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Early Seedling Growth and Accumulation of Proline and Phenol in *Trigonella foenum-graecum* Under Heavy Metal Stress

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Abstract: ***In the present investigation a study was conducted on germination, early seedling growth and accumulation of biomarkers like proline and total phenols in Trigonella foenum-graecum L. in response to stress induced by treating the seeds with different concentrations (5, 10, 15 mg/l) of two heavy metals, viz., lead and cadmium along with the control (0) under laboratory condition. Both the metals showed toxic effects on germination and seedling dry biomass with their increasing concentrations as compared to control. Significant and gradual inhibitory effects of lead and cadmium on lengths of root and shoot, number of leaves per plant and leaf area were also observed in a dose dependent manner. Cadmium treatments showed lowest index of tolerance and maximum inhibition of growth parameters in seedlings of T. foenum-graecum at 15mg/l concentration as compared to lead. Although gradual accumulation of proline and total phenols in seedling leaves increased significantly with increasing concentrations of treated heavy metals as compared to control, cadmium exhibited more positive effect than lead.***

Keywords: *Trigonella foenum-graecum* L., germination, heavy metals, tolerance indices, proline

1. Introduction

The environmental pollution with heavy metals is increasing day by day due to modern human activities, like mining, industrial production, transportation, agriculture etc. Lead and cadmium are the most hazardous, important and non-nutrient pollutants among different heavy metals. These two metal ions get absorbed by the root system of the plant and affect adversely the growth and metabolism. Interaction of lead and cadmium ions with the functional groups of proteins, nucleic acids and polysaccharides and substitution of other metal ions already bound to these functional groups by these two ions can lead to metabolic disorders and reduction in growth (Costa and Spitz, 1997; Seregin and Ivanov, 2001).

Trigonella foenum-graecum L., also known as fenugreek or methi, an important medicinal plant belonging to family Fabaceae, is cultivated in India, Pakistan, Egypt, France, Spain, Turkey, China and some other countries. India is the largest producer of fenugreek in the world (Zohary and Hopf, 2000). It is used as spice, leafy vegetables and in medicine, as anti-inflammatory, analgesic, carminative and antidiabetic (Bhat et al., 2012). The seeds contain major alkaloid trigonelline which produced hypoglycemic activity (Shani et al., 1974) and diosgenin. Fenugreek seeds can be considered as good supplement to cereals because of its high protein (25%), lysine (5.7g / 16g N), soluble (20%) and insoluble (28%) dietary fiber and also rich in Ca, Fe and beta-carotene (NIN Report, 1987).

Understanding the importance of fenugreek it is worthwhile to study the effects of lead and cadmium, two important heavy metals, on physio-biochemical attributes of this crop. In the present investigation seed germination, growth and accumulation of free proline and total phenols in seedling stage of *Trigonella foenum-graecum* were assessed under stress condition induced by different concentrations of lead and cadmium.

2. Materials and Methods

The seeds of *Trigonella foenum-graecum* L. were obtained from the farmer's field of Burdwan, West Bengal. Different concentrations (5, 10, 15 mg/l) of lead (Pb) and cadmium (Cd) were prepared with sterile double distilled water by using lead nitrate and cadmium chloride, respectively. Dry and healthy seeds of uniform size were taken and surface sterilized with 0.1% mercuric chloride for 90 seconds followed by repeated washings in sterile distilled water and then soaked in test solutions separately for 11hours at $23^0 \pm 1^0$C. Only double distilled water, instead of heavy metal solutions, was used for control set. Pretreated seeds were placed in the Petri dishes lined with two layers of filter paper (Whatman no.1) and kept at $23^0 \pm 1^0$C. Three replicates were made for each treatment. Germination percentage was calculated after 72 hours of incubation and the germinated seeds were transferred to the plastic pots containing sterilized sand - saw dust (6:4) mixture. Data on growth parameters (length of root and shoot, dry weight accumulation, number of leaves/plant and leaf area) were recorded in 15 days old seedlings. The contents of free proline and total phenols in the leaves of seedlings on 15th day were determined by the methods of Bates et al., (1973) and Bray and Thorpe (1954), respectively. Tolerance indices at different treatments of heavy metals were determined by the following formula as mentioned by Kabir et al., (2010). T.I. = [Mean root length in metal solution/Mean root length in distilled water (control)] ×100. Data were analyzed statistically for determining mean and standard errors and statistical significance using analyses of variance (ANOVA) following Panse and Sukhatme (1978).

3. Results and Discussion

Toxic effects of Pb and Cd were observed on seed germination percentage and growth parameters in *Trigonella foenum-graecum* as compared to control (Table 1). The

effect of different doses of Pb was less in all the cases than those of Cd. Marked suppression in germination percentage and dry weight accumulation was found at 15mg/l Cd as compared to control, although both the metals showed negative effect. The results are in conformity with the findings of Hasnain et al., (1995), Ayaz and Kadioglu (1997) and Muhammad et al., (2008).

Reduction in seed germination is caused due to enhanced breakdown of stored food materials in seed by treatments of heavy metals (Kalimuthu and Shiva, 1990). Gradual decline in dry biomass with increasing doses of Pb and Cd was resulted due to poor growth of root and shoot of the seedlings. Both Pb and Cd showed significant negative effects on lengths of root and shoot, number of leaves/plant and leaf area. The most inhibitory effect was seen with the treatment of Cd in comparison to Pb (Table 1).

The results of tolerance test of seedlings of *T. foenum-graecum* raised from seeds pretreated with different doses of metal solution showed high tolerance index at 5mg/l Pb as compared to control. Although a gradual decline in the percentage of tolerance was observed with the treatment of both the metals in a dose dependent manner, *T. foenum-graecum* seedlings showed the lowest percentage of tolerance at 15mg/l Cd indicating its more tolerance to lead than Cd at the same concentration. The results are in agreement with the reports of Muhammad et al., (2008) and Suganthi et al., (2013). The metal tolerance capacity of plants is contributed by some metal induced cellular changes due to accumulation of heavy metals in different parts of higher plants (Devi and Prasad, 1998). The changes in the physiological mechanism in seedling growth might be the cause for low tolerance (Kabir et al., 2010).

Significant increase in contents of proline and total phenols was noted in seedlings of *T. foenum-graecum* treated with different concentrations of Pb and Cd as compared to control with maximum accumulation in 15mg/l Cd treated seedlings (Table 2). Both proline and phenols are considered as biomarkers of heavy metal stress. Similar results were also reported by El-Beltagi and Mohamed (2013). The role of proline accumulated in higher amount in plants under heavy metal stress, as pointed out by them, includes protection from desiccation, contribution to osmotic adjustment at the cellular level, protection of enzymes stabilizing the structure of macromolecules and organelles, helps the plant to survive short periods and recover from stress and acting as scavenger of ROS. Pervin et al., (2011) also obtained higher accumulation of proline in cadmium treated *T. foenum-graecum.*

Lavid et al., (2001) suggested *de novo* synthesis of phenols in response to heavy metal stress. The phenols are involved as intermediates in lignin biosynthesis causing anatomical change resulting cell wall endurance and protecting cells from harmful action of heavy metals. Moreover, many phenolic compounds in plants are potential antioxidants.

4. Conclusion

The study shows that the Cd causes more toxic effect than Pb for growth of *Trigonella foenum-graecum* which has better tolerance to Pb. Detailed investigation is required at the cellular and molecular level for better understanding of the adaptive mechanism of this important plant under heavy metal polluted environment.

Table 1: Effect of various concentrations of lead and cadmium on germination percentage (after 72 hrs.) and dry weight accumulation (g), length (cm) of root and shoot, number of leaves/plant and leaf area (cm^2) of 15 days old seedlings of *Trigonella foenum-graecum* L. Each value is mean (n=3) ±S.E.
*: Significant at P 0.05, **: significant at P 0.01.

Treatment	Concentration (mg/L)	Germination (%)	Dry weight accumulation (Dry weight/g of fresh wt.)[g.]	Root length(cm)	Shoot length(cm)	Number of leaves/plant	Leaf area (cm^2)
Control	0	95.55±0.47	0.34 ±0.0032	8.46**±0.06	10.34**±0.01	2.81*±0.03	2.18**±0.04
Lead	5	91.11 ±0.47	0.32 ±0.0021	7.85**±0.04	9. 27**±0.02	2.50*±0.04	1.97**±0.04
	10	86.67 ±0.81	0.31 ±0.0042	6.55**±0.03	9.03**±0.03	2.31*±0.07	1.72**±004
	15	80.0 ±0.81	0.29 ±0.0032	5.82**±0.03	8.53**±0.09	2.47*±0.04	1.70**±0.03
Cadmium	5	80.0 ±0.81	0.32 ±0.0021	5.83**±0.01	8.63**±0.01	2.44*±0.03	1.47**±0.04
	10	73.33 ±0.81	0.30 ±0.0032	5.63**±0.02	8.05**±0.04	2.19*±0.03	1.20**±0.02
	15	68.89 ±0.47	0.27 ±0.0092	5.43**±0.01	7.92**0.03	2.11*±0.02	1.03**±0.02

Table 2: Effects of various concentrations of lead and cadmium on contents of free proline and total phenol [mg/g of fresh tissue] of leaves of 15 days old seedlings of *Trigonella foenum-graecum* L. Each value is mean (n=3) ±S.E.

Treatment	*Concentration*	*Free proline content (mg/g of fresh tissue)*	*Total phenol content (mg/g of fresh tissue)*
Control	0	0.08**±0.0053	3.17**±0.06
Lead	5	0.10**±0.0032	4.33**±0.06
	10	0.14**±0.0074	6.50**±0.11
	15	0.28**±0.0074	8.33**±0.06
Cadmium	5	0.14**±0.0074	3.67**±0.06
	10	0.22**±0.0074	4.33**±0.06
	15	0.32**±0.0080	9.83**±0.06

**: significant at P 0.01.

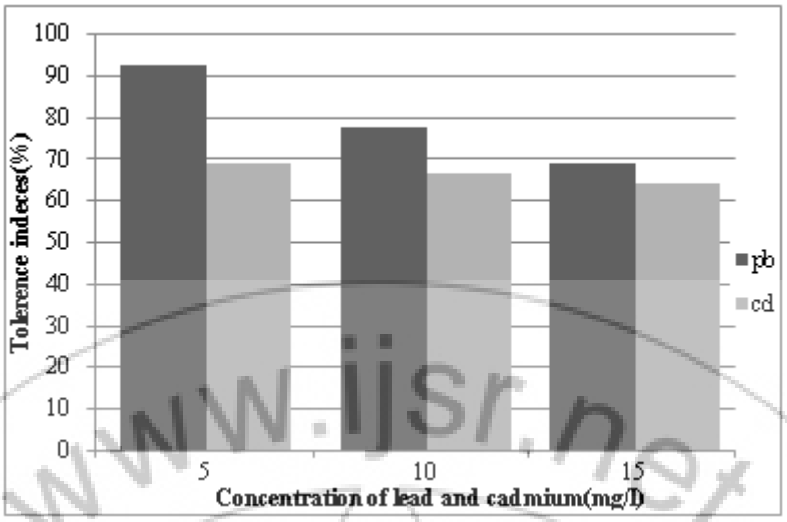

Figure 1: Tolerance indices of *Trigonella foenum-graecum* L. at different concentrations of lead and cadmium

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