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Full Length Research Article

Effect of Lead Nitrate on Germination and Growth of *Cicer arietinum* Linn Seeds

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Abstract

Cicer arietinum Linn (Bengal gram, Chick pea) of the family Leguminosae is an important nutritive pulse extensively used as a protein adjunct to starchy diets in India. It is widely cultivated in India; however, the presence of heavy metals like lead causes a decrease in yield of the crop. In the current situation, lead contamination in soil is resulted on a worldwide scale, causing a major threat to the agriculture sector reducing crop productions. Presence of lead affects different plants differently-some plants can tolerate lead stress whereas others are sensitive to lead. Lead stress significantly affects the early stage of plant growth such as germination and seedling growth parameters. Here in our work, the effect of lead nitrate on phenotypic parameters such as the seed germination percentage, seedling growth, fresh weight and dry weight of cotyledons and seedlings of Bengal gram were evaluated. Lead nitrate didn't affect seed germination percentage however the seedling growth, fresh and dry weight of cotyledons and seedlings were affected significantly.

***Key words*:** *Cicer arietinum* L, Heavy metal, Lead nitrate, Germination, Seedling growth

One of the most hazardous, and enduring heavy metal pollutants in agricultural soil is lead [1-2]. Toxic heavy metals like lead (Pb) causes adverse effects on seed germination and seedling growth parameters of various crops [3-4]. According to a recent report, about 10% of all pollution caused by heavy metals is contributed by Pb and around 42% decrease in root growth might result from the excessive accumulation of Pb [5]. In a case, at 10 µmol/L concentration of lead nitrate have affected the percentage of seed germination, growth of seedling and the dry weight of *Thespesia populnea* L. [6]. However, there are certain plant species having abilities to tolerate toxic amounts of non-essential Pb, Cd, Ag and of several essential micronutrient cations [7-8]. In some Species the elements are absorbed only to a limited extent; so, this represents a case more of avoidance than true tolerance. In other species, the elements accumulate in roots with little transport to shoots. In still others, both roots and shoots contain much higher amount of such elements [9]. People should be aware of edible plants that accumulate relatively high concentrations of elements in the edible parts. There is present concern, for example, that certain vegetables grown on soils formally used as mining areas in Colorado contain excess lead and cadmium [10]. Presence of heavy metals like Pb affects plant growth and germination widely. Percentage of germination significantly changes in many cases when seeds are treated with heavy metals like Al, Pb, Hg etc. Kalimuthu *et al*. [11] noticed that maize seeds treated with Hg and Pb for 24 hours showed inhibitions of germination, seedling growth. However, although Pb does not show any significant effect for any short time exposure it is highly toxic for any long-time exposure and interestingly organic Pb is more harmful for plants than inorganic one. For short time exposure blue-green algae do not show any positive effect to Pb as faras the ultra-structural effects is concerned but Pb effects considerably in mitochondrial and folding of unclear envelops in unicellular alga *Poterioochromonas malhamensis* [12]. Lee *et al*. [13] have noticed the effect of Pb on *Analytic indulines*. They observed the toxic effect of $Pb(NO_3)_2$ on cellular or original structure is appositively correlated phenomena, when the effect increases with the increase of Pb concentration the growth in 50 ppm Pb concentration is almost similar to the controls. At 200ppm concentration growth of cells reduced considerably when 1000ppm concentration, the growth totally halted regardless of the pH value in concentration of 200 ppm and above cells either degrade or combined with cellular debris. Seedlings, both mycorrhizal and non-mycorrhizal, do not get dramatically affected by Pb however the shoot to root ratio and relative number of root tips were affected. Here the main goal of the present work is to study the effect of lead nitrate on seed germination and seedling growth of *Cicer arietinum* Linn and to understand the concentration of lead nitrate that the crop plant of *Cicer arietinum* Linn can tolerate during the germination and early growth condition.

MATERIALS AND METHODS

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Plant materials: Certified healthy seeds of *Cicer arietinum* Linn were collected from Seed Corporation of Assam, Guwahati branch for experimentation. Clean and healthy seeds of uniform size were selected and surface sterilized by using 0.01% mercuric chloride solution, later on the seeds were rinsed three times with sterile distilled water. Mercuric chloride solution (0.01%) was used as surface sterilizer [14] since it checks microbial growth effectively and do not show adverse effects on seed germination and growth.

Experimental plant growth condition and treatment: After surface sterilization, 20g of seeds numbering around 28-30 were dispersed on two layers of Whatman No.1 filter paper placed inside the sterile petri plates. The seeds were soaked with 0.025%, 0.05% and 0.1% Lead nitrate solution along with a control (0%) for 24 hours. The papers and seeds were kept moist for different intervals of time by spraying distilled water and respective test solution of $PbNO_3$ of different concentrations. This way the seeds were allowed to germinate at room temperature where the seeds were exposed to 12 hours light and 12 hours dark period in a 24 hours cycle. The different period of germination for the experiment have been taken as 24 hrs, 48 hrs, 72 hrs, 96 hrs, and 144 hrs. The experiment was replicated thrice.

Analysis of seed germination percentage: The seeds were considered to have germinated when radical emerged through the seed coat. Total number of seeds present in each plate and the number of seeds germinated in each plate was counted to calculate the seed germination percentage. The percentage of seed germination was recorded at different hours.

Seedling growth measurement: The length of the seedlings in each plate was measured in cm after the treatment of the seeds with different concentration of $PbNO_3$ and recorded the data at different time intervals (24, 48, 72, 96 and 144 hrs).

Fresh and dry weight analysis: After treating the seeds with different concentration of $PbNO_3$ for different periods (24, 48, 72, 96 and 144 hrs), the seeds were taken out and soaked to remove residual lead nitrate being absorbed and then the seeds were dried by clean blotting paper. Cotyledons and seedlings were separated and their fresh weight and dry weight were recorded separately. Dry weight was recorded by putting them into hot air oven at 180℃ for two hours for cotyledons and thirty minutes for radicles.

Statistical analysis of the data: To get greater reliance of the finding data collected in all experiments were statistically analyzed. The data obtained from the experiments were subjected to statistical analysis to study the effects of various factors and their interaction using Fisher's method for determination of variance ratio. It is necessary to compare the calculated value of 'F' the respective degree of freedom so that the significance can be tested.

Critical difference (CD): The analysis of variance table gives only broad indication of performance of the concentration and time as well as their growth rate. In order to get clear picture of specific phenomena of the different levels of main factors, the calculation of critical difference was considered necessary. CD was evaluated as follows:

$$CD = \sqrt{[(EMSS \times 2)/n]} \times \text{'t' value at 5\% or 1\% level for error df.}$$

Where, n= total number used for calculating the means.

The calculated value of CD was utilized in testing the difference between the two mean values as significant or not.

Standard Error: In the case of growth, fresh weight and dry weight for each treatment, the mean and standard error was calculated from the three replicates.

RESULTS AND DISCUSSION

Germination percentage

Excess Pb in plants can disrupt a variety of physiological and biochemical processes which thereby affects seed germination parameters [15-16], however there is a limit up to which each plant species can tolerate the Pb concentration. Here the mean germination percentage of seeds is presented in (Fig 1). Mean percentage of germination of seeds treated with 0% lead nitrate was recorded as 100% after 24 hours, while in those treated with 0.025%, 0.05% and 0.1% lead nitrate the mean germination percentage recorded were 98, 96 and 95% respectively. 100% germination was recorded for all the concentration of lead nitrate at 144 hours of germination. It can be concluded that, though the germination speed is slightly affected, above concentrations of lead nitrate do not affect germination percentage significantly at 144 hours.

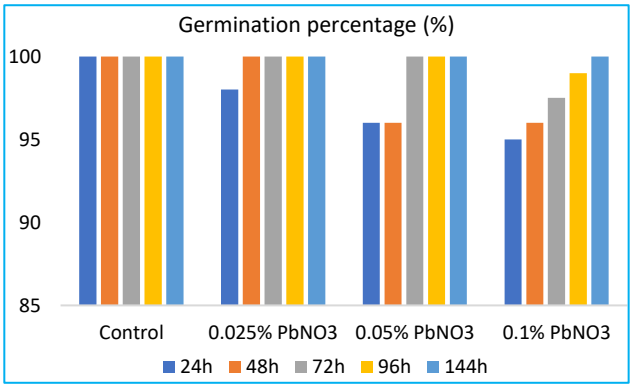
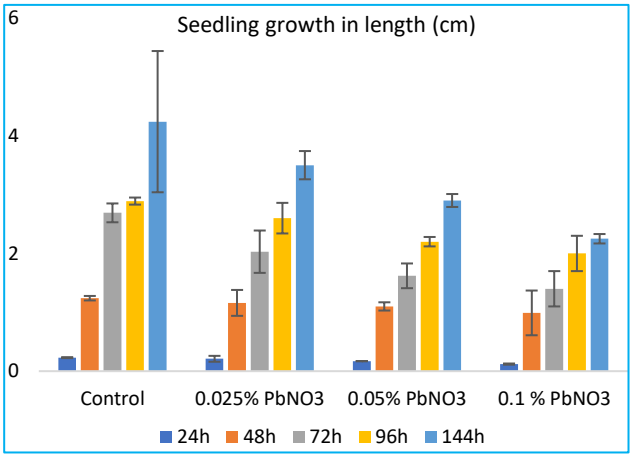


Fig 1 Germination percentage of seeds treated with lead nitrate



C.D. for lead nitrate at 1% level (n=12) = 0.27823
C.D. for lead nitrate at 5% level (n=12) = 0.2117042
C.D. for time period at 1% level (n=15) = 0.24886
C.D. for time period at 5% level (n=15) = 0.189353

Fig 2 Seedling growth (cm) during germination period treated with $PbNO_3$

Seedling growth

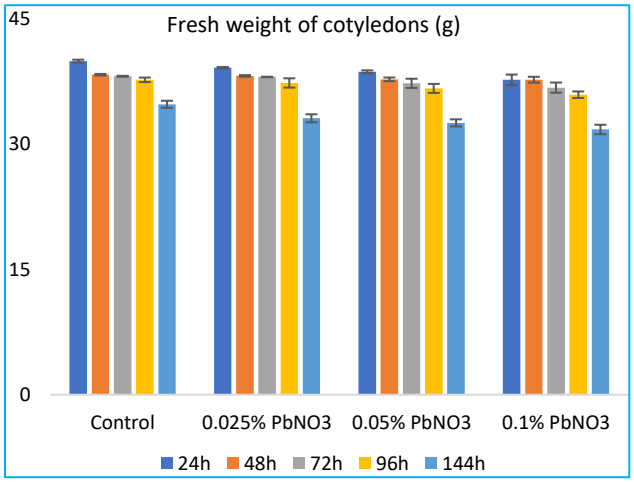
The mean growth (length) of seedling is presented in (Fig 2). The investigation revealed that the length of seedlings increases with the progress of germination period. The effect of lead nitrate on inhibition of seedling growth was found to be

very much prominent at 0.1% concentration of lead nitrate after 144 hours of treatment, though during all the periods under observations seedling growth was inhibited by lead nitrate. At 0.1% lead nitrate mean length of the seedling was 2.25 cm after 144 hours against 4.24 cm at the control. When the data was analyzed statistically (Table 1), the variance table reveals the effect of $PbNO_3$ and time on growth to be significant. The interaction between time and treatments is also significant and this indicated that $PbNO_3$ highly inhibited the growth and growth varied significantly during the period. The interaction between $PbNO_3$ and time period also emerged as significant, indicating that the effect of lead nitrate was different at different time periods. In earlier studies, lead nitrate at concentration 200-1000 mg/L, affected the seedling growth of *Vigna mungo* L. [16] and lead nitrate at concentration 1-1000 mM affected the seedling growth of *Zea mays* L. [17].

Table 1 Analysis of variance for seedling growth

Sources of variation	Degrees of freedom (df)	Sum of squares (ss)	Mean sum of squares (mss)	Variance ratio
$PbNO_3$ (Conc.)	3	6.62	2.21	31.57 **
Time period	4	61.31	15.33	219 **
Interaction	12	4.27	0.355	5.07*
Error	40	2.80	0.07	
Total	59			

**Significant at 1% level



C.D. for Lead nitrate at 1% level (n=12) = 0.7436271
C.D. for Lead nitrate at 5% level (n=12) = 0.5658032
C.D. for Lead nitrate at 1% level (n=15) = 0.6651201
C.D. for Lead nitrate at 5% level (n=15) = 0.506098

Fig 3 Fresh weight of cotyledons affected by lead nitrate

Fresh weight of cotyledons during germination

The mean fresh weight of cotyledons is presented in (Fig 3). It is seen that with increasing time period the fresh weight gradually declines. The decrease in fresh weight is noticed with the increase in $PbNO_3$ concentration. At 144 hours of germination at 0.1% the fresh weight of cotyledon was found to be reduced by 21% of that at 0% concentration. The initial weight of the seeds was 20 g but after soaking in 0%, 0.025%, 0.05% and 0.1% lead nitrate solution, significant increase in fresh weight of cotyledons was recorded. The fresh weight of cotyledons after treatment of 144 hours 0.1% lead nitrate was 31.770 g as against 34.792 g at the 0% lead nitrate (control). Increase in fresh weight might be due to the imbibition's by the seeds but with the increasing time period of germination the reduction in the fresh weight of the cotyledon was seen. This might be due to the consumption of accumulated food material by the growing seedling during the increasing time period of germination. When the data was analyzed statistically, the variance table (Table 2) reveals the effect of lead nitrate and time period to be significant thereby indicating that fresh weight varied during different time intervals.

Table 2 Analysis of variance for fresh weight of cotyledons

Sources of variation	Degrees of freedom (df)	Sum of squares (ss)	Mean sum of squares (mss)	Variance ratio
$PbNO_3$ (Conc.)	3	29.74	9.91	19.82 **
Time period	4	233.13	58.23	116.46 **
Interaction	12	4.14	0.345	0.69
Error	40	19.91	0.50	
Total	59			

**Significant at 1% level

Table 3 Analysis of variance for dry weight of cotyledons

Sources of variation	Degrees of freedom (df)	Sum of squares (ss)	Mean sum of squares (mss)	Variance ratio
$PbNO_3$ (Conc.)	3	28.36	9.45	5.43 *
Time period	4	209.92	52.48	30.16 **
Interaction	12	2.62	0.218	0.125
Error	40	69.63	1.74	
Total	59			

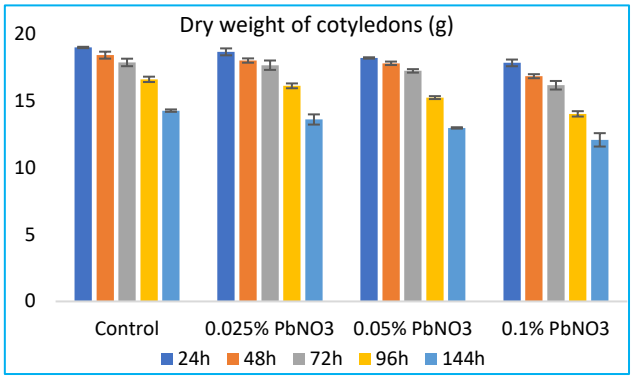
**Significant at 1% level

Dry weight of cotyledons during germination

The mean dry weight is presented in (Fig 4). It is seen that percentage of dry weight of cotyledons gradually decreases with increasing time period. The data was analyzed statistically (Table 3). The variance table shows that the effects of chemicals and time period is significant, revealing the fact that dry weight varied with time. The interaction is not significant. With the increase in time period the dry weight of the cotyledon decreased for all the concentrations of lead nitrate. Changes in dry weight might show that some material was lost from the endosperm during germination. The early dry weight loss from the endosperm may occur at the expense of fat and starch. This type of result was also suggested by Noggle and Fritz [18] working with maize seeds. The effect of lead nitrate on dry weight of cotyledon was found to be very significant revealing the fact that the dry weight of cotyledon varied with time. The

effect was found to be prominent as the concentration of lead nitrate increased; the dry weight of the cotyledon was subsequently reduced.

From (Table 4) it can be seen that there is more decrease in fresh and dry weight of cotyledons in lead nitrate treated conditions compared to the control. The highest reduction in cotyledon fresh and dry weight was noted at 0.05% lead nitrate treatment and 0.1% lead nitrate treatment respectively.



C.D. for Lead nitrate at 1% level (n=12) = 1.3872185
C.D. for Lead nitrate at 5% level (n=12) =1.0554921
C.D. for Lead nitrate at 1% level (n=15) =1.3872185
C.D. for Lead nitrate at 5% level (n=15) =0.9440608

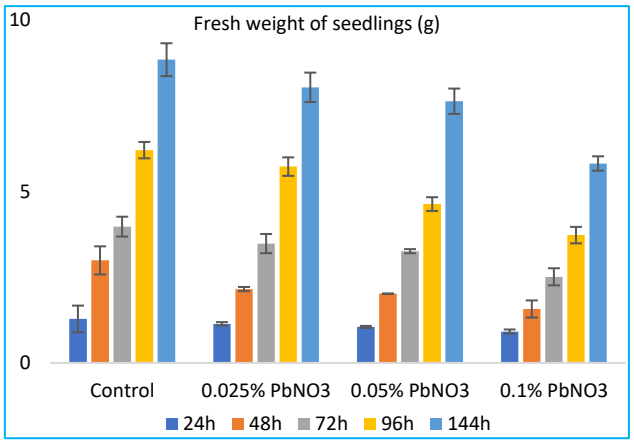
Fig 4 Dry weight of cotyledons affected by lead nitrate

Fresh weight of seedlings

The mean fresh weight (g) of seedlings is presented in (Fig 5). It is observed from the data that with increasing time period the fresh weight of seedling increased, and fresh weight decreased with the rise in concentration of $PbNO_3$.When the data was statistically analyzed (Table 5), the variance table shows the effect of lead nitrate and time period on fresh weight to be significant indicating adverse effects of lead nitrate which varied with time while the interaction was not significant. In some soybean varieties also, lead nitrate at concentration 100, 200 and 400 mg/L have significantly decreased the seedling fresh weight, however in some varieties it was increased [19].

Table 4 Decrease in cotyledon weight (g) at different treatment condition

Treatments	Difference in cotyledon weight (Weight at 24 hour - weight at 144 hour)	
	Fresh weight (g)	Dry weight (g)
Control	5.162	4.72
0.025%	6.059	5.054
0.05 %	6.126	5.23
0.1%	5.941	5.752



C.D. for Lead nitrate at 1% level (n=12) = 0.7986555
C.D. for Lead nitrate at 5% level (n=12) = 6.7896391
C.D. for Lead nitrate at 1% level (n=15) = 0.6892906
C.D. for Lead nitrate at 5% level (n=15) = 0.5249213

Fig 5 Fresh weight of seedlings affected by lead nitrate

Table 5 Analysis of variance for fresh weight of seedlings

Sources of variation	Degrees of freedom (df)	Sum of squares (ss)	Mean sum of squares (mss)	Variance ratio
$PbNO_3$ (Conc.)	3	23.93	7.98	14.86 **
Time period	4	311.70	77.93	145.12 **
Interaction	12	7.71	0.642	1.196
Error	40	21.48	0.537	
Total	59			

**Significant at 1% level

Table 6 Analysis of variance for dry weight of seedlings

Sources of variation	Degrees of freedom (df)	Sum of squares (ss)	Mean sum of squares (mss)	Variance ratio
$PbNO_3$ (Conc.)	3	3.44	1.15	17.69**
Time period	4	20.53	5.13	78.92 **
Interaction	12	1.84	0.15	2.30
Error	40	21.48	0.065	
Total	59			

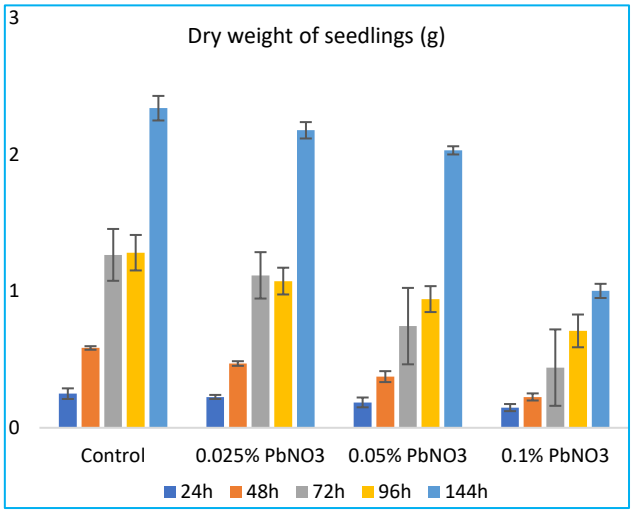
**Significant at 1% level

Dry weight of seedlings during germination

The mean dry weight of seedlings is presented in (Fig 6). After analyzing the data, it was observed that with increasing time period the dry weight of seedling increased. After 24 hour of germination dry weight of seedling at 0.025%, 0.05%, 0.1% lead nitrate was 0.226 g, 0.186 g and 0.148 g respectively against 0.250 g at 0% lead nitrate (control). After 144 hours of germination the dry weight at 0.1% lead nitrate was 1.003 g as against 2.342 g at control. Hence, dry weight of seedling increased with increasing time period. The increase in dry weight in seedling was the result of increase in growth rate of seedling with time. Simultaneously as the concentrations rose up dry weight declined.

The analysis of variance (Table 6) of the data shows the effect of lead nitrate and time period on dry weight of seedling to be significant, it shows adverse effect of lead nitrate and time period on dry weight of seedling to be significant, it shows adverse effect of lead nitrate on dry weight of seedling of seedling which varied during different time period. The interaction of time period and chemical is also found to be significant at 5% level. In a study, the seedling dry weight in *Thespesia populnea* L. was also significantly reduced by the

treatment of lead nitrate at concentration 10, 30, 50 and 70 µmol/L [6].



C.D. for Lead nitrate at 1% level (n=12) = 0.8478651
C.D. for Lead nitrate at 5% level (n=12) = 0.6451149
C.D. for Lead nitrate at 1% level (n=15) = 0.2398125
C.D. for Lead nitrate at 5% level (n=15) = 0.182466

Fig 6 Dry weight of seedlings affected by lead nitrate

CONCLUSION

The effect of lead nitrate on germination, growth, fresh weight, dry weight of gram was studied. The germination percentage is not affected much by lead nitrate treatment. However, all other studied parameters such as seedling growth, fresh and dry weight of cotyledons and seedlings are adversely affected by $PbNO_3$.The increasing concentration of $PbNO_3$ showed adverse effect on growth. With increasing $PbNO_3$ concentration the seedling growth was gradually decreased. The loss of fresh weight in cotyledon during germination occurred with an increase in fresh weight in seedling. It can be concluded that during the course of germination the stored food material in the cotyledon was being consumed by the growing seedling resulting in the loss of fresh weight in the cotyledon while the fresh weight in the seedling increased with increasing time period.

Conflict of interest: The authors declare that there is no conflict of interest exists in this research work.

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