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EFFECTS OF LEAD ACETATE ON BENGAL GRAM SEEDS

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Abstract

Keeping in view the effects of industrial effluents mainly the bio-accumulative heavy metals on plants, we propose the present study. Experiments showed increasing concentrations of lead acetate treatments suppress germination of Bengal gram seeds. Root development in control seeds were appropriate compared to the seeds in 1mM concentration whereas seeds in 2mM and 4mM were more affected by lead acetate. Total carbohydrate and starch content in treated samples increased but reducing sugar content decreased with the progression of higher toxic concentration. Antioxidant enzymes like peroxidase and catalase activities were observed higher in treated samples than control. Seeds in 1mM concentration were considered to be the highest tolerant (45.8%) against the toxicity of lead acetate. Awareness needs to be generated for maintaining toxic free atmosphere and to avoid heavy metal contamination in food for safe and healthy future.

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1. INTRODUCTION

Rapid industrialization leads to the production of heavy loads of wastes. These wastes (effluents) are either released into some water body or directly on to the lands, which are mostly agricultural without any treatments. Sometimes these effluents are used for irrigation due to scarcity of water, especially for raising vegetables and fodder [1]. These effluents containing heavy metals affect plant and soil in variety of ways [2]. In case of plants, heavy metals accumulate in the cells, causing a reduction of cell activities, inhibition of growth and various diseases [3]. The most common problem causing cationic metals (metallic elements whose forms in soil are positively charged cations e.g., Pb^{2+}) are mercury, cadmium, lead, nickel, copper, zinc, chromium, and manganese. The most common anionic compounds (elements whose forms in soil are combined with oxygen and are negatively charged e.g., MoO_4^{2-}) are arsenic, molybdenum, selenium, and boron. The heavy metals like cadmium, lead, zinc are known to produce ROS (Reactive Oxygen Species) and induce oxidative stress in certain plant species [4]. These ROS include superoxide radical, hydroxyl radical and hydrogen peroxide which intern damage biomolecules like proteins, enzymes, sugars, nucleic acids etc [5].

Animals and we, the human beings, are dependent upon these agricultural crops as food source. Bengal gram is one of the major pulse crops in India and widely appreciated as health food. India is the major growing country of the world, accounting for 61.65 % of the total world area under Bengal gram and 68.13 % of the total world production. Bengal gram also called as chick pea (Cicer arietinum) (also garbanzo bean, kalachana (north India), Indian pea, ceci bean) is an edible legume of the family Leguminaceae and subfamily Fabaceae. These are green when raw and turn brown when dried. Chick peas are nutritious and rich in protein that makes the diet healthy to the poor in developing countries, where people are vegetarians or cannot afford animal protein. It offers the most practical means of eradicating protein malnutrition among vegetarian children and nursing mothers.

Figure 1: Bengal gram or Desi (meaning country or local in Hindi), which has small, darker seeds and a rough coat, cultivated mostly in the Indian subcontinent, Ethiopia, Mexico, and Iran.

Desi Bengal gram (Figure 1) has markedly higher fiber content than Kabulis and hence a very low glycemic index which is known to make them suitable for people with blood sugar problems. Bengal gram also exhibited significant antioxidant capability [6].

As agricultural crops vary widely in their tolerance to toxic metals, this study was undertaken to evaluate the toxic effects of Bengal gram seeds germinated under different concentration of lead acetate and to identify the tolerant sample so that its cultivation can be recommended in a particular environment. It is already discussed about the nutritive proportions in edible Bengal gram seeds and this study aimed to check in what level lead toxicity modify these proportions. Ultimately it is related to human health as lead may enters to our body through contaminated seed samples unknowingly. Thus

this study was also proposed to address environmental pollutions.

2. MATERIALS AND METHODS

2.1 Germination protocol

The healthy seeds of Bengal grams were collected randomly from the market. The experiment was conducted in laboratory conditions. Seeds were air dried and stored in room temperature before treating with the heavy metal solutions. Stored seeds were allowed to soak in potassium permanganate solution to avoid fungal contaminations [7]. Soaked sample seeds were washed four to five times with double distilled water and scattered in filter papers kept on petri plates. Three different concentrations of lead acetate solutions were prepared and 5 ml of each treatment was added in order to moisten the filter paper in respective petri plates. In control, distilled water was used in place of metal solutions. All the Petri dishes were kept at room temperature (25±3°C).

2.2 Root length

After 8 days sample seeds were collected and root lengths were measured.

2.3 Total carbohydrate estimation

0.02gm samples were hydrolyzed by keeping in boiling water bath for three hours with 1ml of 2.5N HCL and cooled in room temperature. HCL was used to break down carbohydrates into simple sugars. Solid sodium carbonate was added to neutralize the sample until effervescences were ceased. Samples were made up to 2ml for centrifugation. Supernatants were collected to estimate total carbohydrate present (using standards and blank) by using anthrone reagent and absorbance was taken at 630 nm [8].

2.4 Starch estimation

0.02gm of samples were homogenized in hot 80% ethanol to remove free sugars and centrifuged. Precipitates were retained and washed with hot 80% ethanol repeatedly followed by centrifugation. 2.5ml of water and 3.5ml of 52% perchloric acid were added in precipitate which helped in starch extraction. Centrifugation was done for 20mins, supernatants were collected for the starch estimation (using standards and blank) by using anthrone reagent and the absorbance was taken at 630 nm [8].

2.5 Reducing sugar estimation

Samples (0.02gm) were homogenized in 0.1M phosphate buffer (pH 7) and centrifuged. Supernatants (crude) were collected for the estimation using the standard and blank. The process of estimation of reducing sugar was done by DNS method [9].

2.6 Protein estimation

The seeds were first freeze dried and grounded in motor pestle. The resulting meals were defatted with diethyl ether. Final air dried powdered form of the defatted meals (0.02gm) were homogenized with 0.1M phosphate buffer (pH 7). Supernatants (crude) were collected for protein estimation and the absorbance was taken at 520nm [10].

2.7 Peroxidase assay

200µg crude samples were added in 3ml of reaction mixture containing 50mM phosphate buffer (pH 7), 0.3% of hydrogen peroxide solution as substrate and 1% guaiacol as hydrogen donor. The increase of absorbance due to guaiacol oxidation was monitored under 430nm [11].

2.8 Catalase assay

200µg crude samples were added in 3ml of reaction mixture containing 0.01M phosphate buffer (pH 7), 0.2M hydrogen peroxide solution and the reaction was stopped at different time intervals by addition of dichromate-acetic acid reagent (1:3 ratio of 5% potassium dichromate was mixed with glacial acetic acid, from this 1ml was diluted again with 4ml acetic acid) [12].The reaction mixtures were kept for boiling for 10min, cooled and absorbance was taken at 620nm.

The data obtained from all above mentioned experiments were statistically analyzed by Analysis of Variance (ANOVA)[13] and Duncan's Multiple Range Test at $p<0.05$[14]

3. RESULTS& DISSCUSSION

3.1 Root length & tolerance index

Lead treatment showed various affects in growth as well as in the properties of biomolecules of Bengal gram seeds. As per the experimental data, length of the roots started decreasing significantly ($p<0.05$) with increasing the concentration of lead acetate treatments (1mM/L, 2mM/L& 4mM/L). The control seeds showed highest root length 4.8cm(Table1, Figure2).

Tolerance Indices (TI): T.I. was determined through use of the following formula [15]:

$$T.I = \frac{\text{Mean root length in metal solution}}{\text{Mean root length in ditilled water}} \times 100$$

The tolerance index gave the highest tolerant sample (1mM/L) with 45.8% of tolerance (Table1, Figure3).

Table1. Effects of different lead acetate concentration on root length of Bengal grams and their tolerance index.

Concentration (mM)	Root length(cm)	Tolerance index (%)
Control	4.8 ± 1.2	100
1	2.2 ± 1.9	45.8
2	1.7 ± 1.7	35.4
4	1.5 ± 1.5	31.2

Root length parameter was proven to be significant, P value = 0.004(<0.05).

Experimented Bengal gram seeds showed inhibitions in the growth of the roots under increasing concentrations of Lead Acetate (Figure2). Comparing the result with scientific article [16] where roots of Albizia lebbeck were similarly affected by lead concentration, it can be said that presence of this toxic metal causes negative impact on plant germination. Excessive amounts of toxic elements usually cause reduction in plant growth [17]. The reductions of root length were observed in treated samples as compared to the control. This may be due to reductions in both new cell formation and cell elongation in the extension region of the roots [18]. Also lead treatment reduces the capacity of mitotic cell division in meristematic zone of roots of Allium cepa[19]. Accumulation of excessive lead in the cells of the meristematiczone, blocks the metaphase cell division and results in undeveloped roots [20, 21].

Tolerance to lead treatments in Bengal grams was lower as compared to control (Figure3). This information can be considered a contributing step in exploring and finding of tolerance limit of Bengal grams at different levels of treated metal. Tolerance limit of Thespesia populnea L. gradually decreases with increasing lead levels [22]. Tolerance to heavy metals in plants may be defined as the ability to survive in a soil that is manifested by an interaction between a genotype and its environment [23]

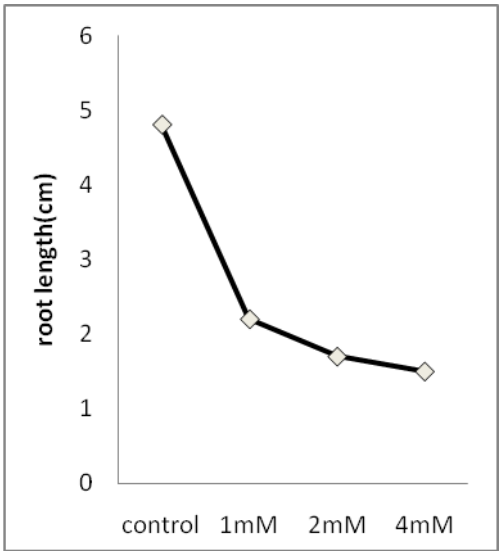

Figure2: Root lengths of different seed samples.

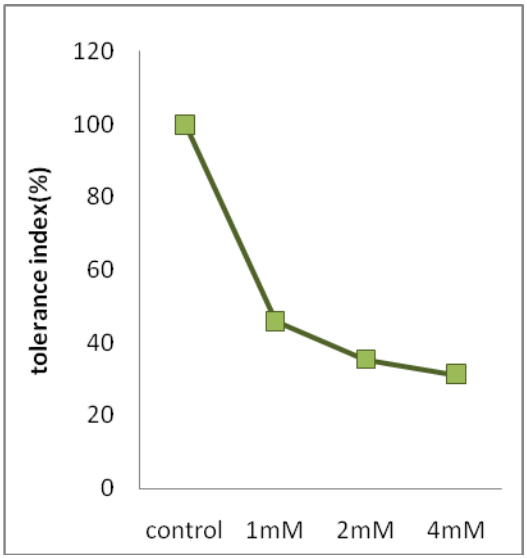

Figure3: Tolerance index of seed samples.

3.2 Total carbohydrates, starch, reducing sugar& protein content

Increasing concentration of lead acetate led to the increase in the carbohydrate and starch content ($p<0.05$) significantly (Table2, Figure4). The concentration of reducing sugar and protein reduced significantly ($p<0.05$) with the increasing concentration of lead acetate (Table2,Figure4).

Table2. Effects of different lead acetate concentration on total carbohydrate, reducing sugar and starch of Bengal gram seeds.

Concentration (mM)	Total carbohydrate* (mg/g)	Reducing Sugar** (mg/g)	Starch*** (mg/g)	Protein**** (mg/g)
Control	350 ± 48.1	205.4 ± 82.3	111 ± 55.5	270 ± 94.4
1	270 ± 49.5	184 ± 79.1	76.3 ± 21.4	168.5 ± 99.5
2	297 ± 54.2	158.3 ± 63.9	115.3 ± 32.8	112 ± 46.5
4	500 ± 117.1	132.3 ± 52.1	320 ± 136.4	50.3 ± 13.9

All the results were significant, P values (<0.05) were 0.03*, 0.02**, 0.01*** & 0.2****respectively.

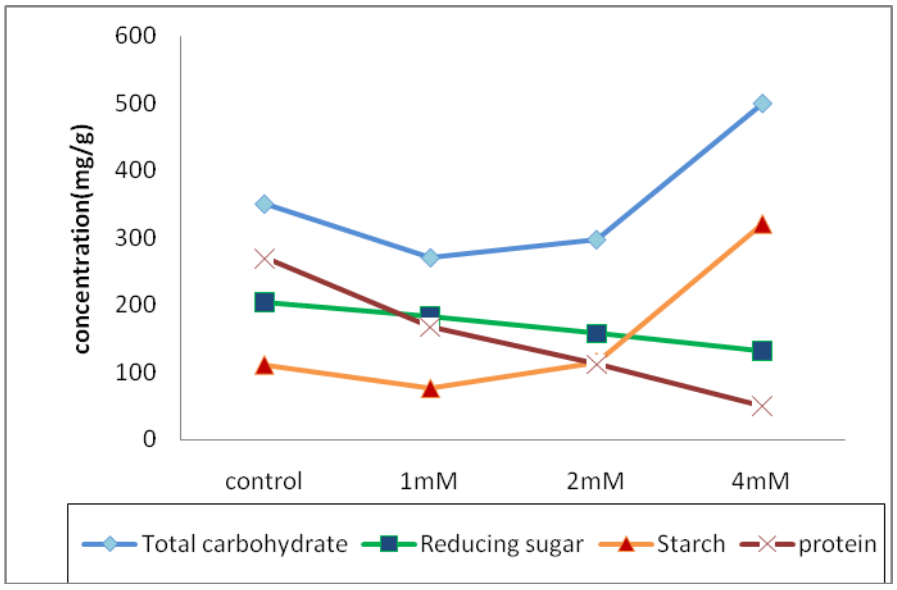

Figure4: Concentration of total carbohydrates, reducing sugar, starch& protein present in different lead acetate treatments.

We can predict that at molecular level lead acetate toxicity directly affects breakdown of starch into simpler sugar during germination of 4mM samples (Figure4). It may attack amylase activities in the seeds. Increasing lead concentration causes inhibition of amylolytic activity progressively [24]. Bhushan et al, also found up to 94% percent retardation in starch utilization in endosperm of oat seeds during germination due to lead treatments. Under abiotic stresses such as heavy metals, water deficit and salt stress, the conversion of starch to sucrose is inhibited [25, 26]. Comparing this result with reducing sugar content it can be said that obstruction in starch breakdown causes limited generation of reducible free sugar. During germination, carbohydrate is used as a source of energy for embryonic growth which results the changes of carbohydrate content after germination [27]. If we consider only 1mM, 2mM and 4mM samples, result gave increasing total carbohydrate and starch but decreasing reducing sugar concentration. Hence, it can be said that as the concentration of the treatment level goes up, starch breakdown reduces and due to production of less free sugars, seeds cannot utilize it as energy source for germination which causes presence of high content of total carbohydrates in 4mM. But relating the result of less starch content in 1mM and 2mM samples with control, it creates a different conclusion. These particular concentrations may induce a positive effect on the enzymes responsible in breakdown of starch. The activity of the sucrose biosynthetic enzyme sucrose phosphate synthase is induced by salt stress [28, 29]. Thus total carbohydrate content is less as compared to control. Hence, it is concluded that in 1mM and 2mM samples lead treatment favor utilization of sugars during germination but not in 4mM.

Concentration of protein in treated Bengal gram samples also decreases (Figure4) which is correlating the result found in in Horse grams with similar treatment [30]. Higher concentration of treatments prohibit breakdown of proteins into amino acids or inactivate the enzymes required for the breakdown.

3.3 Enzyme activities

The enzyme activities showed quite different results (Table3, Figure5). Peroxidase activity was more in treated samples than control but the activity went down with the increase in concentration. 1mM samples were highest in the activity. On the other side only control and 1mM seed samples showed catalase activity where 1mM with higher activity. Both the enzymatic assay gave significant results ($p<0.05$).

Table3. Effects of different lead acetate concentration on protein content, peroxidase and catalase activity.

Concentration(mM)	Peroxidase activity^{+} (Unit/ml)	Catalase activity^{++} (Unit/ml)
Control	0.13 ± 0.004	1.21 ± 0.02
1	0.24 ± 0.004	1.53 ± 0.12
2	0.19 ± 0.016	0
4	0.16 ± 0.003	0

P values (<0.05) of above mentioned readings are significant, 0.0002^{+}& 0.01^{++}

Peroxidases are widely accepted as 'stress enzymes' [31]. It is being activated for the defensive mechanism against ROS to get rid of stress generated in the plant. As peroxidases are located in cytosol, cell wall, vacuole and in extracellular spaces, increased peroxidase activity in lead stressed samples might be possibly due to increased release of localized peroxidases [32]. It is suggested that the elevated peroxidase activity in 1mM than control can be the consequence of either ionic microenvironment or the tissue specific gene expression in the leave and root [33]. Furthermore, peroxidase participating in the lignin biosynthesis may build up a physical barrier against poisoning heavy metals [34]. In addition, our results (while comparing treated

samples with control) are in agreement with many researchers who have reported that, lead enhances peroxidase activity in many plants such as Phaseolusvulgarisand Salix acmophylla[35, 36]. Therefore, an increase in peroxidase activity prevents plant from toxic effects of ROS. It is assumed that the hyper activity of antioxidant enzyme peroxidase in leaves and roots of radish may be consequence of strategy adapted by plant for its survival under stress by metals like lead [36]. Under metal toxicity conditions, level of peroxidase activity has been used as potential biomarker to evaluate the intensity of stress [37]. Above 1mM, the activity goes down due to higher toxicity.

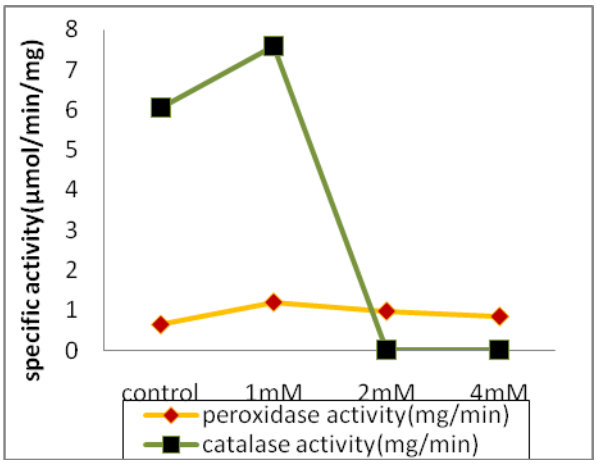

Figure5: Peroxidase &Catalase activity of the seed samples under different treatments. Seed samples of 2mM & 4mMconcentrations showed zero catalase activity.

Similar to peroxidase, catalase is meant for neutralizing the toxicity of superoxide generated due to stress. Catalase is universally present oxidoreductase that decomposes H_2O_2 to water and molecular oxygen and it is one of the key enzymes involved in removal of toxic peroxides [38]. Earlier also higher catalase activity was noticed in the treated samples than control [39]. The reason behind this is the scavenging role of catalase for H_2O_2. An increase in catalase activity in radish plant (Raphanussativus) was reported in various articles [40, 41, 42]. According to our results, 1mM concentration was least tolerable to the seed samples and beyond that lead toxicity was intolerant thus the catalase activity was nil in other two samples (Fig.5). Lead treatment resulted in a decline in catalase activity was also reported in roots of two different rice seedlings [32]. A reduction in catalase activity under stressful conditions has been attributed to the inactivation of enzyme protein due to ROS [43], decrease in enzyme synthesis or change in assembly of enzyme subunits [44, 45]. Similar decline in catalase activity was reported under salinity [46].

It is concluded that the maximum concentration in which Bengal gram seeds can withstand the level of lead acetate toxicity is 1mM. Samples of 2mM and 4mM concentrations are being modified by lead acetate badly which reduce their nutritive proportions. Intakes of these contaminated seeds will harm human health. So awareness need to be generated for eradication of pollutions created due to heavy metal toxicity for the sake of better future and healthy, disease free long life.

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