

Phytotoxic effect of zinc on seed germination and seedling growth of rice plants

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SUMMARY : Experiments were conducted in plastic Petridishes containing well mixed cow dung manure and garden soil in laboratory conditions in order to find out the phytotoxic effect of zinc on seed germination and seedling growth of three cultivars of rice (Gayatri, Ratna and Heera). The test heavy metal enhanced the seed germination and seedling growth at lower concentrations whereas, higher concentrations caused retarding effects. The optimum concentrations for maximum percentage of seed germination were found in 0.01 mg/l followed by 0.1 mg/l and 1.0 mg/l. More or less similar results were also noticed in seedling growth of all test cultivars of rice tested. Lower concentration of zinc 0.01 and 0.1 mg/l exhibited stimulatory effect on seed germination and seedling growth. Concentration of zinc > 1.0 mg/l acted as phytotoxic to the parameters studied. Among the rice cultivars, Ratna was found more resistant to zinc followed by Heera and Gayatri.

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Zinc being a non-biodegradable heavy metal, persists in the environment and accumulates in different parts of living and non-living matter and has been created a significant ecological disbalance. The accumulation of zinc components which do not constitute a part of any biogeochemical cycle is obviously harmful. Total value of zinc in soil depends on chemical composition of raw materials in the soil. Zinc value in soil is usually 10 to 300 mg/kg with an average of 50 mg/kg (Mousavi, 2011). Zinc is in different forms in the soil as water soluble, exchange, connected to organic matter and stabilized by the secondary clay minerals (Alloway, 2008). Zinc is one of the essential micronutrients playing a significant role in many vital metabolic processes (Rout and Das, 2003). Zinc toxicity has been reported in several plants when excessive levels of zinc was added (Brawn and Rasmussen, 1971). Zinc is known to play a significant role in chlorophyll synthesis, protein synthesis, carbohydrate metabolism and activation of oxidation process and enzymes (Singh, 1969). Deficiency of zinc has been

established as a major cause of poor yields or crop failures in various parts of India (Fakkar and Randhawa, 1978). Zn could also increase the biosynthesis of chlorophyll and carotenoids ultimately providing beneficial for the photosynthetic machinery of the plant system (Aravind and Prasad, 2004). Zinc is a must for plant development, its above critical limit and deficiency is a major global problem hindering plant cultivation. Basing on the above facts, the present investigation deals with the identification of tolerance concentration of zinc for rice cultivation, find out resistant variety and rationalize the Zn phytotoxicity.

EXPERIMENTAL METHODOLOGY

Test chemicals:

ZnSO₄·7H₂O was chosen as test chemical. Stock solution of the test chemical (1g/l) concentration was prepared by dissolving 4.397g of ZnSO₄·7H₂O in 100 ml of distilled water. From that stock solution, concentration of 0.01, 0.1, 1.0, 10, 20 and 30 mg/l were prepared by proportional

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dilution with distilled water which were used for various treatments.

Test cultivars:

Three different cultivars (Gayatri, Ratna and Heera) of rice (*Oryza sativa* L.) were chosen as experimental plant materials for present investigation. Pure line seeds of above mentioned 3 varieties of rice were procured from the Central Rice Research Institute (CRRI), Cuttack. Selected seeds of uniform size and colour of each variety were surface sterilized with 0.03 per cent (v/v) formalin solution for 30 min. and then washed thoroughly with tap water for use in experiments.

Seed germination and seedling growth:

In order to study the percentage of germination influenced by different concentrations of heavy metals, the surface sterilised seeds were allowed to germinate in plastic Petridishes containing well mixed cow dung manure and garden soil mixed in a proportion 2:8 (v/v). The Petridishes were divided into twenty one sets (each set with 5 replicate) for individual concentration of test chemicals and control. Before sowing the seeds, equal volume of different concentrations of respective test chemicals were supplied to all the Petridishes and distilled water to control sets. The soil and manure mixture of each Petridish were well padded and allowed to settle before sowing of the seeds @20 seeds per Petridish.

All the Petridishes of both treated and control sets containing seeds were kept in a B.O.D. incubator maintained at $30\pm 2^{\circ}\text{C}$. Appearance of sprouts (coleoptile) from the seeds were considered as the criteria of germination. The per cent of seed germination was calculated at the interval of 24 hours after 2 days of sowing. Then, all the Petridishes of control and treated sets containing germinated seeds were transferred into the seedling growth chamber maintained at $30\pm 2^{\circ}\text{C}$ and provided with continuous illumination from two fluorescent tube lights supplying $2\pm 0.5\text{k}$ lux intensity of light. The seedlings were provided with equal amount of respective test chemicals and distilled water as per the experimental design at an interval of 24 hours. This procedure was continued upto 15 days. The seedlings were collected at the age of 15 days at random and studied for different parameters viz., seedling length, fresh weight and dry weight. 15 days old seedlings collected @5 seedlings from each Petridish. Petridish were washed separately with tap water and surface dried by soaking with blotting papers. Shoot and root lengths of seedlings of both control and treated sets were measured and recorded. The sampling seedlings after measurement of shoot and root length were separated into shoot and root portion. Then fresh weight of shoot and root were measured separately with an electrically operated balance, then the weighed shoots and roots were kept in an oven maintained at $40\pm 2^{\circ}\text{C}$ for 48 hours and there after their dry weights were recorded.

EXPERIMENTAL FINDINGS AND DISCUSSION

The experimental findings of the present study have been presented in the following sub heads:

Seed germination:

All the concentrations of Zn except 0.01 mg/l considerably checked the rate of seed germination in all the test cultivars of rice. 100 per cent germination was marked at 4 DAS in seeds influenced by 0.01 mg/L concentration of zinc in all test cultivars while 97.2 per cent, 98.6 per cent and 94.8 per cent germination were noticed in seeds of Gayatri, Ratna and Heera, respectively in control sets. The least germination of seeds 4 DAS were 85.6 per cent, 88.1 per cent and 81.5 per cent in Gayatri, Ratna and Heera, respectively influenced by 30 mg/l concentration of zinc. There was a positive correlation between percentage of seed germination and metal concentration at 0.01 mg/l. Further increase of metal concentration showed a negative correlation. Increase of incubation period marked a positive correlation with seed

Table 1: Effect of different concentrations of Zn on seed germination of Gayatri, Ratna and Heera cultivars of rice

Concentration. of Zn (mg/l)	Per cent germination at different days after sowing			
	2	3	4	5
Gayatri				
Control	76.8±0.38	91.0±0.46	97.2±0.18	100.0
0.01	80.4±0.26	93.3±0.14	100.0	100.0
0.1	75.3±0.13	90.2±0.38	98.0±0.17	100.0
1	70.2±0.26	86.6±0.19	94.3±0.36	98.3±0.23
10	68.6±0.21	78.8±0.23	86.4±0.23	96.4±0.21
20	59.4±0.11	68.7±0.24	76.6±0.24	90.5±0.24
30	52.3±0.15	65.3±0.25	72.5±0.13	85.6±0.23
Ratna				
Control	76.3±0.18	93.3±0.21	98.6±0.18	100.0
0.01	80.4±0.26	96.4±0.26	100.0	100.0
0.1	75.5±0.21	92.6±0.29	96.4±0.18	100.0
1	72.6±0.38	88.7±0.31	98.6±0.26	100.0
10	70.3±0.34	82.8±0.18	92.5±0.25	96.2±0.22
20	65.4±0.41	72.3±0.26	79.6±0.23	92.3±0.38
30	60.3±0.32	68.3±0.26	76.5±0.36	88.1±0.26
Heera				
Control	74.3±0.31	88.8±0.28	94.8±0.18	100.0
0.01	80.4±0.43	92.4±0.26	100.0	100.0
0.1	73.3±0.33	90.3±0.26	96.3±0.43	100.0
1	70.4±0.41	83.4±0.23	88.4±0.46	100.0
10	68.3±0.28	81.1±0.27	86.4±0.38	92.3±0.32
30	59.5±0.29	62.8±0.18	66.7±0.16	81.5±0.43

Each value is mean of 5 replicates \pm SE

germination throughout the period of observation in all the three test cultivars of rice (Table 1). Present findings corroborate with report of several workers in the other plants and metals Sharma and Agrawal (2008) in mustard, Peraita *et al.* (2001) in alfalfa, Ganeson and Manoharan (1983) in *Abelmoschus esculentus*, Adhikary *et al.* (2011) in rice plant, Gupta (1991) in bean and mustard, Mishra and Choudhury (1997) in rice cultivars and Padhi (1990) in rice. In the present investigation, it was marked that all the concentrations of zinc altered the process of germination. Lower concentration (0.01 mg/l) of zinc enhanced the per cent germination but concentration >1.0 mg/l inhibited the germination. It was reported that osmotic potential in seeds helps in making all the preparatory for germination (Saxena, 1979). Further, Singh and Saxena (1991) have reported that osmotic potential of seeds regulates the activation of hydrolytic enzymes (amylase and protease) during seed germination which controls the rate of germination. In the present investigation, the osmotic potential in seeds of 3 test cultivars of rice might have altered by different concentrations of zinc resulting variation in percentage of germination. Higher concentration of Zn (>1.0

mg/l) might have checked or indirectly inhibited the synthesis of plant hormones which usually induce or activate the synthesis of hydrolysing enzymes responsible for better germination.

During the process of germination, the reserved food materials present in the endosperm for the development of embryo are being converted to simpler forms by the action of various enzymes. Formation of L-amylase from aleuron layer in endosperm and secretion of hormones are stimulated or inhibited by the application of different concentrations of Zn as a result of which the reserved starch or formation of new complex of starch derivatives in seeds during germination are greatly affected. Seeds treated with solution of different concentrations of Zn showed that at higher concentration (above critical limit), the amylase activity might be unable to solubilize the starch content of endosperm and hence the sugars derived from the hydrolysis of endosperm starch might have not consumed by the embryo that is why embryo deprived of food could not developed. Inhibition of starch breaks down caused by zinc rather than due to increase synthesis or accumulation of new compounds in them. The highest content

Table 2: Effect of different concentrations of Zn on seedling growth of Gayatri, Ratna and Heera cultivars of rice on 15 days after sowing

Concentration of Zn (mg/l)	Seedling length (cm)		Fresh weight (mg)		Dry weight (mg)	
	Shoot	Root	Shoot	Root	Shoot	Root
Gayatri						
Control	21.4±0.023	9.8±0.026	148.2±0.038	69.4±0.023	36.2±0.051	19.8±0.036
0.01	24.2±0.021	10.9±0.007	162.3±0.034	83.3±0.024	43.3±0.061	23.4±0.032
0.1	23.6±0.026	10.7±0.029	158.0±0.039	76.2±0.025	42.6±0.042	22.6±0.028
1	22.1±0.031	10.1±0.033	152.3±0.023	73.1±0.057	41.4±0.032	20.1±0.029
10	20.2±0.033	9.6±0.036	143.0±0.041	68.6±0.041	38.3±0.031	19.9±0.030
20	18.6±0.036	9.2±0.038	137.1±0.045	61.2±0.038	33.4±0.034	18.3±0.028
30	18.0±0.038	8.8±0.041	126.3±0.061	52.8±0.036	31.3±0.016	17.4±0.024
Ratna						
Control	23.2±0.023	10.6±0.034	156.8±0.016	82.1±0.036	38.3±0.051	22.2±0.031
0.01	31.4±0.041	14.2±0.012	169.2±0.018	92.2±0.039	46.6±0.046	25.1±0.036
0.1	29.6±0.049	13.6±0.051	161.0±0.021	86.2±0.043	45.0±0.041	24.2±0.021
1	27.2±0.062	12.2±0.062	156.3±0.027	80.3±0.048	43.2±0.038	21.9±0.026
10	25.3±0.029	11.3±0.041	148.0±0.044	70.3±0.048	41.2±0.019	21.1±0.021
20	24.1±0.028	10.9±0.018	137.0±0.048	62.1±0.041	38.9±0.033	20.3±0.023
30	22.6±0.025	9.8±0.023	129.0±0.037	51.4±0.061	36.1±0.036	18.2±0.048
Heera						
Control	22.5±0.041	9.9±0.031	146.2±0.062	75.2±0.026	37.3±0.023	21.1±0.048
0.01	27.4±0.036	10.9±0.036	168.3±0.051	96.4±0.028	46.2±0.039	23.9±0.031
0.1	26.3±0.018	10.6±0.042	162.4±0.033	91.3±0.023	43.8±0.037	23.2±0.026
1	25.6±0.051	10.3±0.046	153.6±0.036	82.4±0.033	41.1±0.031	21.6±0.028
10	24.5±0.056	10.0±0.051	148.7±0.039	71.6±0.036	38.3±0.019	20.1±0.022
20	22.4±0.048	9.8±0.021	142.8±0.028	63.3±0.057	36.1±0.015	18.3±0.029
30	21.3±0.037	9.6±0.026	132.9±0.018	58.1±0.062	34.2±0.009	17.1±0.013

Each value is mean of 5 samples ± SE

of starch concomitant with lower content of soluble carbohydrate in zinc treated endosperm is a clear indication of the inhibitory effect on the process of hydrolysis of starch to sugars, eventually depriving embryos food and resulting in inhibition of embryo growth. It may be suggested that the morphogenetic events inducing seed germination depend upon the environmental conditions and the genotype of the cultivars. The rate of germination depends upon the mobilization of food from centre of preservation towards centre of utilization (Yadav and Srivastava, 1997).

Seedling growth:

Seedling growth is the second important event of plant life after germination. It is crucial stage where all necessary constituents of the cell, cell wall and cell inclusions are synthesized which ultimately reflect on seedling vigour. In this piece of investigation, it was noticed that lower concentration of zinc (0.01 to 0.1 mg/l) exhibited increased values in various parameters of seedling growth such as shoot and root length fresh and dry weights of both shoot and root of all the test cultivars studied over control. However, a negative correlation was marked the concentration of zinc >1.0 mg/l (Table 2). Similar findings were reported by Rudraskha *et al.* (1992) where the conjugated form of zinc at lower concentration significantly increased the height and dry matter of sorghum and wheat over control, Baruah and Bharat (1996) and Bulbule and Dashpande (1989) influence of Fe in growth of rice plant, Yadav and Srivastava (1997) effect of Cd on seedling of some crops and Adhikary *et al.* (2011) effect of Hg on rice seedling.

It seems that the lower concentration of zinc acts as vital role on synthesis of auxins that might have promoted the growth and development. Nag *et al.* (1984) reported that there was an increase in the levels of a few oxidizing enzymes and a distinct inhibition in the activities of a number of hydrolysing enzymes in rice seedlings exposed to toxic dose of zinc. Lower concentration of zinc (0.01 to 0.1 mg/l) favours the growth and development of plants whereas, higher zinc concentration >1.0mg/l acts as phytotoxic. Hence, it is concluded that lower concentration of zinc might have synthesized requisite amount of IAA and other precursors which induced various parameters of seedling growth. Among the test cultivars, Ratna was found more resistant followed by Heera and Gayatri.

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