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INFLUENCE OF ELECTROMAGNETIC FIELD (E.M FIELD) AND LEAD CHLORIDE ON THE SEED GERMINATION, GROWTH AND CHLOROPHYLL CONTENT OF *VIGNA RADIATA* (L.) R. WILCZEK (MUNG BEAN)

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ABSTRACT

The present study deals with the individual and combined effect of two stresses. First is the effect of heavy metal (lead) and other is the effect of Electromagnetic field on the germination, growth and chlorophyll contents of test crop mung bean seedlings (*Vigna radiata*). Experiments were performed in three different series, *i.e.* i. Study the individual effect of lead chloride ($PbCl_2$), ii. Study the effect of Electromagnetic field and iii. Study the combined effect of lead chloride and Electromagnetic field. The results showed: i. a significant decline in seed germination, growth and chlorophyll content due to the increase of concentrations of lead, ii. A significant increase in seed germination and total chlorophyll contents and a decrease in root and shoot growth due to exposure to Electromagnetic field, iii. Whereas the combined treatment of lead chloride and Electromagnetic field cause a considerable decline in the germination of seeds, growth of seedling and total chlorophyll content of mung bean and this showed an increase with the enhancement of lead chloride concentration and Electromagnetic field exposure time.

Keywords: Seed germination, seedling growth, chlorophyll contents, carotenoids, Pb (lead), Electromagnetic field (E.M field).

INTRODUCTION

Lead is one of the heavy metals which is not required in considerable amount. It causes toxic for living beings (Duffus 2002). The contamination of lead in soil is introduced by metallic lead, inorganic ions and salt (Harrison, 2001). The toxicity of lead decline the germination, growth and other physiological processes and yield of wheat crop (Rashid and Mukharji, 1993), effected seedlings of *Sesamum indicum* and decreased growth and biomass individually and with combination of other metals (Singh *et al*., 1994).The content of chlorophyll, protein and protease also decrease in weed plants of *Cyperus difformis, Chenopodium ambrosioides* and *Digiteria sanguinales* (Ewais, 1997), similarly, the chlorophyll contents, rate of photosynthesis and biomass decreased in rice plants (Oncel *et al.*, 2000; Zeng *et al*., 2007). According to Ahmed *et al*., (2008) the mung variety seriously negatively affected by higher concentration of lead (500mg/l). Lead also inhibited the germination in *Lens culinaris* (Azmat *et al.*, 2009). Different varieties of maize are found to be susceptible to lead which caused inhibition of growth and decline chlorophyll content (Ghani 2010). Wheat germination, root and shoot growth and chlorophyll contents decreased was observed by Bhatti *et al*., (2013). Similar results were observed by Patil and Umadevi (2014) when they studied different species of Eucalyptus (*E. tereticornis, E. camaldulensis, E. globulus and E. citriodora*).

Electromagnetic field is one the fastest spreading radiation across the globe. All populations are now exposed to varying degree of Electromagnetic field because of advancement of technology in recent decades. The Electromagnetic field effects on plants in both positive and negative manner. Different schools of thoughts are given on related effect of exposure of Electromagnetic field (Alexander and Doijode, 1995; Harichand *et al*., 2002; Fischer *et al.,* 2004 and Rajendra *et al*., 2005). Rate of germination of seeds of tomato increased due to the exposure of Electromagnetic field as compared of control, but when the intensity increased the growth and germination decreased (Moon and Chung 2000). An increase in the germination of seeds of *Oryza sativa* due to exposure of 150mT and 250mT of Electromagnetic field was observed by Carbonell *et al*., (2000). Germination, shoot development and fresh weight of seedling of maize were positively affected by Electromagnetic field (Aladjadjiyan, 2002). On the other hand, root growth decrease in *Lens culinaris* seedlings with enhanced exposure of Magnetic field (Penuelas *et al*., 2004). Short term exposure of Electromagnetic field increase the germination and growth of wheat plants while when the intensity increased all growth parameters reduced (Faeghi and Syedepoure 2013). The effect of Electromagnetic field due to high tension wires was studied on the meiotic behaviour and pollen fertility and it was observed that the Electromagnetic field cause an increase in the meiotic abnormalities at each level of meiosis and an increase in the number of sterile pollen grains (Zaidi and Khatoon, 2003, 2012; Zaidi *et al.*, 2012, 2013a and b). A considerable change in morphology of leaves of *Aleo vera* was observed due to the exposure of Electromagnetic field (Sheikh *et al.*, 2013) and a decline in germination of *Urtica dioica* seeds was observed upon exposure to Electromagnetic field (Zedeh *et al*., 2014).

From the literature survey it is observed that scientists worked on different stresses individually which harm the crops in different manner but not a single study is available which deals with the combined effects of these two stresses. Therefore the present study is designed to study the possible effect of both theses stresses (heavy metal and Electromagnetic field) on mung bean.

MATERIALS AND METHODS

The seeds of mung bean were surface sterilized by soaking them in 0.5% $Ca(OCl)_2$ for 10-15minutes. The seeds were then rinsed several times with distilled water. The Petri plates and all the glass wares were also sterilized in autoclave to avoid any contamination. All the Petri plates containing double layer of Whatman filter paper (I). All concentrations were prepared in milimolar (mM) and milimolal (mm) units. The Petri plates were labelled as T_1 (Electromagnetic field), T_2 (250mM), T_3 (500mM), T_4 (750mM), T_5 (250mM + Electromagnetic field), T_6 (500mM + Electromagnetic field), T_7 (750mM + Electromagnetic field) and C(control). In the similar way pots were also prepared and labelled as their respective treatments. 500g of soil was placed in each pot with desirable amount of $PbCl_2$ in their respective pots. The experiments were in following ways:

i. Study the effect of Electromagnetic field

To study the effect of Electromagnetic field, the exposure of Electromagnetic field applied by a stand coiled with wires which created upto199.9 mG (milligauss) Electromagnetic field was prepared and the intensity of Electromagnetic field was measured for confirmation with the help of a gauss meter (Lutron Electromagnetic field 822-A Tester). The sterilized and imbibed seeds (10 seeds) were first exposed to Electromagnetic field for half an hour and then placed in sterilized Petri plates labelled as T_1. The Petri plates were moistened with 3ml of distilled water. On the other hand, 10 exposed seeds were sown in pots also and irrigated with distilled water. Whereas, three replicates were prepared in each case.

ii. Study the effect of lead chloride ($PbCl_2$)

For the study three different concentrations of lead *i.e* 250 milimolar (6.95 g of $PbCl_2$ in 100 mL of distilled water), 500milimolar (13.91 g in 100 mL) and 750milimolar (20.85 g in 100 mL) were prepared. The sterilized and imbibed seeds (10 seeds) were placed in each Petri plate labelled as T_2 (250 mM), T_3 (500 mM) and T_4 (750 mM). The Petri plates were moistened with their respective concentrations of heavy metals. Each treatment has three replicates. For pots experiment, Molal concentrations of lead prepared by adding 34.75g of lead chloride in soil for 250milimolal, 69.5g of lead chloride in soil, for 500milimolal and 104.25g of lead chloride in soil for 750milimolal in 500g of soil to make desired molal concentrations, then 10 seeds were sown in each treatment pot, whereas irrigated with tape water regularly.

iii. Study the combined effect of Electromagnetic field and lead chloride ($PbCl_2$)

To study the combined effect three treatments T_5(250mM and Electromagnetic field), T_6(500mM and Electromagnetic field) and T_7(750mM and Electromagnetic field) and control were established. The petri plates were prepared in the same way. They were moistened with their respective lead concentrations and then all the petri plates containing 10 seeds each exposed to Electromagnetic field (180mT) for half an hour. The control seeds moistened with distilled water. Replicates were made in each case. Similarly, pots treatments were prepared in the same ways as above mentioned. Each treated pot received 10 exposed seeds by Electromagnetic field for half an hour.

The petri plates were placed in undisturbed place having constant and moderate temperature. The treatments were then moistened with different concentrations and distilled water in alternate days and Electromagnetic field treated petri plates exposed for half an hour in alternate days. Similarly, pots were placed in undisturbed environment of greenhouse and irrigated with tape water regularly, and exposed by Electromagnetic field in alternate days.

The germination of seeds was recorded regularly up to 5^{th} day. The experiment in Petri plates was terminated at the 15^{th} day. After that, root length, shoot length, fresh weight and dry weight were noted down. Whereas in case of pots the experiment was terminated at the 30^{th} day. The leaf material was collected for the analysis of chlorophyll content. Chlorophyll estimation was done by following method. Leaves of each treated plants were properly cut into small pieces and weighed 0.25g and were taken for chlorophyll and carotenoid estimation. Chlorophyll a, chlorophyll b and total chlorophyll were estimated following Arnon's method (Arnon, 1949) and total carotenoid contents were estimated by Duxbury and Yentsch (1956). The absorbance of the solution was read at 645, 663 and 652 nm for Chlorophyll a, Chlorophyll b and total chlorophyll and absorbance read at 480 and 510nm for total carotenoid contents.

Calculation

Chlorophyll a (mg g^{-1}) = 12.7 (D_{663}) - 2.69 (D_{645}) × $V \div 1000 \times w$

Chlorophyll b (mg g^{-1}) = 22.9 (D_{645}) - 4.68 (D_{663}) × $V \div 1000 \times w$

Total chlorophyll (mg g^{-1}) = 20.2 (D_{645}) + 8.02 (D_{663}) × $V \div 1000 \times w$

Total carotenoids (mg g^{-1}) = 7.6 (D_{480}) – 1.49 (D_{510}) × $V \div 1000 \times w$

Where D = optical density, V = final volume of 80% acetone (ml) w = dry weight of sample taken (g)

The final germination percentage and speed of germination were calculated following Khandakar and Bradbear (1983).

S = [N1/1+N2/2+N3/3…………….Nn/n] ×100/1

Where S stand for speed of germination, N1, N2, N3… Nn stand for number of seeds germinated per day separately.

Whereas, the rate of germination calculated by

$$\text{Germination\%} = \frac{Total\ no.\ of\ seeds\ germinated\ in\ a\ particular\ treatment}{Total\ no.\ of\ seeds\ treated\ in\ a\ particular\ treatment} * 100$$

Mean and standard error of root shoot length were calculated with the help of computer data analysis program at M/S excel program. The data were further subjected to statistical analysis and post hoc test was performed by using COSTAT, DMRT at 5% confidence level (Gomez and Gomez, 1984).

Plant tolerance index (T.I %) was determined for total plant height using following formula.

$$T.I = \frac{Gt}{G^{\circ}} \times 100$$

Gt = Mean plant growth in metal enriched medium and G^0 = Mean plant growth in control treatment.

RESULTS

Collected data evaluated the result for the germination, growth and biomass and chlorophyll content of mung seedling against the individual and combined treatment of lead and Electromagnetic field. The results of germination percentage, speed of germination, root length, shoot length, fresh weight, dry weight and tolerance index were shown in Table 1, whereas their chlorophyll contents shown in Table 2.

i. Effect of exposure of Electromagnetic field

The results are summarized in Table 1. Rate of germination was not affected due to the exposure of Electromagnetic field. But the speed of germination was affected positively as compared to control. The Electromagnetic field also significantly decline shoot growth and fresh weight of seedlings as compared to control at the level of $p < 0.05$ (Table 3).

Tolerance index of mung seedling against the exposure of induced Electromagnetic field is 60% in root, whereas the shoots extensively tolerated the Electromagnetic field exposure. Chlorophyll contents also increase as compared to control that's show non-significant results while carotenoid content decrease (Table 2).

ii. Effect of different concentrations of $PbCl_2$

The results are presented in Table 1. Germination speed of mung seeds were affected more by lead concentration as compared to control and Electromagnetic field. The speed and rate of germination decline with increased of concentration. Similarly root shoot and fresh weight also significantly decreased at most 5% ($p \leq 0.05$) with the increase of concentration (Table 3).

Tolerance index of mung seeds against lead concentration is 60-19% in root and 100-50% in shoot. On the other hand total chlorophyll content increase when concentration increase but the carotenoid content decreased as compared to control (Table 2).

Table 1. The effect of Electromagnetic field and lead concentrations on the speed, percentage germination, seedling growth and biomass and tolerance index % of mung seedling.

Treatment	Speed of Germination	Germination %	Root Length (cm)	Tolerance Index %	Shoot length (cm)	Tolerance Index %	Fresh weight (g)	Dry weight (g)
Control	98.9a ± 0.01	100a ± 0.00	2.53a ± 0.50	100a	10.01ab ± 0.04	100ab	0.22b ± 0.008	0.038ab ± 0.004
E.M field	100a ± .00	100a ± 0.00	2.52a ± 0.64	100a	11.38a ± 0.53	105.6a	0.242a ± 0.03	0.025b ± 0.007
Pb 250mM	100a ±.00	98.8b ± .002	1.52a ± 0.27	60c	10.05a ± 1.08	100a	0.226b ± 0.01	0.040a ± 0.020
Pb 500mM	92.56b ± .002	92.05c ± 0.01	1.49a ± 0.23	58.8d	8.5b ± 1.08	89.2c	0.21b ± 0.02	0.023b ± 0.003
Pb 750mM	91b ±.01	79.14d ± .002	0.5a ± 0.04	19.7e	4.58e ± 0.24	50.8d	0.216b ± 0.02	0.011c ± 0.002
Pb 250mm+ E.M field	100a ±.00	100a ± 1.00	1.41b ± 0.10	55.7d	4.51e ± 0.68	47.4e	0.212b ± 0.06	0.032d ± 0.005
Pb 500mm+ E.M field	80c ±.01	73.69d ± .21	1.36c ± 0.28	53.7d	5.52d ± 0.79	58.0d	0.13c ± 0.33	0.018d ± 0.01
Pb 750mm+ E.M field	69d ±.003	50e ±.13	0.79d ± 0.58	51.2d	3.37e ± 0.24	35.4e	.066d ± 0.005	0.006e ± 0.050

Each mean have three replicates. Mean ± SD have three replicates. Means not according by the same letter are significantly different from each other at $p \leq 0.05$.

Table 2. Effect of Electromagnetic field and lead concentration on Chlorophyll contents of mung seedling.

Mung Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids
Control	0.64a ± 0.1	1.341b ± 0.09	1.99b ± 0.01	0.60a ± 0.04
E.M field	0.17f ± 0.02	2.17a ± 0.03	2.34a ± 0.04	0.53b ± 0.03
Pb 250mm	0.47d ± 0.03	0.63d ± 0.04	1.1b ± 0.09	0.63 ± 0.02
Pb 500mm	0.60b ± 0.03	0.68d ± 0.01	1.28ab ± 0.07	0.55b ± 0.03
Pb 750mm	0.30e ± 0.09	0.83c ± 0.02	1.13ab ± 0.01	0.56b ± 0.01
Pb 250mm+E.M field	0.51c ± 0.01	0.785bc ± 0.02	1.29ab ± 0.01	0.64a ± 0.03
Pb 500mm+E.M field	0.50c ± 0.07	0.084e ± 0.001	0.64d ± 0.04	0.052d ± 0.001
Pb 750mm+E.M field	0.34e ± 0.1	0.295e ± 0.01	0.64d ± 0.01	0.03e ± 0.001

Each mean have three replicates. Means not according by the same letter are significantly different from each other at $p \leq 0.05$.

iii. Combined effect of Electromagnetic field and different concentrations of $PbCl_2$

The results are summarized in Table 1. Germination speed and rate of germination decline as concentration increase in combination of Electromagnetic field. While at lower concentration of lead with Electromagnetic field the root shoot growth and biomass increase but due to the higher concentration of lead with Electromagnetic field exposure caused highly significant decrease in shoot growth, fresh weight and carotenoid content (Table 3).

Tolerance index of seedling of mung against the combined treatment were 55-50% in roots and 47-35 % in shoot this index is lower than individual tolerance index of Electromagnetic field treatment and higher than individual index of lead treatment. On the other hand highly significant (Table 3) decrease observed in total chlorophyll contents and carotenoid content of mung bean plants in combined treatment of Electromagnetic field and lead (Table 2).

Table 3. ANOVA on the effect of Electromagnetic field and lead on growth parameters of mung bean seedling.

Dependent Variable: ROOT LENGHT					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	34.213[a]	11	3.110	5.141	0.000
Intercept	79.078	1	79.078	130.701	0.000
CONTROL	.667	2	.334	.552	0.583
EMF	6.055	3	2.018	3.336	0.036
lead	23.299	3	7.766	12.836	0.000
EMF * lead	4.895	3	1.632	2.697	0.068
Error	14.521	24	.605		
Dependent Variable: SHOOT LENGHT					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	357.615[a]	11	32.510	32.772	0.000
Intercept	1460.898	1	1460.898	1472.667	0.000
CONTROL	2.788	2	1.394	1.405	0.265
EMF	50.761	3	16.920	17.056	0.000
lead	268.008	3	89.336	90.056	0.000
EMF * lead	90.928	3	30.309	30.554	0.000
Error	23.808	24	.992		
Dependent Variable: FRESH WEIGHT					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.814[a]	11	.074	16.199	0.000
Intercept	1.925	1	1.925	421.067	0.000
CONTROL	.011	2	.005	1.169	0.328
EMF	.156	3	.052	11.413	0.000
lead	.186	3	.062	13.541	0.000
EMF * lead	.263	3	.088	19.196	0.000
Error	.110	24	.005		
Dependent Variable: DRY WEIGHT					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.012[a]	11	.001	2.934	0.013
Intercept	.026	1	.026	72.835	0.000
CONTROL	.002	2	.001	2.968	0.071
EMF	.006	3	.002	5.282	0.006
lead	.003	3	.001	2.335	0.099
EMF * lead	.001	3	.000	.819	0.496
Error	.009	24	.000		
Dependent Variable: TOTAL CHLOROPHYLL CONTENT					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.514[a]	11	.410	13.941	0.000
Intercept	13.042	1	13.042	443.073	0.000
CONTROL	.164	2	.082	2.793	0.081
EMF	.271	3	.090	3.073	0.047
lead	3.098	3	1.033	35.084	0.000
EMF * lead	.065	3	.022	.733	0.542
Error	.706	24	.029		

Cont'd….Table 3

Dependent Variable: CARETINOID CONTENT					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.663[a]	11	.151	3.324	0.007
Intercept	5.999	1	5.999	131.859	0.000
CONTROL	.245	2	.122	2.689	0.088
EMF	.464	3	.155	3.401	0.034
lead	.397	3	.132	2.907	0.055
EMF * lead	.346	3	.115	2.537	0.081
Error	1.092	24	.045		

DISCUSSION

The present work deals with the study of the effect of lead and Electromagnetic field on the germination and growth in mung bean seeds and seedlings. We observed individual and combined effect of both these two stresses. Individually the germination rate, root shoot growth, biomass and chlorophyll content with gradual increase of concentration of lead. Our results are contrasting with previous finding regarding the effect of lead on germination and growth of different plants, like lead inhibit the root shoot growth of *Thespesia populnea* (Kabir*et al*., 2010), lead inhibit the germination, growth and chlorophyll content in Wheat (*Triticum aestivum* L.) (Hasnain *et al*., 1998 and Bhati *et al*., 2013), in barley (Stobert *et al*., 1985) and in mung bean (*Phaseolus vulgaris*) (Somashekaraiah *et al*., 1992). Lead also decrease the growth of *Arachis hypogea* L. (Abraham *et al*., 2013), different concentration of lead decrease the germination rate, growth of root and shoot and biomass of seedling of *Albizia lebbeck* (Farooqi *et al*., 2009), in spinach (Khan *et al*.,2013), in *Cucumis sativus* (An *et al*., 2004). A significant decrease occurred in germination rate and growth parameters including rate of photosynthesis and enzyme activity due to high concentration treatment of lead in *Sinapis arvensis* (wild mustard) (Heidari and Sarani 2011).

On the other hand, individual effect of Electromagnetic field also studied on the germination, growth and chlorophyll contents in seed and seedlings of mung bean. Exposure of Electromagnetic field increase the germination rate but decrease root, shoot length and chlorophyll contents of seedling of mung bean plants. Our result also corroborate with the findings of earlier researchers like a significant decrease of shoot length, biomass and total chlorophyll contents in *Satureja bachtiarica* (Ramezani *et al*., 2012) and in wheat (Faeghi and Sayedapour 2013). Rate of germination and speed of germination triggered fast due to the Electromagnetic field exposure in *Helianthus annuus*, but the root shoot growth decrease when exposure o Electromagnetic field expanded for the long interval of time (Vashisth and Nagarajan 2010).

In this study, the effect of combined treatment of lead and Electromagnetic field on the seeds and seedlings of mung bean plants indicated a highly significant decrease in germination, growth of root, shoot, biomass and chlorophyll contents including carotenoids (Table 4) whereas, germination percentage decrease up to 50% which indicate that combined treatment of these two stresses affect more adversely to the seeds and seedlings of mung bean plants as compared to their individual effect..

Therefore, it is concluded that, interaction between the treatments and growth parameters are highly significant with each other (Table 4). Individually, rate of germination increase due to exposure of Electromagnetic field and decrease with increase of lead concentrations. Hence the germination rate decline in combined treatment, growth and chlorophyll contents more pronounced decrease in combined treatment as compared to individual treatment.

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