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LEAD NITRATE INDUCED CHANGES ON GERMINATION OF GREEN GRAM [*Vigna radiata* (L.) Wilczek]

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Abstract: In the present study the seed germination, radicle length, length of the root, shoot and other morphological characters were analyzed in *Vigna radiata* induced by the heavy metal Pb $(No_3)_2$ at different concentrations such as 0, 20, 40, 60, 80 and 100 mg/l. The germination study was carried out in petriplates lined with filter paper and were watered with different concentration of Pb $(No_3)_2$. The percentage of seed germination, radicle length, length of the root and shoot and number of seed showing emerged leaves were taken into consideration for seasonal screening experiment. The germination percentage was found highest in summer (91%) than winter (81%). The tolerance index of *Vigna radiata* was found to be more in summer [(20 ppm-0.81), (40 ppm-0.66), (60 ppm-0.58), (80 ppm-0.45), (100 ppm-0.33)] than in winter season [(20 ppm-0.78), (40 ppm-0.72), (60 ppm-0.64), (80 ppm-0.46), (100 ppm-0.22)]. The maximum Vigour index was recorded in case of control (567-summer, 424- winter) as compared to 100 ppm concentration of Pb (88- summer, 68- winter). The percentage of Phytotoxicity was also found slightly higher in summer (72) than winter season (71). The present findings emphasized that the summer season yielded better result than winter with regard to the rate of germination, thereby indicating that temperature played a major role in the process of germination. Green gram showed lowest percentage of tolerance at higher concentrations of the heavy metal solution.

Keywords: Pb $(No_3)_2$, *Vigna radiata*, germination studies, vigour index, tolerance index, phytotoxicity.

INTRODUCTION

Pulse crops have been shown to be an excellent source of dietary protein important for the human diet and play a key role in crop rotation due to their ability to fix nitrogen. In India, pulses are cultivated to an extent of 22.37 million hectares of area with an average production of 14.66 million tonnes by an average productivity of 655 kg per hectare during the year of 2008-2009. Among the pulses, pigeon pea, black gram and green gram are the major contributors of the total pulses production. Mung bean [*Vigna radiata* (L.) Wilczek] also known as green gram, green bean, mash bean, golden gram and green soy is an excellent

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source of easily digestible proteins with low flatulence which complements the staple rice diet in Asia (Saminathan, 2013). In India, nearly 8% of the area is occupied by green gram, which is the third important pulse crop of India in terms of area under cultivation and production next to pigeon pea and black gram. The Indian subcontinent covers up to 55% of the total world acreage and 45% of total production leading to Pakistan, Sri Lanka, Thailand, and China (Rishi, 2009).

Heavy metal contamination of soil and water is a serious problem for ecosystem which poses strong negative effects on plant growth and development (Kabata, 2001). It is known that metal sensitivity and toxicity to plants are influenced by not only the concentration and the toxicant types, but are also dependent to several developmental stages of the plants (Liu *et al*., 2005). Seed germination being the most highly sensitive physiological processes in plants is regulated by several hormonal interaction and environmental factors (Hatamzadeh *et al*., 2012). In addition, seed germination is more sensitive to metal pollution because of lack of adequate defence mechanisms (Liu *et al*., 2005; Xiong *et al*., 2005). Therefore, seed germination behaviour is necessary to be understood under adverse environmental conditions. During the present investigation attempts were made to find the effect of different concentrations of lead nitrate (0, 20, 40, 60, 80 and 100 ppm) on seed germination, growth and other morphological features in *Vigna radiata* in laboratory conditions. A seasonal varietal screening test was also conducted to find out the tolerant and susceptible varieties under the heavy metal stress.

MATERIALS AND METHODS

The experiments relating to germination studies were carried out by top paper method as recommended by International seed Testing Association (ISTA, 1976). The seeds were first surface sterilized with 0.2% mercuric chloride ($HgCl_2$) for two minutes and then were thoroughly washed with distilled water. The sterilized seeds were arranged equi-spacially on the periphery of sterilized petridishes lined with filter paper. Each petridish was irrigated uniformly by various concentrations of lead (Pb) solution respectively. In addition, petridishes containing seeds were irrigated with distilled water and was maintained as control. Each treatment including the control was replicated three times. All the petridishes were kept under diffused light at room temperature (28 ± 2 ^{0}C). The numbers of seeds germinated in each treatment were counted at every 24 hours for about 5 days. The emergence of radicle was taken as a criterion for germination. Five seedlings from each replica were selected for recording the morphological parameters such as shoot length and root length in a centimetre scale.

The following formulae were followed to estimate percentage of germination besides other related parameters:

Germination % = number of seed germinate/total number of seed × 100

The % of phytotoxicity of the metal was calculated by the formulae suggested by Chou *et al*., 1978

Percentage of phytotoxicity = radicle length of the control - radicle length of test seeds ÷ radicle length of control × 100

Vigour index of the seedlings were calculated by using the formulae proposed by Baki and Anderson, 1973.

Vigour index = germination percentage × length of the embryonic axis

The tolerance indexes of the seedlings were calculated by using the formulae proposed by Turner and Marshal, 1972.

Tolerance index = mean length of longest root in treatment/ mean length of longest root in control

RESULTS AND DISCUSSION

The data on germination percentage of seeds of *V. radiata* in winter and summer season under lead nitrate treatment is presented in Table 1. The Fig.1 also shows the effect of different concentrations of Pb on seed germination in green gram. The maximum percentage of germination was observed at control (81±1) in summer than in winter (91±1). The germination percentage was observed to be decreased gradually with progressive increase in Pb $(No_3)_2$ concentrations irrespective of seasons. The minimum percentage of germination was recorded at 100 ppm concentration of Pb (45.66±1.33 in winter and 51±2 in summer). When compared between the seasons, the percentage of germination was observed to be more in summer at all the treatments. The decrease in seed germination may be due to the breakdown of stored food material in seed or the toxic effect of Pb (Kalimuthu and Siva, 1990). Like wise the length of the radicle and growth of the seedlings showed a decreasing trend with the increase in Pb concentration (Fig. 2). Similar experiments were carried out in green gram under the influence of mercury (Jagatheeswari and Ranganathan, 2012), mung bean under lead acetate (Gautam *et al.,* 2008), in green gram under the effect of cobalt (Hussain *et al*., 2007).

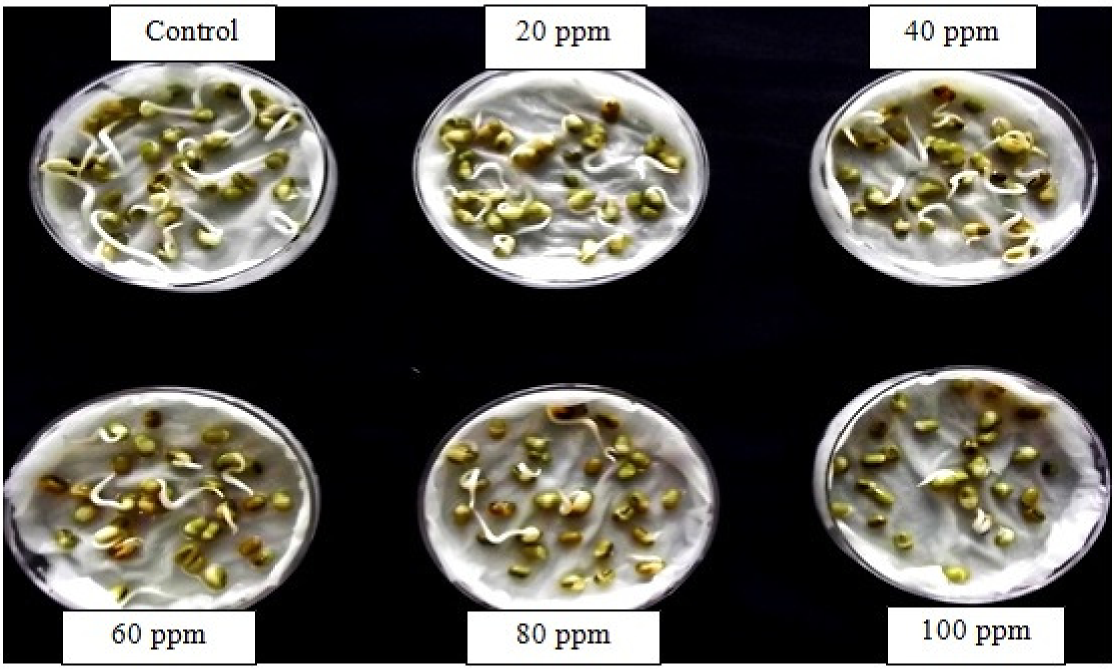

Fig. 1: Effect of various concentrations of Pb on seed germination of [*Vigna radiata* (L.) Wilczek]

Table 1: Effect of various concentrations of Pb on seed germination and radicle length of [*Vigna radiata* (L.) Wilczek] in winter and summer season after 72 hours (values are mean ±SEM of three replicates)

Conc. of Pb (in ppm)	Winter season			Summer season		
	Number of seed germination	Percentage of seed germination	Radicle length (in cm)	Number of seed germination	Percentage of germination	Radicle length (in cm)
0	24.33±0.33	81.0±1	5.23±0.14	27.33±0.33	91.0±1	6.23±0.08
20	21.66±0.33	72.0±1	4.1±0.05	25.66±0.33	85.0±1	4.6±0.05
40	20.66±0.33	69.0±1	3.46±0.14	24.33±0.33	81.0±1	3.66±0.08
60	19.66±0.33	65.67±1.33	2.83±0.06	20.66±0.33	69.0±1	3.23±0.14
80	17.66±0.33	58.66±1.33	2.23±0.03	18.33±0.67	60.66±1	2.36±0.14
100	13.66±0.33	45.66±1.33	1.5±0.05	15.33±0.66	51.0±2	1.73±0.12

The root length was observed to be maximum at control in both the seasons (2.83±0.06 cm in winter and 4.93±0.03 cm in summer) and minimum when treated with Pb at 100 ppm (0.63±0.08 cm in winter and 1.63±0.08 cm in summer). The maximum shoot length was found at control (7.7±0.11 cm) of summer and minimum was observed at 100 ppm (3.4±0.05 cm) of winter. There was a gradual decrease in shoot and root length with the increase in Pb concentration from 0 to 100 ppm irrespective of seasons.

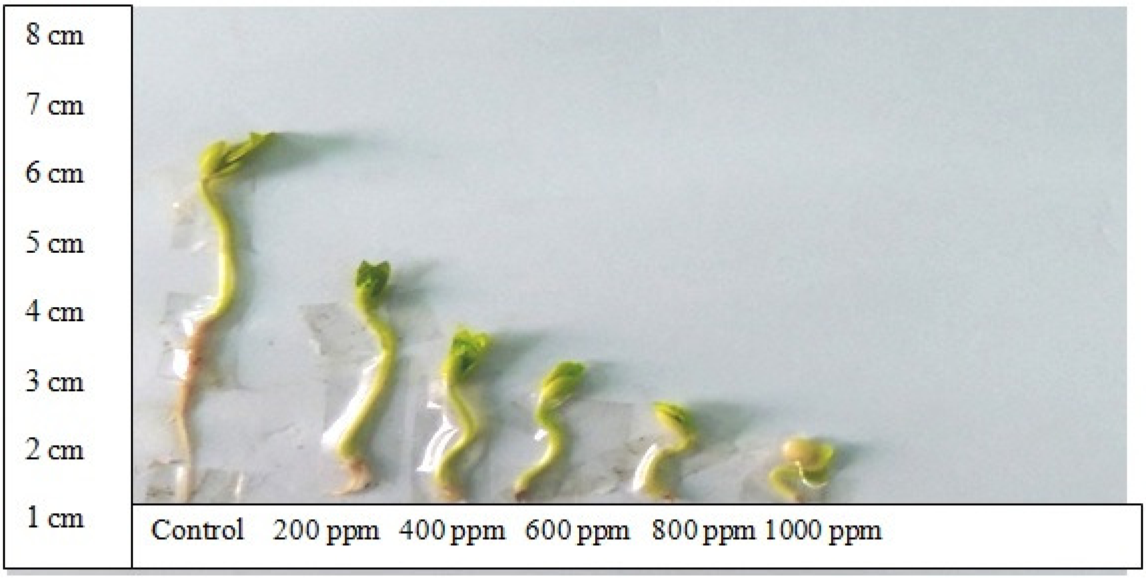

Fig. 2: Effect of various concentrations of Pb on *Vigna radiata* seedling after 5 days of germination

The number of seeds which emerged leaves were affected by different concentrations of Pb and followed the same trend as that of root and shoot length (Table 2). Similar results were obtained in the experiment conducted on black gram under the treatment of Pb and Cr (Xiong *et al.,* 2005).

The vigour index was found to be higher at control (424 in winter and 567 in summer). The minimum value was recorded at 100 ppm (68 in winter and 88 in summer). In both the season the vigour index and tolerance index values showed a gradual decline with increase in concentration of lead (Pb), while the percentage of phytotoxicity value showed a reverse trend (Table 3). This result is in conformity with similar experiment conducted on the effect of different concentrations of mercury on the germination in green gram (Saminathan, 2013).

Table 2: Effect of various concentrations of Pb on root and shoot length of [*Vigna radiata* (L.) Wilczek] in winter and summer season after 5 days (values are mean ±SEM of three replicates)

Conc. of Pb (in ppm)	Winter season			Summer season		
	Number of seed emerged leaves	Root length (in cm)	Shoot length (in cm)	Number of seed emerged leaves	Root length (in cm)	Shoot length (in cm)
0	11.66±0.88	2.83±0.06	6.9±0.05	15.33±0.33	4.93±0.03	7.7±0.11
20	7.0±0.57	2.23±0.08	6.2±0.05	12.33±0.66	4.0±0.05	7.2±0.15
40	5.33±0.33	2.06±0.03	5.7±0.05	10.33±0.33	3.3±0.11	6.53±0.08

60	4.33±0.33	1.83±0.06	4.5±0.05	8.0±0.57	2.86±0.06	5.56±0.14
80	2.66±0.33	1.33±0.08	4.0±0.05	6.0±0.57	2.23±0.08	5.1±0.1
100	0.33±0.33	0.63±0.08	3.4±0.05	0.66±0.33	1.63±0.08	4.2±0.11

Table 3: Effect of various concentrations of Pb on vigour index, tolerance index, percentage of phytotoxicity on [*Vigna radiata* (L.) Wilczek] in winter and summer

Conc. of Pb (in ppm)	Winter season			Summer season		
	Vigour index	Tolerance index	Percentage of phytotoxicity	Vigour index	Tolerance index	Percentage of phytotoxicity
0	424	-	-	567	-	-
20	295	0.78	22	391	0.81	26
40	239	0.72	34	296	0.66	41
60	186	0.64	46	223	0.58	48
80	131	0.46	57	143	0.45	62
100	68	0.22	71	88	0.33	72

CONCLUSION

The present investigation revealed the detrimental effect of heavy metal Pb by showing its toxic impact on germination and growth of *Vigna radiata.* Increase in the concentration led to changes in most of the growth parameters of plants studied during the investigation. It was observed that seasons also play an important role which may be due to the temperature effect, since the summer season is more conducive for seed germination as compared to winter. Further there is a need to impose certain rules for environmental management in order to reduce the heavy metal level especially Pb from a wide range of sources like metal processing industries and captive power plants.

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