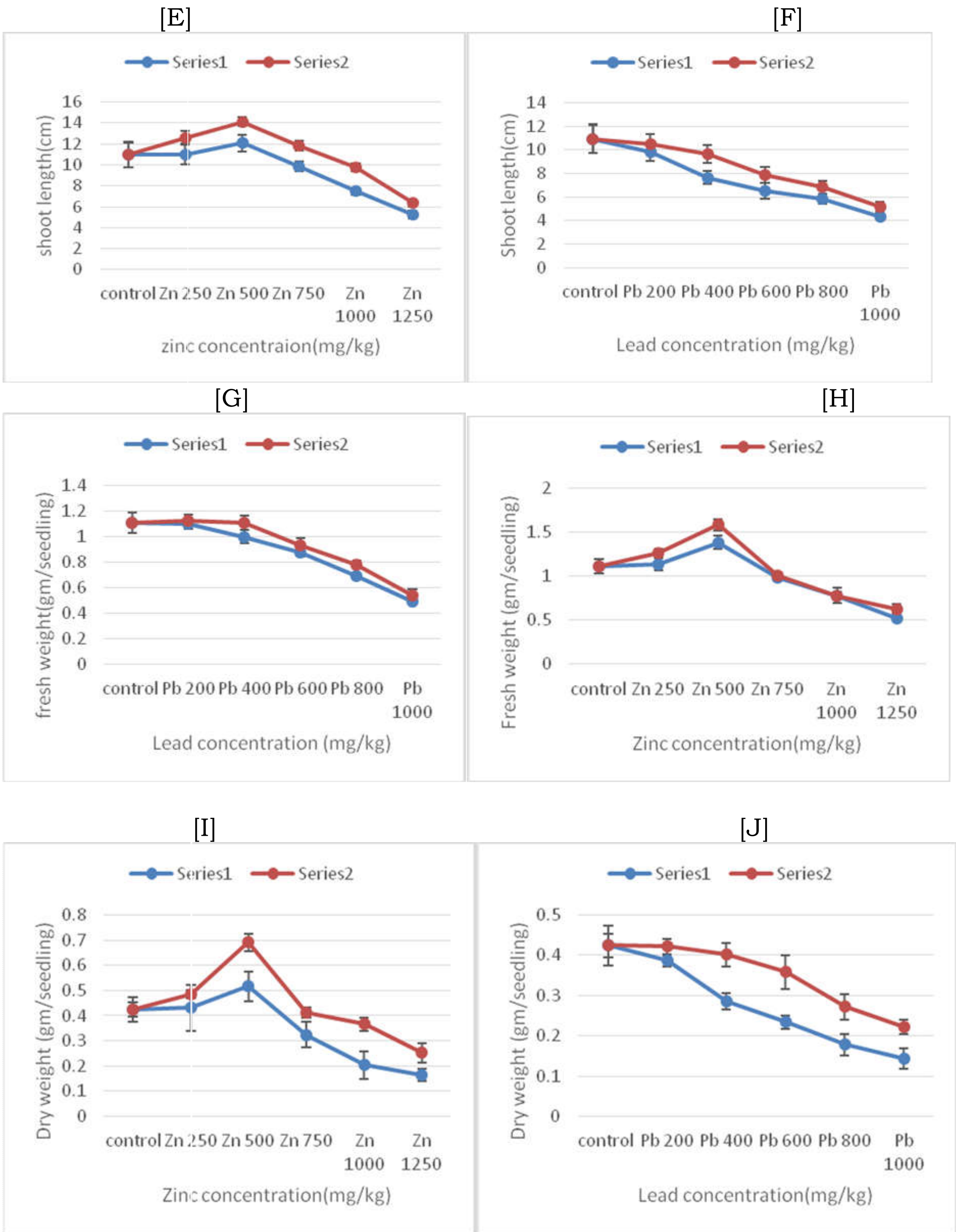


Fig 1: A: seedling germination percentage under influence of Zn, **B:** seedling germination percentage under influence of Pb, **C:** effect of Zn on root length, **D:** effect of Pb on root length **E**: shoot length of seedling under influence of Zn, F: shoot length of seedling under influence of Pb, **G:** effect of Zn on fresh weight and **H:** effect of Pb on fresh weight, I: effect of Zn on Dry weight, J: effect of Pb on Dry weight.
Series1 : effect of heavy metal without salicylic acid (2)
Series 2: effect of heavy metal with salicylic acid

Tolerance index

Siddhi *et. al.,* 2016 showed that the higher lead and zinc treatment lowers the percentage of tolerance in *Glycine max* but application of .5mm salicylic acid increase the tolerance index under stress condition. In zinc stress condition, Tolerance index higher at 250 and 500mg/kg but lower at 750-1250mg/kg with SA acid. The highest value of RLSTI, SLSTI, SFSTI and SDSTI was recorded 160.07%, 128.67%, 142.53% and 162.97% respectively at zinc 500mg/kg with SA acid. Minimum value at zinc 1250 mg/kg, RLSTI, SLSTI, SFSTI and SDSTI was recorded 61.21%, 57.80%, 56.74% and 59.43% respectively with SA acid. In lead stress condition, when lead concentration was increased, the Tolerance Index significantly declined. The lowest value of RLSTI, SLSTI, SFSTI and SDSTI was recorded 42.33%, 47.03%, 48.92% and 52.59% respectively at lead 1000mg/kg with 0.5mM SA (Table1). Similar effects were observed in earlier studies of (*Glycine max* L.) Exposed to chromium stress [7].

CONCLUSION

The data reported in the previous study suggest that increasing concentrations of lead had an increased inhibitory effect on seed germination percentage, root length, shoot length, tolerance index, fresh weight and dry weight of soybean seedlings. It is inferred from the results of present investigation that exogenous application of SA mitigated the adverse effects of lead and zinc stress on growth parameter of soybean plants in early seedlings growth stage. Further protection under heavy metal stress was achieved through enhanced activities of antioxidant enzymes, SOD and POD etc. SA being economic and environmental friendly can be recommended for farmers to use in their fields for alleviating heavy metal stress; In addition SA induces systemic resistance against diseases and this can also benefit the crop productivity of the region. Effect of SA on soybean growth up to maturity can be further studied to understand its role for longer periods in soil plants.

REFERENCES

1. Chaudhary, S.S., Datta, S. (2016). Impact of organic manure amendment on growth of *Withania somnifera* (L.) Dunal var JA-20.Wor. J. Pharm. Res., 5(6):925-935.
2. Gupta, S., Meena, M.K., Datta, S. (2016). Effect of selected heavy metals (lead and Zinc) on seedling growth of soybean *Glycine max* (L.) MERR. Int. J. Pharm. Pharm. Sci., 8(8):302-305.
3. Akinci, I.E., Akinci, S., Yilmaz, K. (2010). Response of tomato *(Solanum lycopersicum L.)* to lead toxicity: Growth, element uptake, chlorophyll and water content. Afr. J. Agric. Res., 5:416-423.
4. Meena, K.C. (2011). An impact assessment of frontline demonstrations (flds) on soybean growers. Raj. J. Extn. Edu. 19:133-138.
5. Song, W., Zheng, A., Shao, H., Chu, L., Brestic, M., Zhang, Z. (2012). The alleviative effect of salicylic acid on the physiological indices of the seedling leaves in six different wheat genotypes under lead stress. Plant omics J. 5(5):486-493.
6. Mishra, A., Choudhuri, M.A. (1999). Effects of salicylic acid on heavy metal induced membrane deterioration mediated by lipoxygenase in rice. Biol. Plant. 42:409–415.
7. Drazic, G., Mihailovic, N. (2005). Modification of cadmium toxicity in soybean seedlings by salicylic acid. Plant Sci. 168:511–517.
8. Gupta, S., Meena, M.K., Datta, S. (2014). Isolation, characterization of plant growth promoting bacteria from the plant *Chlorophytum borivilianum* and *in-vitro* screening for activity of nitrogen fixation, phospthate solubilization and IAA production. Int. J. Curr. Microbiol. App. Sci. 3(7):1082-1090.
9. Meena, M.K., Gupta, S., Datta, S. (2016). Antifungal Potential of PGPR, their Growth Promoting Activity on Seed Germination and Seedling Growth of Winter Wheat and Genetic Variabilities among Bacterial Isolates. Int.J.Curr.Microbiol.App.Sci. 5(1):235-243.
10. Parashar, A., Yusuf, M., Fariduddin, Q., Ahmad, A. (2014). Salicylic acid enhances antioxidant system in *Brassica juncea* grown under different levels of manganese. Int. J. Bio. Macro. 70:551-558.
11. Zhang, Y., Xu, S., Yang, S., Chen, Y. (2015). Salicylic acid alleviates cadmium-induced inhibition of growth and photosynthesis through up regulating antioxidant defense system in two melon cultivars (*Cucumis melo* L.). Protoplasma. 252(3):911–924.
12. Jazi, B.S., Yazdi, L.H., Ranjbar, M. (2011). Effect of salicylic acid on some plant growth parameters under lead stress in *Brassica napus* var. Okapi. Iran. J. Plant Physiol. 1(3):177-185.
13. Fatima, R.N., Javed, F., Wahid, A. (2014). Salicylic Acid Modifies Growth Performance and Nutrient Status of Rice (*Oryza sativa*) under Cadmium Stress. Int. J. Agric. Biol., 16:1083-1090.
14. Metwally, A., Finkemeier, I., Georgi, M., Dietz, K.J. (2003). Plant Physiol., 132:272-281.

15. Popova, L., Maslenkova, L., Yordanova, L., Krantev, A., Szalai, G., Janda, T. (2008). Gen. Appl. Plant Physiol., 34(3-4):133-148.

CITATION OF THIS ARTICLE

S Gupta, M Kr. Meena, S Datta.The Alleviating Effect of Salicylic Acid on Early Seedling Growth Of Soybean Under Zinc and Lead Stress. Bull. Env. Pharmacol. Life Sci., Vol 6[4] March 2017: 48-54