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In vitro effect of chromium, lead and manganese on seed germination, growth and mineral composition of finger millet (*Eleusine coracana*)

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ABSTRACT

*It is a well-known fact that heavy metal stress is one of the serious threats to agricultural yield. In the present work, heavy metal screening was carried out using chromium (Cr), lead (Pb) and manganese (Mn) independently on finger millet [*Eleusine coracana *(L.) Gaertn.] seed and seedling growth under* in vitro *conditions. Adverse effects of chromium on finger millet seed germination as well as seedling architectural damage were noticed from 10 ppm onwards. Particularly, 30 and 15 % of seed germination was noticed at 50 and 100 ppm concentrations of chromium, respectively. But the extremely toxic lead did not prove its toxic nature when compared to chromium and even at 100 ppm concentration the reduction of seed germination and seedling growth were minor. Manganese promoted the seed germination even at 50 and 100 ppm concentrations. In ICP-OES (inductively coupled plasma-optical emission spectrometer) analysis, contents of residual nitrogen, phosphorous, potassium and sulphur were reduced in seedlings grown on media containing high concentrations of chromium which indicated the toxic levels of this particular metal in this crop. In addition, the sulphur content slightly increased in seedlings treated with higher concentration of lead and showed almost double amount in seedlings treated with high concentration of manganese. The contents of microelements varied with different treatments and as expected content of managense increased in seedlings treated with high concentration of manganese.*

Keywords: Finger millet, chromium, lead, manganese, ICP-OES, growth, nutrient composition

INTRODUCTION

Heavy metal stress is one of the serious concerns, causing mostly adverse impacts on crop growth and development. Specifically, soil pollution has become a major threat for agriculture yield recently (Antoine *et al.*, 2017). Inclusion of heavy metals in the soil leads to alteration in plant metabolic pathways including production of reactive oxygen species (ROS) which in turn damages the plant growth (Stambulska *et al.*, 2018). Chromium is second most contaminant and seventh most abundant heavy metal in earth and is highly toxic in its valence state Cr (VI) when compared to Cr (III) state. Studies on the deleterious effect of chromium on different metabolic pathways were done in various crops such as pea, sunflower, rice, brassica etc. and found reduced yield levels (Ozdener *et al.*, 2011; Ma *et al.*, 2016). Similarly, lead is one of the major pollutants in soil and is more toxic than chromium. Lead is absorbed and accumulated in different parts of plants easily and regulated by pH, calcium channels based on particle size and root exudation. Though Pb does not hold any biological worth, its accumulation may lead to decreased seed germination capacity, inhibition of chlorophyll biosynthesis and biomass, chromosomal lesions, root elongation etc., as observed previously in different plants (Chand *et al.*, 2015; Wang *et al.*, 2015). Essential manganese forms II, III and IV are found in biological systems and specifically form II is the most soluble one. In contrast, high accumulation of Mn in plants exhibits deleterious effects on photosynthesis and causes unfavorable growth (Anjum *et al.*, 2015).

It is a well-known fact that finger millet is a nutritious food crop grown mostly for its grain and especially valuable as it contains the amino acid methionine besides rich sources of vitamin A, B1, B2 and B3 (Subastri *et al.*, 2015). In addition, finger millet is an energy feed valuable for high carbohydrate content and considered as

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a helpful famine crop due to its easy storage. Further this crop serves as a best source of feed livestock and used in the production of liquors. Few research works conducted with finger millet using different heavy metals such as cobalt, mercury, zinc and cadmium and noticed the adverse impacts on seed germination along with callus growth in high concentrations (Krishania and Agarwal, 2015). The effects of various heavy metals such as Cd, Cr, Co, Pb and Sn on seed germination and morphological and physiological variations in seedling growth in both pearl millet, green gram, great millet and horse gram crops have been reported by various workers (Gangaiah *et al.*, 2013; Neelesh *et al.*, 2014; Keerthi Kumari *et al.*, 2016; Keerthi Kumari *et al.*, 2017). Hence the present work was conducted to study the effect of heavy metal on finger millet seed germination and seedling growth and development.

MATERIALS AND METHODS

Initially, all the seeds of finger millet were washed in running tap water for 5 min to get them free from dust particles. Further surface sterilization of the seeds was carried out by treatment with 70% ethanol for 1 min and later treated with a solution of 0.1% mercuric chloride [$HgCl_2$ (w/v)] for 10 min. Surface sterilization was followed by three rinses with sterile distilled water. Later the moist seeds were placed on sterile filter paper to remove excess moisture before inoculation into the respective medium.

Media preparation and growth conditions

The composition of the media includes distilled water along with 1, 10, 20, 30 (only for chromium), 50 and 100 ppm concentrations of respective heavy metal i.e. Cr or Pb or Mn along with 0.8 percent agar. Media prepared with tap or distilled water (T.W. or D.W.) were used as controls without the inclusion of any metal. All the media were maintained at a pH of 5.8 and each test tube consisted of 15 ml of medium. The sources of Cr, Pb and Mn were potassium dichromate, lead nitrate and manganese sulphate monohydrate, respectively.

Inoculation and in vitro *growth conditions*

Culture tubes containing media were autoclaved at 15 lbs/in^2 for 20 min in an autoclave and later inoculation of seed material was carried out in the laminar airflow chamber (LAF). The culture room was maintained at 25 ± 2°C with a relative humidity of 50-60% in which incubation of inoculated test tubes was carried out along with 16 h photo period. All the data was collected on 15th day (after two weeks) and a minimum of three replicates was involved in each experiment which was conducted thrice. Statistical part of the work i.e. the mean and standard error was done using standard excel program in the personal computer.

ICP-OES analysis to estimate residual elements

Determination of remnant elements from *in vitro* grown heavy metal treated seedlings along with untreated seedling materials was carried out using ICP-OES (Prodigy, Teledyne Leeman, Hudson, N.H., USA) through the modified method of Chen and Ma (2001).

RESULTS AND DISCUSSION

Effect of Chromium

The present study elevates the adverse impacts of Cr metal on finger millet seed germination and seedling growth (Fig. 1). About 90% of germination in both the controls and 80% in 1 ppm Cr medium was noticed when compared to germination at high concentrations. Seed germination and growth inhibition were observed from 10 ppm concentration of chromium. Particularly, 30% and 15 % of seed germination were observed at 50 and 100 ppm of chromium (Fig. 1 A and B). Hence, seedlings treated with 30 ppm were considered for various analyses due to lack of 50 and 100 ppm treated seedling materials. Length of both shoot and root was also affected with high doses of chromium. When compared to control, increasing chromium concentration from 1 to 100 ppm exhibited decreased length of both shoots and roots (Fig. 1). Specifically, negligible root length values were noticed in seeds treated with high concentrations (Fig. 1 D). Thus, high concentration of chromium damages the seed germination and growth of finger millet seedlings but does not show any impact at low concentrations. These results also indicate that finger millet has the capacity to grow in chromium rich regions up to certain concentrations. Apart from seed germination, morphology of seedlings was also affected by chromium in different crops, specifically with respect to decline in shoot and root growth as noticed by Kabir (2016), Gangaiah *et al.* (2013) and Neelesh *et al.* (2014).

Effect of Lead

In lead treatment, unexpectedly there was a minor reduction in percentage of germination compared to control cultures (Fig. 1). In fact, minor increase in the seed germination at 50 ppm and after that reduction in the percentage of germination at 100 ppm was noticed. Similarly, negligible amount of reduction in the lengths of shoot and root at 100 ppm concentration was observed (Fig. 1). There were uneven modifications in shoot and root lengths occurred with lead but overall there was no much toxic effect was noticed (Fig. 1). Generally, toxic symptoms of lead such as stunted growth, blackening of root system, chlorosis etc., were not observed in the present work. Moreover, an increasing pattern of seed germination at 50 ppm of Pb (Fig. 1) indicates that this crop has tolerance to lead.

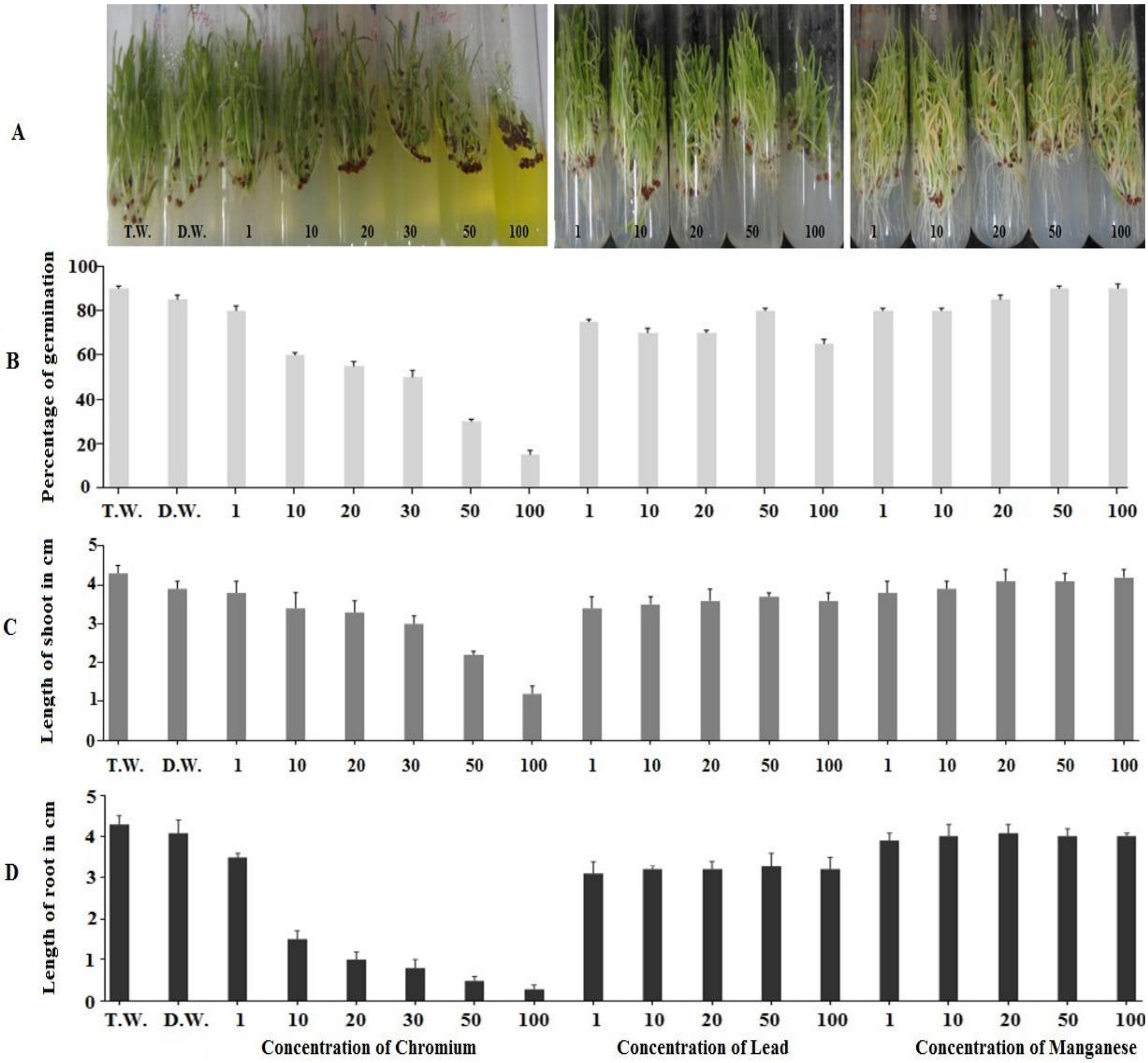

Fig.1: Effect of chromium, lead and manganese on seed germination and seedling growth of finger millet

Effect of Manganese

Gradual promotion in seed germination and seedling growth with increasing Mn concentrations was observed (Fig. 1). Percentage of germination was high in all the treatments ranging from 80 to 90 which are almost equal to controls. Moreover, increasing Mn concentration exhibited increase in length of shoot in our investigation (Fig. 1). Morphology of all the manganese treated seedlings was almost same, indicating the growth promoting nature of manganese in this crop. In contrast, number of roots were slightly reduced in samples under high concentration of manganese (Fig. 1). Any phytotoxic nature even in excess concentrations (excluding minor root variation) was not observed with Mn in this crop. In contrast, excess of manganese causes reduction in the seedling growth in horse gram was noticed (Keerthi Kumari *et al.*, 2016). High concentration of Mn in plant tissue causes alterations in enzyme activity, absorption and mineral elements usage indicated that the finger millet has ability to grow in these heavy metal rich areas.

Table 1: Effect of heavy metals on contents (%) of macroelements in seedlings using ICP-OES analysis

Elements	Controls		Seedling on Cr medium (ppm)						Seedling on Pb medium (ppm)					Seedling on Mn medium (ppm)				
	T.W	D.W	1	10	20	30	50	100	1	10	20	50	100	1	10	20	50	100
N	2.6	2.6	2.7	2.6	2.6	2.4												
P	0.82	0.78	0.96	0.76	0.74				1.45	1.42	1.47	1.36	1.28	1.29	1.33	1.35	1.29	1.38
K	2.4	2.2	2.5	1.9	1.9				1.41	1.40	1.42	1.51	1.59	1.76	1.74	1.68	1.83	1.82
S	0.63	0.59	0.62	0.48	0.44				0.29	0.29	0.29	0.29	0.30	0.32	0.39	0.44	0.49	0.59

ICP-OES analysis

Macroelements

Negligible difference in nitrogen contents between controls (2.6) and 1 ppm chromium treated seedlings (2.7) was noticed (Table 1). Also minor reduction of nitrogen content from 1 to 30 ppm was observed, which indicates the essentiality of this macronutrient in seedling growth and development. Residual phosphorus and potassium contents were reduced with increasing concentration of chromium due to damage caused by this heavy metal. Uneven phosphorus and potassium values were observed in lead and manganese treated seedlings. Overall, content of phosphorous was more and content of potassium was less when compared to control seedlings. Significant decrease of sulphur was observed in chromium treatment and it may be attributed to the breakage of protein bonding and less sulphur will be absorbed from the limited air due to severe damage of seedlings. In addition, gradual increase in sulphur content was noticed with increased Mn concentration in the medium. Overall, the most significant observation in ICP-OES analysis is that chromium treated seedlings differ from lead and manganese treated seedlings with respect to the contents of residual macroelements (Table 1). Particularly, macroelements are reduced with increasing chromium concentration due to severe noxious nature of this particular metal with this crop. This damage probably releases these elements from the plant and sent in to the environment. These results correlated with the report of Shobana *et al.* (2013) that generally among millets, finger millet contains more macroelements but Cr treatment reduces the levels to some extent indicating the toxic effect of this particular heavy metal. Irregular macroelement values (except sulphur) were observed in lead and manganese treated seedlings.

Microelements

Contents of Fe in chromium treated seedlings were progressively increased from 1 ppm to 20 ppm and uneven values were observed in lead and manganese treated seedlings (Table 2). In agreement with these results, increasing chromium concentration increases iron levels and this may be function less iron as per Singh *etal*. (2011). Zinc content

Table 2: Effect of heavy metals on contents (ppm) of microelements in seedlings using ICP-OES analysis

Elements	Controls		Seedling on Cr medium (ppm)						Seedling on Pb medium (ppm)					Seedling on Mn medium (ppm)				
	T.W	D.W	1	10	20	30	50	100	1	10	20	50	100	1	10	20	50	100
Fe	118.6	73	75.7	86.3	107				78	93	96	84	88	97	98	86	76	80
Zn	38.5	36.5	42.8	37.3	38.3				39.55	41.07	41.12	39.48	40.27	47.48	47.14	46.74	46.8	46.9
Cu	32.9	31.6	33.7	32.8	32				14.28	12.58	15.26	13.32	12.12	17.26	17.6	16.44	14.1	15.76
Mn	366.2	394.9	459	350.9	264.8				159	150	157	258	131	376	1281	2182	3519	5083

in chromium treated seedlings was uneven but overall content was more in Pb and Mn treatments when compared to control seedlings. Overall, copper values decreased in lead and manganese treated seedlings when compared to controls. As expected manganese values were increased in Mn treated seedlings and were decreased in high Cr treated seedlings. Decreased copper and manganese values in high chromium treatments may be due to their release from the seedlings because of high toxicity. Particularly increased manganese in Mn treatments is not surprise in the present work.

Among heavy metals used in the present investigation, chromium proved to be more toxic than lead and manganese promoted the seed germination as well growth of the seedlings under *in vitro* conditions. Specifically, only 30% and 15 % of seed germination was achieved at 50 and 100 ppm chromium concentrations. Similarly, both shoot and root lengths were also reduced in seedlings grown on high concentration of chromium and particularly lengths of roots were drastically decreased. Moreover, there was minor reduction with respect to percentage of seed germination and seedling growth even at 100 ppm concentration of lead. Manganese promoted the seed germination and seedling growth even in high doses with negligible errors. In ICP-OES data, content of remnant macronutrients were reduced in seedlings grown on high chromium concentration media indicates the lethal nature of this metal in this crop. Furthermore, this work may be useful to deal with the various morphological, biochemical and molecular insights of heavy metal stress on crop plants and outline the scope for future research.

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