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Growth and Macronutrients Status of Mung Bean [*Vigna radiata* (L.) Wilczek] Grown under Lead (Pb) Stress and Exposed to Foliar Spray of Indole Acetic Acid (IAA)

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ABSTRACT

Background: Growth and nutritional status of plant are affected adversely by heavy metals while improved by plant growth regulators. The experiment was aimed to explore the ameliorative potential of indole acetic acid (IAA) for toxicity of Pb.

Methods: Two varieties *i.e.*, M-8 and MN-92 of mung bean were grown in pots arranged under complete randomization. Fifteen days after germination, Pb was added @ 10 and 20 mg/kg soil as solutions of $PbNO_3$. Indole acetic acid @100.0 mM was sprayed twice at 15 and 30 days of age. Stem length, root length, leaf area and nutrient ions were recorded at physiological maturity. Three replicates from each treatment were evaluated.

Result: The alleviation role of IAA for stem length was recorded as 21.46 and 26.14% for low and high lead stress. The root length was compensated to 34.78 and 31.40% for the respective low and high lead toxicity. Leaf area was affected as 18.11 and 17.32% by IAA. Similarly, Nitrogen contents were nullified to the extents of 15.34 and 8.13%. Phosphorus contents were changed as 11.62 and 4.65% while potassium contents were as 10.14 and 19.36%. The rectifying potential of IAA for leaf biomasses were as 7.64 and 7.43%.

Key words: Indole acetic acid, Lead, Leaf area, Mung, Nitrogen, Stem length.

INTRODUCTION

Rapidly growing trend of urbanization accompanied with more industrialization, has increased the heavy metals in the environment (Ashraf *et al.* 2019). The effects of heavy metals toxicity are increasing worldwide particularly due to anthropogenic causes. Among the heavy metals pollution soil contamination is one of the most important as it enters into the food chain through plants. During the course of evolution, some plants have developed mechanisms to overcome such stresses. However, metals like Zn, Al, Cu, Pb, As, Cd exert severe effects on plants (Angulo-Bejarano *et al.* 2021).

Heavy metals pollution in the environment is a threatening problem for life of all livings (Heckathorn *et al.* 2004). Heavy metals of environment finally the food chains and cause stunted growth of plants (Moustaka *et al.* 2016). Some remedial actions are mandatory to check the emission of heavy metals and their incorporation into various ecosystems (Hasan *et al.* 2019). These measures can be of physical, chemical or of mechanical types (Wuana and Okieimen, 2011).

Mining activities, municipal sewage sludge are the main sources of Pb (Kumar *et al.* 1995). Lead being the nonessential element for plants accumulates in cell causing interruptions in physiological phenomenon. In plants, Pb toxicity disrupts many metabolic activities like organelles stability, electron transport chain, membrane integrity, photosystems complex, mineral metabolism, enzymatic activity and oxygen-evolving complex (Aslam *et al.* 2021). Exogenous Pb causes increases leaf H_2O_2, lipid peroxidation, growth reduction, pigments reduction and photosynthesis decline due to oxidative stress (Giannakoula *et al.* 2021).

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Phytohormones, also called as Plant Growth Regulators, are biological molecules which are synthesized within plants and can act at the site of production or are transported to distant cells to promote growth and developmental process in normal and stressed plants (Iqbal *et al.* 2014). In many countries like EU, USA and Japan PGRs are commercially marketed (Jahan *et al.* 2019). Exogenous application of several PGRs including auxin promotes growth also (Khan *et al.* 2004). Exogenous PGRs can mitigate the adverse effects of stress (Bielach *et al.* 2017). Of these PGRs, auxin is one of the most common. Indole-3-acetic acid (IAA), is the first identified auxin

(Fahad *et al.* 2015). IAA is produced by many plants, yeast (Bunsangiam *et al.* 2021) and *Escherichia coli* (Wu *et al.* 2021). Auxins act as an active signaling molecule (Weijers and Wagner, 2016). Indole-3-acetic acid (IAA) mitigates the adverse effects of stress (Abdel Latef *et al.* 2021).

Mung bean [*Vigna radiata* (L.) R.Wilczek[originated in 1,500 BC. Its origin was in subcontinent and thereafter spread to Asian, African, Australian and American countries. Its cultivation extended later on to Himalayas at elevations of 1,850 m (Lambrides and Godwin, 2006). In Pakistan, Mung bean is is cultivated for its use as fodder and green manure (Rehman *et al.* 2017), as vegetables (AVRDC, 2012) and for improvement of soil fertility due to nitrogen fixing capacity (Ilyas *et al.* 2018).

MATERIALS AND METHODS

The experiment was for the purpose of finding mitigating capacity of indole acetic acid (IAA) for two mung bean [*Vigna radiata* (L.) Wickzek] varieties grown in Pb contaminated soil. The experiment was conducted in Bahauddin Zakaryia University, Multan Pakistan during 2016-17.

Sandy loam soil never irrigated with effluents was chosen after an initial survey. Pots of plastic material measuring 30 cm diameter were filled with this soil. Two mung bean varieties *i.e.* M-8 and MN-92 were selected and obtained from pulses department of a research Institute. IAA and Lead nitrate salt of Sigma Aldrich, Japan were acquired from dealer.

Soil, after filling of pots, was moisturized with water and left till getting of field capacity moisture contents. Seepage from pots was prevented by lining of pots with polyethylene sheath. Healthy and of uniform size seeds after sorting were sterilized with 1% H_2O_2. Five seeds in each pots were sown. After germination thinning was carried out to maintain three seedlings per pot to ensure uniform nutrients supply. Normal irrigation and pesticides application were practiced for plant protection. Pots arrangement was by complete randomization of all treatments. According to reviewed literature, Lead stress @10 and 20 mg Pb/kg soil was induced by adding lead nitrate. Pots left without addition of metal salt were as control. IAA (100.0 mM) solution was prepared in water pre-estimated by foliar spray as trial. Surfactant of Tween-20 (0.1%) was mixed with solution. Plants were sprayed after fifteen and thirty days of germination. One group of plants without addition of metal was sprayed with just distilled water.

The treatments applied were as: Normal soil (Without addition of metal salts) +Distilled water spray; Normal soil (Without addition of metal salts) + 100.0 mM IAA spray; Lead (10.0 mg/kg soil) + Distilled water spray; Lead (20.0mg/kg soil) + Distilled water spray; Lead (10.0 mg/kg soil) + 100.0 mM IAA spray; Lead (20.0mg/kg soil) + 100.0 mM IAA spray Data for three plants as replicates were recorded from every group of treatment. Stem length, root length, leaf number and leaf area were recorded for each. After these recordings respective plants were harvested carefully for NPK determination. NPK were quantified from digested material of plants with the help of spectrophotometer.

Using software of COSTAT, results were analyzed at significance level of 5%, Duncan multiple range test was applied for comparison of means (Duncan, 1955). MSTAT-C Computer Statistical Programm was used for significant results to test means by LSD tests.

RESULTS AND DISCUSSION

Stem length (cm)

Stem length significantly differed among treatments. Varieties and treatments both revealed highly significant differences. While, interactive response among treatment and varieties were non significantly different (Table 1). IAA increased stem length as 18.61%. Under stress of low and high lead stem length was declined as 38.61 and 46.49% respectively. IAA alleviated the toxic effect of lead, therefore, stem length reduction was limited to 17.15 and 20.34% respectively (Table 2).

Root length (cm)

Highly significant differences were observed in root length by treatments, varieties and also by their interactions (Table 1). IAA increased root length upto 14.46%. Low and high lead decreased root length by 45.96 and 59.45% respectively, IAA mitigated toxic effect of lead. Hence, reduction by metal was 11.18 and 28.05% respectively (Table 3).

Table 1: Mean sum of squares for stem length, root length, leaf area, nitrogen contents, phosphorus contents and potassium contents of 40 days age mung bean [*Vigna radiata* (L.) Wickzek] plants grown in lead (10, 20 mg/kg soil) and exposed to foliar spray of IAA (100 mM)] at 15 and 30 days of age.

Source	Df	MSS					
		Stem length	Root length	Leaf area	Nitrogen contents	Phosphorus contents	Potassium contents
Treatment (T)	5	130.850***	344.175***	65447.628***	0.005^{ns}	0.254^{ns}	0.405^{ns}
Variety (V)	1	52.804***	518.852***	16995.902***	0.012^{ns}	0.138^{ns}	6.012**
T × V	5	0.635^{ns}	9.378**	2388.625***	2.944^{ns}	0.001^{ns}	0.025^{ns}
Error	24	1.928	2.067	18.719	0.004	0.777	0.439
Total	35						

***= Highly significant; *= Significant; ns= Not significant.

Leaf area (cm^2)

Results revealed that variety, treatments and their interactions highly significantly different (Table 1). Leaf area was increased by IAA treatment (53.54%). Low and high lead treatments reduced leaf area as 38.61 and 62.20% respectively. IAA alleviated lead stress effects and limited reductions to18.50 and 44.88% respectively (Table 4).

Nitrogen (N) contents (mg/g)

The data related nitrogen contents showed that highly significant differences were found among different treatments. Varieties and collective response in varieties and treatments showed non-significant differences (Table 1). Increased nitrogen contents (0.09%) were observed by IAA treatment. Low and high lead treatments decreased the

Table 2: Mean values (± SE) for lead toxicity effect on stem length (cm) of 40 days age mung bean [*Vigna radiata* (L.) Wickzek] plants grown in lead (10, 20 mg/kg soil) and exposed to foliar spray of IAA (100 mM)] at 15 and 30 days of age.

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.95)
MN-92	18.26±2.17[cd]	21.60±1.90[ab]	11.03±0.55[fg]	9.20±0.80[g]	15.66±0.37[de]	13.71±1.93[ef]	14.91±4.98[b]
%age		+18.29	-39.57	-49.61	-14.23	-24.90	
M-8	20.23±1.55[bc]	24.06±1.50[a]	12.60±0.600[fg]	11.40±1.05[g]	18.77±1.46[d]	16.96±1.30[de]	16.12±4.64[a]
%age		+18.95	-37.71	-43.650	-7.20	-16.13	+8.12
Treatment mean LSD = (1.65)	19.25±2.00[b]	22.83±2.04[a]	11.81±1.000[e]	10.30±1.46[e]	17.20±1.93[c]	15.33±2.31[d]	17.33±4.68
%age		+18.61	-38.61	-46.49	-17.15 {21.46}	-20.34 {26.14}	

[Values represent means ± SE; Values of %age differences are increase (+)/decrease (-) over column 1(Untreated or V1) (LSD=2.86). Value sharing the same letter within rows and columns differ non significantly (P≤0.05). Values in {} represents amelioration %age by IAA.

Table 3: Mean values (±SE) for lead toxicity effect on root length of 40 days age mung bean [*Vigna radiata* (L.) Wickzek] plants grown in lead (10, 20 mg/kg soil) and exposed to foliar spray of IAA (100 mM) at 15 and 30 days of age.

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.98)
MN-92	22.66±1.44[e]	25.33±1.52[d]	11.30±1.36[hi]	9.06±1.24[i]	19.66±1.44[f]	16.00±1.59[g]	17.31±6.10[b]
%age		+11.78	-50.13	-59.99	-13.21	-29.39	
M-8	31.33±1.52[b]	36.40±1.24[a]	17.80±1.65[fg]	12.81±1.70[h]	28.33±1.37[c]	22.80±1.10[e]	24.91±8.34[a]
%age		+16.00	-42.73	-59.11	-9.58	-27.233	+43.88
Treatment mean (LSD=1.71)	26.96±4.95[b]	30.86±6.18[a]	14.57±3.82[e]	10.93±2.44[f]	23.95±4.92[c]	19.40±3.91[d]	21.11±8.17
%age		+14.46	-45.96	-59.45	-11.18 {34.78}	-28.05 {31.40}	

Values represent means ± SE; Values of %age differences are increase(+)/decrease(-) over column 1(Untreated or V1)(LSD = 2.42). Values sharing the same letter within rows and columns differ non significantly (P≤0.05). Values in {} represents amelioration %age by IAA

Table 4: Means values (±SE) for lead toxicity effect on leaf area of 40 days age mung bean [*Vigna radiata* (L.) Wickzek] plants grown in lead (10, 20 mg/kg soil) and exposed to foliar spray of IAA (100 mM) at 15 and 30 days of age.

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.98)
MN-92	213±2.64	346± 8.71	147±2.64	98± 1.15	175±1.52	139±2.61	186±82.11[b]
%age		+62.44	-30.985	-53.99	-17.84	-34.74	
M-8	295±1.47[c]	433±1.29[a]	175±1.52[f]	94± 3.37[i]	240±10.14[d]	142±1.90[gh]	230±115.04[a]
%age		+46.779	-40.67	-68.13	-18.64	-51.864	+22.03
Treatment mean (LSD=1.71)	254± 45.28[ab]	390± 47.62[a]	161±15.63[abc]	96± 3.242[c]	207± 36.00[ab]	140± 2.63[bc]	208± 100.94
%age		+53.54	-36.61	-62.20	-18.50{18.111}	-44.88{17.32}	

Values represent means ± SE; Values of %age differences are increase (+)/decrease (-) over column 1(Untreated or V1) (LSD = 15.57). Values sharing the same letter within rows and columns differ non significantly (P≤0.05). Values in {} represents amelioration %age by IAA.

nitrogen values 23.25 and 29.96% respectively. IAA ameliorated the lead stress. Therefore, reductions by metal treatments were lowered to 7.90 and 21.83% respectively (Table 5).

Phosphorus (P) contents (mg/g)

The data revealed that phosphorus contents showed that treatments, varieties and their interactions showed non-significant differences (Table 1). Increased phosphorus contents (6.04%) were observed by IAA treatment. Low and high lead treatments decreased the nitrogen values as 25.11 and 27.90% respectively. IAA ameliorated the lead stress therefore phosphorus was reduced up to 13.48 and 23.23% respectively (Table 6).

Potassium (K) contents (mg/g)

The data related to potassium contents showed that varieties revealed significant differences while treatments and their interactions with varieties treatments showed non-significant differences (Table 1). Increased potassium contents (4.14%) were observed by IAA treatment. Low and high lead treatments decreased the potassium values 12.99 and 26.66% respectively. IAA ameliorated the lead stress therefore metal induced reductions were limited to 2.84 and 6.70% respectively (Table 7).

Well grown plants of mung bean with adequate amount of nutrients provide more energy in term of food and fodder. Growth was reduced in stem, root and leaves by Pb and enhanced by IAA (Table 2-4). Growth reduction might be owed to a decline of water potential (Atteya, 2002). Water contents of metal stressed plants are lowered by enhanced resistance in its flow (Poschenrieder *et al.1989*) or it might be due to metal induced change in cell wall structure (Poschenrieder *et al. 1989*). Growth and morphology of mung bean is influenced by plant growth promoting rhizobia (PGPR) and other external factors (Neha *et al.* 2021). Plant growth regulators can influence the productivity of mung bean (Bhadane *et al.* 2021).

Growth reduction might be due to metal induced chlorophyll decrease by denaturation of its biosynthetic enzymes *i.e.*, 6-amino laevulinic acid dehydratase and porphobilinogenase by metal (Hampp *et al.* 1974). Lead toxicity affects the rate of photosynthesis (Carlson *et al.* 1975); changes in the thylakoid structure (Fodor *et al.* 1996); reduction of mitochondrial cristae (Samardakiewicz, 2000) and phosphorylation process (Wozny, 1995).

Reactive oxygen species (ROS) production by metal lowers concentration of osmotica such as carbohydrates and amino acids (Zhang *et al.* 1999); causes protein and lipid oxidation/ degradation of mitochondrial membrane and

Table 5: Means values (±SE) for lead toxicity effect on Nitrogen content of 40 days age mung bean [*Vigna radiata* (L.) Wickzek] plants grown in lead (10, 20 mg/kg soil) and exposed to foliar spray of IAA (100 mM) at 15 and 30 days of age.

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=11.11)
MN-92	2.06±0.06[ab]	2.16±0.11[a]	1.63±0.12[cd]	1.45±0.25[d]	2.01±0.03[abc]	1.61±0.30[cd]	1.82±0.31[a]
%age		+4.85	-20.72	-29.61	-2.42	-21.79	
M-8	2.11±0.24[ab]	2.21±0.41[a]	1.720±0.25[bcd]	1.61±0.44[cd]	2.01±0.07[abc]	1.85±0.05[abcd]	1.92±0.32[a]
%age		+4.73	-18.48	-23.69	-4.73	-12.32	+8.86
Treatment mean (LSD=1.71)	2.18±0.16[a]	2.19± 0.27[a]	1.68±0.19[c]	1.53±0.33[c]	2.01±0.05[ab]	1.73±0.23[bc]	1.87±0.32[bc]
%age		+0.09	-23.25	-29.96	-7.90 {15.34}	-21.83 {8.13}	

[Values represent means ± SE; Values of %age differences are increase (+)/decrease (-) over column 1(Untreated or V1) (LSD = 27.22). Values sharing the same letter within rows and columns differ non significantly (P≤0.05). Values in {} represents amelioration %age by IAA.

Table 6: Means values (±SE) for lead toxicity effect on phosphorus of 40 days age mung bean [*Vigna radiata* (L.) Wickzek] plants grown in lead (10, 20 mg/kg soil) and exposed to foliar spray of IAA (100 mM)] at 15 and 30 days of age.

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.43)
MN-92	0.19±0.09	0.20±0.08	0.14±0.05	0.13±0.06	0.16±0.03	0.15±0.04	0.16±0.06
%age		+5.26	-26.31	-31.57	-15.78	-21.05	
M-8	0.13±0.09	0.25±0.10	0.18±0.02	0.17±0.03	0.20±0.08	0.18±0.02	0.20±0.06
%age		+92.30	+38.46	+30.76	+53.84	+38.46	+22.28
Treatment mean (LSD=0.75)	0.21±0.08	0.22±0.08	0.16±0.04	0.15±0.04	0.18±0.05	0.16 0.03	0.18±0.06
%age		+6.04	25.11	27.90	13.48 {11.62}	23.25 {4.65}	

Values represent means ± SE; Values of %age differences are increase (+)/decrease (-) over column 1(Untreated or V1) (LSD=1.07). Values sharing the same letter within rows and columns differ non significantly (P≤0.05). Values in {} represents amelioration %age by IAA.

Table 7: Means values (±SE) for lead toxicity effect on potassium of 40 days age mung bean [*Vigna radiata* (L.) Wickzek] plants grown in lead (10, 20 mg/kg soil) and exposed to foliar spray of IAA (100 mM) at 15 and 30 days of age.

	No metal +Distilled water spray	No metal +IAA spray	10 mg/kg Pb + Distilled water spray	20 mg/kg Pb + Distilled water spray	10 mg/kg Pb + IAA spray	20 mg/kg Pb + IAA spray	Variety mean (LSD=0.98)
MN-92	2.00± 0.08	2.07± 0.07	1.70± 0.54	1.20± 0.16	1.93±0.05	1.85± 0.15	1.80± 0.35
%age		+3.50	-15.00	-40.00	-3.50	-7.50	
M-8	2.76± 0.02	2.90± 1.40	2.45± 1.15	2.30± 0.70	2.69 ±0.30	2.60±1.0	2.61 ±0.78
%age		+5.07	-11.23	-16.66	-2.53	-5.79	+45.38
Treatment mean (LSD=1.71)	2.38± 0.43	2.48± 0.99	2.07± 0.93	1.76±0.77	2.31± 0.45	2.22±0.75	2.20±0.73
%age		+4.14	-12.99	-26.06	-2.849 {10.14}	-6.70 {19.36}	

Values represent means ± SE; Values of %age differences are increase (+)/decrease (-) over column 1(Untreated or V1) (LSD = 1.81) Values sharing the same letter within rows and columns differ non significantly (P≤0.05). Values in {} represents amelioration %age by IAA.

inhibition of photosynthetic ETC (Kappus, 1985). Peroxidase reduction by ROS results in shoot growth retardation (Stoeva and Bineva, 2003). However, IAA treated plants showed better expression in all above parameters.

Lead exerted a negative impact on N, P, K content in lead treated mung bean plants (Table 5-7). Lead reduces the uptake and transportation of mineral nutrients in plants (Goldbold and Kettner, 1991). Saygideger *et al.* (2004) observed that high level of Pb could decrease the nitrogen contents in *Typha ceratophyllum*. The decline in nitrogen concentration due to Pb may be as a result of moisture stress which is created by Pb (Burzynisky and Grabowski, 1984). Application of Pb decreases the nitrogen and phosphorus contents (Kibria *et al.* 2009). According to Orhue and Innch (2010), concentrations of phosphorous and potassium were significantly decrease in *Celosia argentia* by treatment of Pb. According to Blatt (1993), transport of ion is regulated by auxin. Auxins cause an increased in concentration of K ion in wheat grain and leaves (Wierzbowska and Bowszys, 2008). Response of the varieties differed to IAA and metal. Yield varies with genetic makeup of mung bean (Priya and Ratna Babu, 2021; Salman *et al.* 2021)

CONCLUSION

IAA treatment ameliorated the PB toxicity effect to significant extent for growth and to a level of non-significant for ionic concentration.

Conflict of interest: None.

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