The Pharma Innovation Journal 2018; 7(7): 465-469

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(7): 465-469

www.thepharmajournal.com
Received: 21-05-2018
Accepted: 24-06-2018

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Mophological analysis of black gram (*Vigna mungo* (L.) with zinc stress

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Abstract
Pulses occupy an area of 68.32 million ha and contribute 57.51 million tonnes to the world's food basket. Out of this, India shares about 35.2 per cent area and 27.65 percent of the global production. The percent contribution of pulses to the total food grain production in India has declined during the last three decades (Anon., 2011). Among the various pulses, black gram or urd bean (*Vigna mungo* [L.] Hepper) is an important grain legume with easily digestible protein and low flatulence contents. It is highly prized pulse, rich in phosphoric acid. Black gram grain contains about 25% protein, 56% carbohydrate, 2% fat, 4% minerals and 0.4%vitamins. Metal pollution is a multi elemental problem, in many cases, it is more appropriate to study the combined effect of sub toxic & toxic concentration of heavy metals. Zinc metal (Zn) is essential mineral element for plant growth but at high concentration these are highly toxic to plants. . Zinc sulfate (25-36% Zinc) is most commonly used source of zinc as plant micro nutrient. Several sources can supply Zn when needed but Zinc sulfate is usually used to supply the needed amount of Zn when dry fertilizer materials are used (in Zn deficiency in soil). Therefore, Zinc sulfate hepta hydrate (ZnSO4.7H2O) used in this study for Zn stress condition. The present work is aimed to study the effects of the heavy metal Zn on the growth of a widely cultivated crop *Vigna mungo* (L.) (OBG-31) Mahuri.

Subsequent dilutions were made using distilled water & stock solution of 0, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800, 900, 1000 mg/L. Sterilized cotton & blotting papers were spread on the petriplates where test chemicals were taken & surface sterilized 10 healthy seeds of *Vigna mungo* (L.) were taken for study of germination. Growth of the seedling was measured by taking the root & shoot on the 7th of day of germination. Lethal concentration (LC_{50}) was calculated by considering shoot emergence. Ratio of Root & Shoot lengths & their percent changes were in decreasing trend corresponding to increase in concentration of the solution. The correlation of the roots to the concentrations of the solution was significant at $P \leq 0.05$ level. The ration of root & shoot length increases with increase in concentrations of solution. The fresh weight and dry weight of shoot & root decreases with increase in concentrations of solution . Our result suggested that the presence of Zinc at higher concentration resulted in growth inhibition, structural damage of *Vigna mungo* (L.) Plant.

Keywords: *Vigna mungo* (L.) (OBG-31) Mahuri, Zinc sulfate hepta hydrate (ZnSO4.7H2O) himedia

Introduction
Pulses are an integral part of Indian agriculture. Quality protein content in pulses is nearly three times as much as cereals, so they are cheaper source to overcome protein malnutrition among human beings. Urd bean (*Vigna mungo* (L.)Hepper) is one of the most popular pulses in South East Asia and a substantial source of dietary protein in India . Heavy metals are of great interest for research purpose with respect toxicological importance to human health, plant and animals (Azevedo & Lea, 2005; Jarup 2003; Almeida *et al*. 2007) [3, 8, 2]. Due to rapid industrialization urbanization & intensive agriculture increasing contamination of heavy metals in soil has a major concern. Excessive level of heavy metals in the soil environment adversely affect the germination of seeds, plant growth, alter the level of bio molecules in the cells & interfere with the activities of many key enzymes related to normal metabolic & developmental processes.

Zinc is one of the essential micro-mineral nutrient (at low concentration) for normal growth & development of all organisms & functioning as a cofactor to perform various physiological processes, including electron in photosynthesis, mitochondrial respiration, superoxide scavenging, lignifications of cell walls & ethylene sensing. Several sources can supply Zn when needed but Zinc sulfate is usually used to supply the needed amount of Zn when dry fertilizer materials are used (in Zn deficiency in soil).

Therefore, Zinc sulfate hepta hydrate (ZnSO4.7H2O) used in this study for Zn stress condition. Zn sulfate comes in the form of colourless, rhombic crystals. It is efflorescent in dry air. Among the heavy metals Zn is an essential micronutrient that affects several metabolic processes of plants (Rout & Das, 2003) [10] & has a long biological half-life. Because of long biological half-life they tend to accumulate in the living systems & get magnified through the food chain (Bioaccumulation). Zn at appropriate concentrations is required for structural & catalytic components of protein & enzymes as cofactors, essential for normal growth & development of plants & a vast number of protein sequences containing Zn-binding structural domains (Steffens 1990). However, excessive accumulation of the micronutrient in plants operate a stress factor causing physiological constraints leading to decreased seed vigor & plant growth (Van Assche & Clijsters 1986,1990, Ali *et al*. 2000). Plants absorb metal toxicants like Zinc from soil & water through the root & may be impacted on to leaf surface but rarely enters leaves directly unless dissolved.

The present work is aimed to study the effects of the heavy metal Zn on the growth of a widely cultivated crop *Vigna mungo* (L.) (OBG-31) Mahuri.

Materials & Methods

The seeds of *Vigna mungo* (L.) Hepper OBG-31(Mahuri) (Black gram) was selected as the test material for this study. The seeds of *Vigna mungo* (L.) was collected from Centre for Pulse Research (CPR) Ratanpur, Berhampur. Healthy seeds of uniform sizes were sorted.

Test Chemicals

Zinc sulfate **(**ZnSO4. 7H2O), was used in the work as reagent from HIMEDIA. First stock solution of 1000 mg/L was prepared in the following proportion, i.e. 1gm of $ZnSO_4$ in 1 lit. Distilled water. Subsequent dilutions were made using distilled water & stock solution of 0, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800, 900, 1000 mg/L. Fresh test solutions were prepared each time experiments were performed.

Germination studies

Sterilized cotton & blotting papers were spread on the petriplates where test chemicals were taken & surface sterilized 10 healthy seeds of *Vigna mungo* (L.) were taken for study of germination. These petriplates were properly labeled. Sterile conditions were maintained. All experiments were carried out for 7 days at 28±2 ˚C in alternate light & dark period of 8 hours & 16 hours respectively. Two replicas of each concentration were taken for experimentation. Distilled water was taken as control. Utmost care should taken to avoid drying & over flooding. Plants were grown in a culture room. The observations were recorded for germination studies.

Morphological Studies

Growth of the seedling was measured by taking the root & shoot on the 7th day germination. Lethal concentration (LC_{50}) was calculated by considering shoot emergence of Urd bean seeds in test solutions 100, 150, 200, 250, 300, 350, 400, 500, 600,700, 800, 900 & 1000 mg/L. Observations were presented in table no. 1. LC_{50} values were calculated from data using HPSS regression analysis.

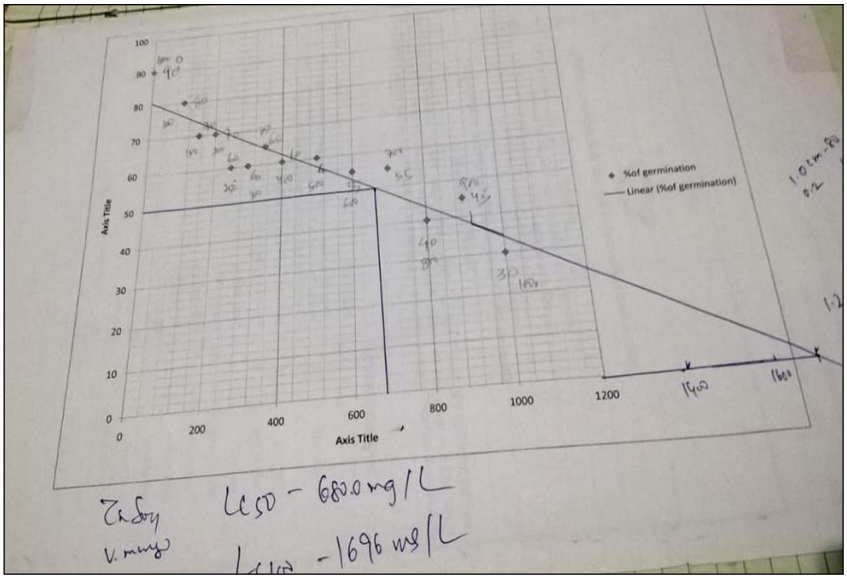
Shoot length & Root length

From each replicate 10 seedlings were selected randomly & length of root and shoot were measured with the help of a scale.

Shoot weight & Root weight

Ten seedling of each replicate were taken. The shoots were separated from the roots.these were washed thoroughly, surface dried by means of blotting paper. Then fresh weight of roots & shoots were taken separately by a single pan in a electronic balance. The weighed materials were kept in an oven for 24 hours at a temperature of 80˚C & their dry weight recorded.

Results



Determination of LC_{50}

Determination of LC_{50}:-The lethal concentration (LC_{50}) had been extensively used to evaluate the solubility of the potential toxic chemicals for laboratory studies with various seeds of cereals, pulses, etc . LC_{50} was determined taking the percentage of shoot emergence. Percent of shoot emergence

was recorded from two replicates of 13 different concentrations of $ZnSO_4$ and distilled water as control observation were recorded. The results obtained from the germination study were given in Table-1 & Fig-1. The XLSTAT 2016 analysis for regression analysis is given in fig.-3. The correlation between the concentrations was found out to be significant at $P \leq 0.05$ level. The correlation and the analysis between germination parameters study showed R value to be -0.983 ($p > 0.05$)

Based on LC50 Value, five different concentrations of $ZnSO_4$ i.e. 200 mg/L, 400 mg/L, 600 mg/L, 800 mg/L, 1000 mg/L were selected for the study of morphological & biochemical parameters. The percentage of germination of seeds and shoot emergence at these five different parameters were shown in Table-1 & Fig-1.. The percentage of germination and shoot emergence was decreased by 10% on exposure of 200 mg/L $ZnSO_4$, there was further significant decrease of about 80% when the concentration of $ZnSO_4$ became 1000 mg/L. The correlation between Zinc treatment & seed germination was found to be negative.

Root & shoot lengths were measured after 7-days of exposure to the test chemical ($ZnSO_4$). Data with respect to root & shoot lengths were presented in Table-2 & Fig-2. to Data clearly indicated a sharp decline in the root & shoot length of seedlings. Ratio of Root & Shoot lengths & their percent changes were given in Table-2 & Fig-2. Decreasing trend was seen in the root & shoot length corresponding to increase in concentration of the $ZnSO_4$. The statistical analysis between concentration & root length of the seedlings showed negative & significant correlation. . The ration of root & shoot length increases constantly from 3.82 control to 5.00 in exposed seedling of 1000 mg/L $ZnSO_4$.The fresh weight and dry weight of shoot & root were measured and their percentage were calculated which decreases from control to the higher concentration of $ZnSO_4$ treated seedlings were given in Table-3 & Fig-3.

There were visible morphological changes seen in the roots which appear different from that of the control. Roots appeared to be swollen in higher concentrations of $ZnSO_4$ and developments of lateral roots were less in comparision to the control. There was marked difference in the development of the lateral branches to the tap roots. The colour of the roots appeared to be blackish/brownish red with swelling / clumping of roots.

Table 1: Effect of different concentrations of $ZnSO_4$ (mg/L) on the% of seed germination and shoot emergence of V*igna mungo* L. noted after 24hrs.of sowing. (Mean value of 10 seeds)

Conc of zinc (mg/L)	Percentage of seed Germination	% of Shoot emergence
0	100	100
100	90	90
150	85	80
200	80	70
250	75	70
300	70	60
350	60	50
400	70	50
500	65	40
600	50	30
700	45	20
800	40	10
900	30	0
1000	20	0

Table 2: Effect of different concentrations of $ZnSO_4$ (mg/L) on length of shoot and root *Vigna mungo L.* seedling of 7-days oldwith R/S ratio value & percentage change with control

Concentration of ZnSo4 mg/L	Shoot Length in cm	Root Length in cm.	R/s ratio
0	14.00 (100)	3.66 (100)	3.82 (100)
200	10.66 (76.14)	0.66 (18.03)	16.15 (422.24)
400	7.00 (50.00)	0.13 (3.55)	53.84 (1407.73)
600	3.00 (21.42)	0.10 (0.27)	30.00 (784.31)
800	1.66 (11.85)	0.10 (0.27)	16.60 (433.98)
1000	0.50 (3.57)	0.1 (0.27)	5.00 (130.71)

Table 3: Effect of different concentrations of $ZnSO_4$ (mg/L) on the weight of shoot and root of*Vigna mungo* L. seedling of 7-days old & percentage change with the control

Conc of $ZnSo_4$ mg/L	Fresh Wt. of shoot in mg	Dry wt. of shoot in mg	Fresh Wt of root in mg	Dry Wt of root in mg
0	0.210 (100)	0.036 (100)	2.00 (100)	0.13 (100)
200	0.080 (36.986)	0.017 (47.222)	1.57 (78.70)	0.10 (76.33)
400	0.062 (28.311)	0.022 (61.111)	1.68 (84.09)	0.12 (92.36)
600	0.038 (17.352)	0.009 (25.000)	1.23 (61.54)	0.10 (77.86)
800	0.076 (34.703)	0.007 (19.444)	0.18 (9.02)	0.07 (58.77)
1000	0.007 (3.196)	0.002 (56.556)	0.11 (5.93)	0.03 (27.48)

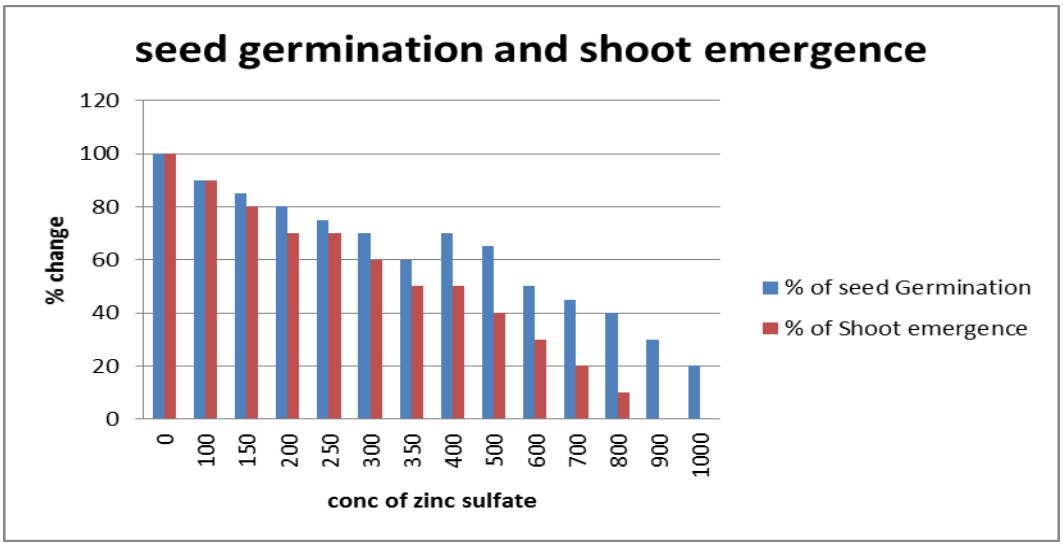


Fig 1: Histograms showing Percentage of change in seed germination and shoot emergence of *Vigna mungo* L. Seeds treated with different concentrations of $ZnSO_4$ (mg/L) 7 days old seedling.

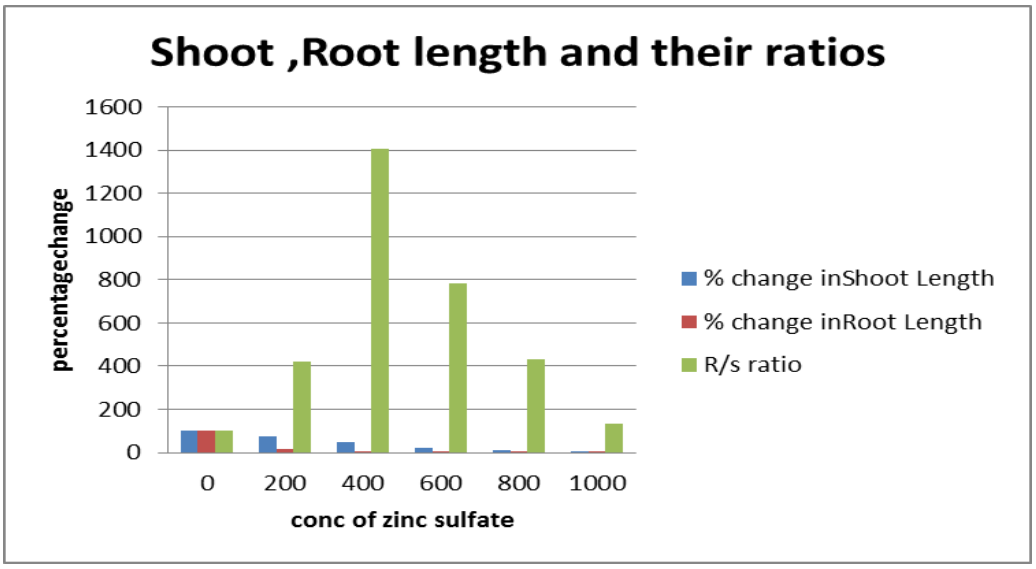


Fig 2: Histograms showing Percentage of change in shoot, root length and their ratios in *Vigna mungo* L. Seeds treated with different concentrations of $ZnSO_4$ (mg/L) Of 7 days old seedling.

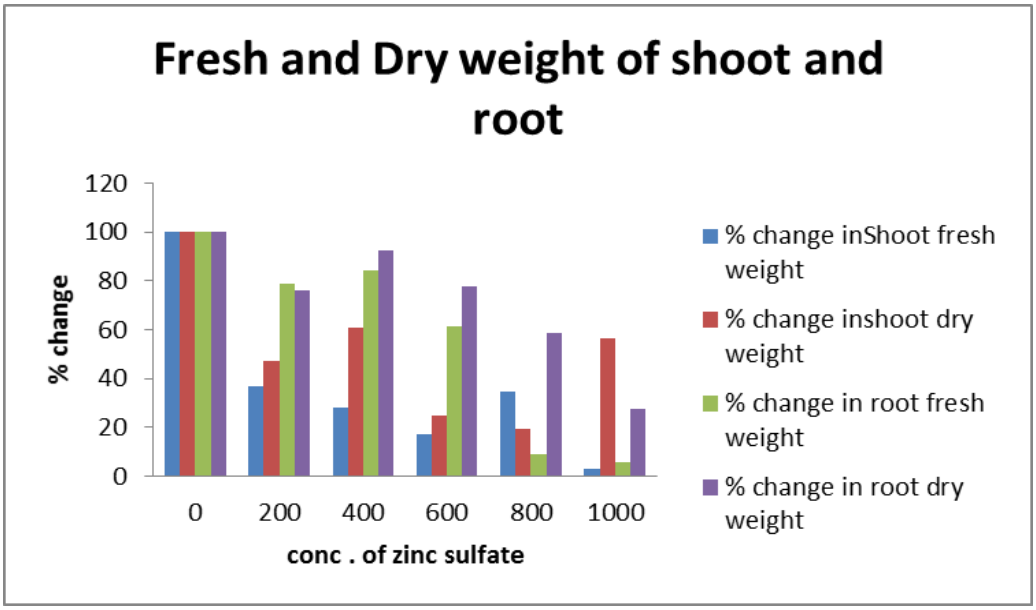


Fig 3: Histograms showing Percentage of change in fresh and dry weight of shoot and root of *Vigna mungo* L. Seeds treated with different concentrations of $ZnSO_4$ (mg/L) Of 7 days old seedling.

Discussion

The phytotoxicity of zinc reduced the growth and development rate of metabolism in various plant species such as *phaseolus vulgaris* (Cakmak and Marshner, 1993), *Brassica Juncea* (Pprasad and Hagmeyer, 1999) and tobacco (Tklec *et al.*, 2014). There might be increase or decrease in macromolecules of plant tissues exposed to toxicants (Malla and Mohaty, 2005 [9]. Zinc induced inhibition in both root and shoot growth. But Dubey and Dwivedi (1987) [6] reported that zinc stimulated radicle growth in soyabean. On the contrary, Shukla and Pandey (1993) [11] reported that a decrease in the root and shoot length of soyabean in response to zinc. Abbasi *et al.*, (1993) reported that considerably higher concentration of zinc causes inhibition in the growth in*Cicer aritinum.* Zinc toxicity in plants limited the growth of both root and shoot (Choi *et al.*, 1996; Ebbs & Kochian, 1997; Fontes and Cox, 1998) [7]. Shukla and Pandey (1993) [11] reported a decrease in fresh weight and dry weight in three varieties of soyabean treated with zinc. Growth inhibition was also reported in zinc treated gram seedlings. Taking into consideration the different growth parameters, it may be concluded that relatively higher toxic effects of zinc on the root systems may be because o its accumulation in the organ. Zinc being an essential element is expected to follow the typical diagnostic curve suggested by Berry and Wallace (1989) [4]. In *Phaseolus radiatus,* trends similar to the typical curve have been reported (Tripathy, 1993) [13].

Summary & Conclusion

Grain legumes occupy unique position in Indian agriculture. Besides forming a sustainable component of Indian agriculture, they are a major source of vegetable protein to the larger mass of the country that are basically vegetarian in their food habit.

In the present study, there is sharp decline in the seedling growth, was observed. There was a linear relationship between the percent of germination and concentration of the zinc i.e. increase in the concentration resulted decrease in the germination percentage. Higher dose of zinc inhibits the development of root and shoot than the normal dose required by plant which caused a decline in fresh and dry weight of both root and shoot.

It shows that if ZnSo4 concentration becomes more in the soil than normal or if it is accumulated plentily, then germination, shoot emergence and leaf development gradually decreases and production becomes very less. It fails to fulfill the human requirements and the economic prosperity of the farmers meet the tragic end.

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