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Influence of tungsten and molybdenum on seed germination and early seedling growth of wheat - a comparative study

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Abstract
Fifty one promising landraces of rice cultivars of three districts viz. Nadia, 24 Parganas(N) and MA comparative study on the effect of tungsten and molybdenum on seed germination and early seedling growth of wheat was carried out. Both W and Mo influenced the seedling growth in similar fashion. W and Mo enhanced the germination relative index at lower application. Relative yield increased at lower applied doses of W as well as Mo whereas at higher application it decreased. Lower applied doses of W and Mo showed maximum index of metal tolerance. Higher applied doses of both metals showed toxic effect on plant growth. A highly significant ($p = 0.01$ in W and $p = 0.001$ in Mo) increment in grade of growth inhibition (GGI) at the higher administration of W and Mo was observed. The toxic effect of tungsten on wheat was more marked than molybdenum.

Keywords: Tungsten, molybdenum, germination, relative yield, tolerance index, GGI

Tungsten (W) and molybdenum (Mo) are trace elements and rank 54th and 53rd in natural abundance. Both metals are similar in chemical properties, electron negativity and atomic and ionic radii. The fact that W and Mo can perform similar functions in different organisms reflects their chemical similarity. Tungsten can compete with molybdenum for incorporation into the enzyme complex and results in enzyme inactivation. Plants absorb W and Mo in the form of WO_4^{2-} and MoO_4^{2-} respectively (Wilson and Cline, 1966; Mengel and Kirkby, 1996) and accumulate it in different plant parts (Aery, 2000). Whereas the role of Mo is well studied in plants and considered as essential mineral nutrient (Arnon and Stout, 1939) W is less studied and generally known to be a competitive inhibitor of Mo function *in vivo* in bacteria (Brill *et al.*, 1974), in green alga *Chlorella* (Cardenas *et al.*, 1972) and the higher plants such as spinach (Notton and Hewitt, 1972).

Though the biological importance of tungsten has been fully proved by the isolation of a number of tungsten containing enzymes (L'vov *et al.*, 2002), it has been found to be beneficial for plant growth only in some instances (Davies and Stockdill, 1956; Kumar and Aery, 2010b).

Tungsten and molybdenum are similar in their natural abundance. Their physical and chemical properties are also almost similar. Tungsten is found as wolframite and scheelite in the nearby areas (Aery, 2000). The information about the effect of tungsten on seed germination and early seedling growth is lacking. A comparative study was carried out to explore the relative effect of tungsten and molybdenum on seed germination, early seedling growth, relative yield, vigour index, index of metal tolerance and grade of growth inhibition in wheat.

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Materials and Method

The experiments were conducted in the month of January under laboratory conditions where the photoperiod was 8 hours day^{-1} with a light intensity of 3600 Lux. Twelve seeds of a certified variety Raj 4037 of *Triticum aestivum* L. were placed in each Petri plate with filter paper. Five concentrations of tungsten (3, 9, 27, 81 and 243 μg ml^{-1}) and molybdenum (0.1, 0.5, 2.5, 12.5 and 62.5 μg ml^{-1}) were applied as sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) (E. Merk) and sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) (E. Merk). The concentrations were prepared separately by taking corresponding amount (calculated on the basis of molecular weight) of chemical per liter of water. No other supplement nutrients were added. Control constituted only distilled water. A fixed amount of solution was poured in each Petri plate to saturate the filter paper. Three replicates were used for each concentration. After the start of seed germination, the speed of seed germination was observed after every hour. Plants were harvested after seven days of treatment and root-shoot length was measured. The plant samples were dried at 80°C in an oven for 48 hours for the measurements of dry-weight.

Germination Relative Index: Germination relative index was computed after Sreevastava and Sareen (1972) by using the following formula: GRI = $\sum [X_n (h - n)]$ where X_n is the number of germinants at n^{th} count; h, the total number of counts and n, the count number.

Tolerance Index: Tolerance index of seedlings were calculated by using the formula given by Turner and Marshall (1972).

Vigour Index: Vigour index was calculated by using the

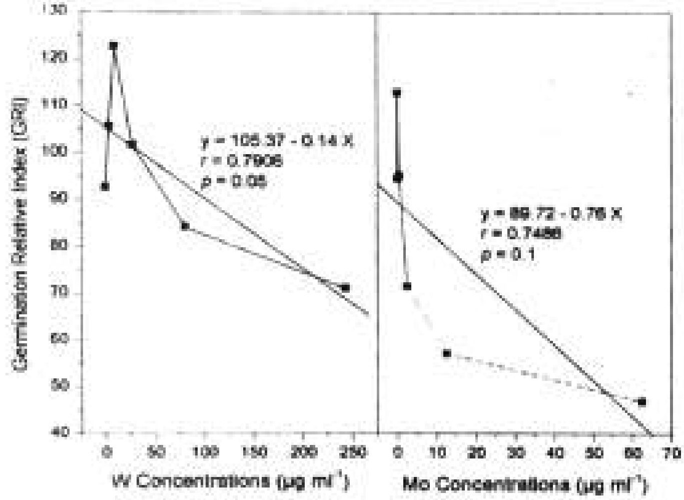

Fig: 1. Effect of various concentrations of W and Mo as sodium tungstate and sodium molybdate, respectively on the germination relative index (GRI) of *Triticum aestivum* L.

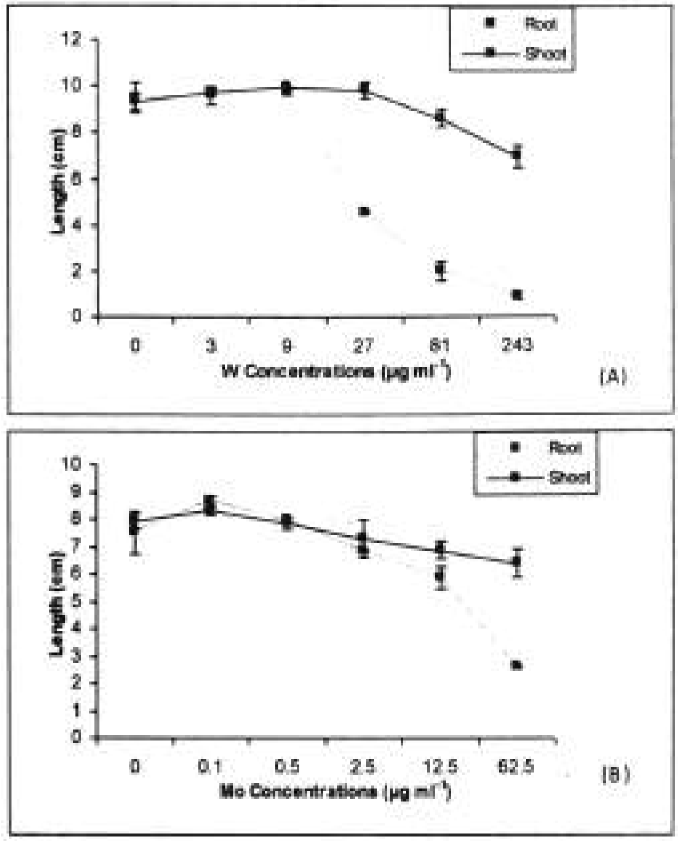

Fig: 2. Effect of various concentrations of W (A) and Mo (B) as sodium tungstate and sodium molybdate on the root-shoot length of *Triticum aestivum* L. (Error bars denote standard error)

formula given by Dhindwal *et al.* (1991).

Grade of Growth Inhibition (GGI): Grade of growth inhibition was calculated by the following formula as described by Aery (2010): Grade of Growth Inhibition (GGI) = (Dry weight of control plant – Dry weight of treated plant/ Dry weight of control plant) × 100.

Results

Results on the effect of W and Mo on germination relative index are shown in Fig. 1. Both W and Mo showed stimulatory effect on the germination relative index which was observed to be maximum in lower applied doses of W (9 $\mu g\ ml^{-1}$) and Mo (0.5 $\mu g\ ml^{-1}$). Beyond the above levels, germination relative index decreased (Fig 1).

Tungsten and molybdenum showed promotory effect on root-shoot length of wheat. Maximum root-shoot length was observed in 9 $\mu g\ ml^{-1}$ and 0.1 $\mu g\ ml^{-1}$ treatments of W and Mo, respectively. The increments over the control respectively, for root and shoot length were 2.32% and 6.42% in tungsten and 15.42% and 5.41% in molybdenum treatment. Beyond the above level, root-shoot length of seedlings decreased regularly (Fig. 2). Minimum root and shoot length was observed at the highest applied doses of W (243 $\mu g\ ml^{-1}$) and Mo (62.5 $\mu g\ ml^{-1}$).

Lower applied doses of W (9 $\mu g\ ml^{-1}$) and Mo (0.1 $\mu g\ ml^{-1}$) resulted in an increment in relative yield. Maximum relative yield was observed at lower applied doses of W (9 $\mu g\ ml^{-1}$) and Mo (0.1 $\mu g\ ml^{-1}$) and were 117 and 105, respectively. Higher applied doses of W and Mo showed a decrement in relative yield. Minimum relative yield was observed at the highest applied doses of W (243 $\mu g\ ml^{-1}$) and Mo (62.5 $\mu g\ ml^{-1}$) and were respectively, 44% and 62% lower, over the control (Table 1).

Table 1: Effect of various concentrations of tungsten and molybdenum as sodium tungstate and sodium molybdate, respectively on relative yield of *T aestivum*

W	Relative yield	Mo	Relative yield
Control	100	Control	100
3	104	0.1	105
9	117	0.5	99
27	64	2.5	93
81	57	12.5	82
243	44	62.5	62

W=W conc. ($\mu g\ ml^{-1}$); Mo=Mo conc. ($\mu g\ ml^{-1}$)

Vigor index of wheat increased in lower concentrations of tungsten and molybdenum. Higher concentrations of both these metals resulted in decreased vigour index. The decrement was more significant in Mo treatment ($p = 0.001$) than W treatment ($p = 0.01$) (Fig 3).

Lower applied doses of W (9 $\mu g\ ml^{-1}$) and Mo (0.1 $\mu g\ ml^{-1}$) positively influenced the index of metal tolerance. Beyond the above level index of metal tolerance concomitantly decreased. Minimum index of metal tolerance was observed at the highest applied doses of W and Mo (Fig 4).

Lower applied doses of W (9 $\mu g\ ml^{-1}$) and Mo (0.1 $\mu g\ ml^{-1}$) showed minimum grade of growth inhibition. It

Table 2: Relationship between applied metal concentrations and relative yield of *T. aestivum*

Parameter	Regression equation	Correlation coeff. (r)	Significance
RY (W)	y=95.9673-0.2473x	-0.7846	S*
RY (Mo)	y=98.0537-0.6059x	-0.9420	S**

RY=relative yield, y = parameter, x = applied metal concentrations, S* = significant at 0.05 probability level, S** = significant at 0.001 level

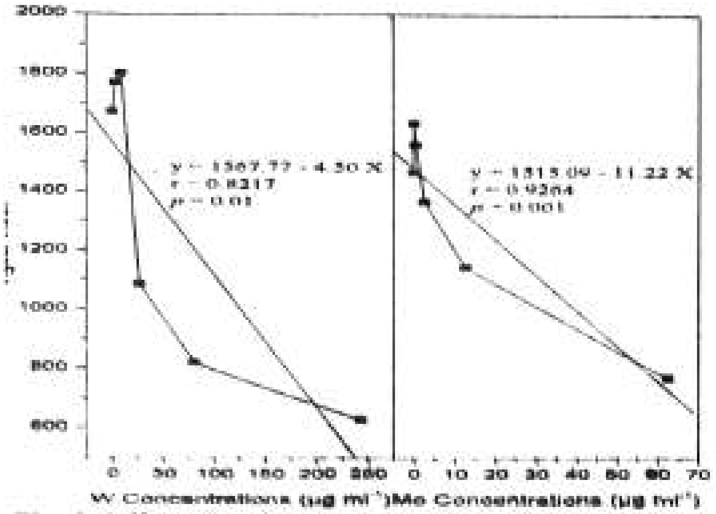

Fig: 3. Effect of various concentrations of W and Mo as sodium tungstate and sodium molybdate, respectively on the vigour index of *Triticum aestivum* L.

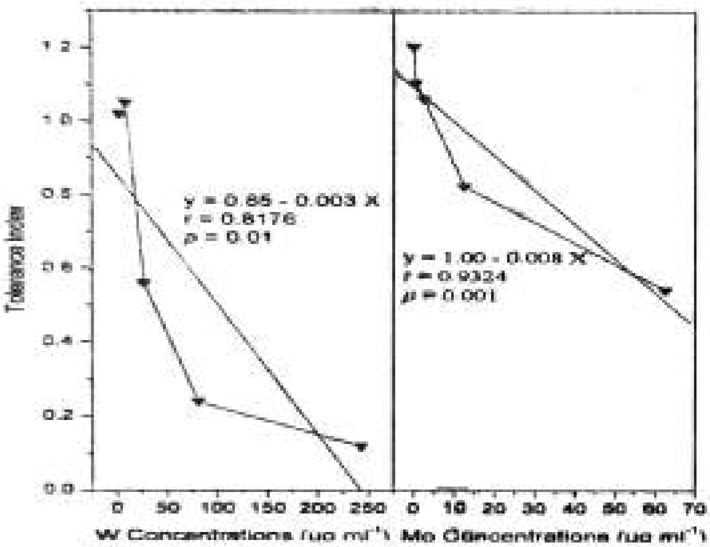

Fig: 4. Effect of various concentrations of W and Mo as sodium tungstate and sodium molybdate, respectively on the tolerance index of *Triticum aestivum* L.

was observed to be maximum at higher applied doses of W (243 µg ml^{-1}) and Mo (62.5 µg ml^{-1}).

Discussion

Tungsten and molybdenum positively influenced the seed germination and seedling growth. Both these metals showed a stimulatory effect on germination relative index at lower applied doses whereas higher applied doses adversely affected the seed germination and resulted in decreased germination relative index. Present results are in conformity to the findings of Rout and Das (2002) on rice who observed that seed germination rate declined at higher concentration (1.6 µM) of molybdenum. Similar findings have been reported on the effect of Li (Surana and Aery, 2005), Si (Mali, 2008), Ni (Jagetiya and Aery, 1994), W (Kumar and Aery, 2010a) and Mo (Chatterjee and Nautiyal, 2001). Results obtained from germination studies indicate that W enhances germination relative index more efficiently compared to Mo (Fig 1).

The increment in dry-matter with the application of Mo to plants is well established (Chatterjee and Nautiyal,

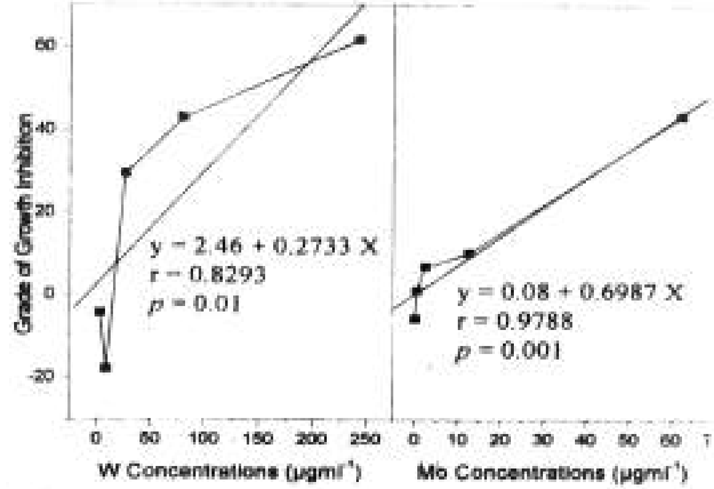

Fig: 5. Effect of various concentrations of W and Mo as sodium tungstate and sodium molybdate, respectively on the grade of growth inhibition of *Triticum aestivum* L.

2001 and Weng *et al.*, 2009). In the present study increased relative yield of wheat was also observed at lower applied doses of W and Mo. It has also been reported that sodium tungstate increases the growth rate and final yield of algae (Tyagi, 1974). Kumar and Aery (2010a, 2010b) reported an increment in dry matter and relative yield with the application of W in cowpea. Higher concentrations of both these metals resulted in decreased dry-matter. This might be due to higher accumulation of metals in cell which inhibits cell enlargement (Aery and Jagetiya, 2000; Mali and Aery, 2008) and/ or due to inhibition of cell division in the meristematic zone (Powell *et al.*, 1986). The decrement in relative yield of seedling was observed to be higher in W than Mo treatment (Table 1 and 2).

The root elongation method developed by Wilkins (1978) to quantify the inhibitory effect of metal ions on root growth has been used widely in ecological studies for testing of tolerance of plants to metals. Jayakumar *et al.* (2008) observed the increment at lower level and decrement at higher level in vigour index and tolerance index in response to cobalt. In the present study both tungsten and molybdenum were found to be inducer for vigour and tolerance indices. Vigour index was observed to be maximum at lower applied doses of tungsten (9 µg ml^{-1}) and molybdenum (0.1 µg ml^{-1}). The increment in vigour index indicates the positive effect of both these metals on the health of wheat. Minimum vigour index was observed at higher applied doses of W and Mo (Fig

Table 3: Relationship between applied metal concentrations and root-shoot length of *Triticum aestivum* L.

Parameter	Regression equation	Correlation coeff. (r)	Significance
Tungsten			
RL	y=8.17-.03x	-0.8207	S*
SL	y=9.42-.01x	-0.9090	S**
Molybdenum			
RL	y=7.68-.08x	-0.9531	S**
SL	y=7.76-.02x	-0.7994	S*

RL=root length, SL=shoot length, y = parameter, x = applied metal concentrations, S* = significant at 0.05 probability level, S** = significant at 0.001 level

3). The magnitude of influence in vigour index of wheat varied in response to W and Mo. W cultured seedlings showed more increment in lower and decrement at higher levels, respectively, compared to Mo cultured seedlings.

A tolerance index of one means that the growth of root in the metal solution was the same as that in the reference solution (Freedman, 1995). In the present study lower applied doses of both tungsten (9 μg ml^{-1}) and molybdenum (0.1 μg ml^{-1}) showed maximum (more than one) tolerance index (Fig. 4). It indicates that lower applied doses of both W and Mo are favorable for plant growth. The increment in tolerance index is observed to be more remarkable in Mo treated plants compared to W treated plants. It reduced concomitantly at higher administration of both the metals. Jayakumar *et al.* (2008) and Pugalvendhan *et al.* (2009) have also observed the same results with the application of Co and Hg, respectively. An increase in the level of prolines has been observed (unpublished work of the authors) which may provide protection by chelating the metals in the cytoplasm and maintaining the water balance which is often disturbed by heavy metals (Xu *et al.*, 2009). Alternatively, plants may achieve metal tolerance by protecting the integrity of plasma membrane against metal damage by the use of heat shock proteins (Lewis *et al.*, 1999) or metallothioneins (Grennan, 2011).

Both W and Mo influence the grade of growth inhibition in the same fashion. A decrement in grade of growth inhibition in both W and Mo cultured seedlings was observed in lower concentrations. But the minimum grade of growth inhibition in tungsten treatment was three times than molybdenum treatment (Fig. 5). Higher concentrations of W and Mo showed toxic effect on seedling growth which resulted in increased grade of growth inhibition. Kumar and Aery (2010a) reported that application of W decreases the grade of growth inhibition at lower level and increases at higher level in cowpea.

Correlation coefficient between applied tungsten and molybdenum concentrations and relative yield and root-shoot length were also computed. In all the cases the negative value of correlation coefficient indicates the degree of toxic effect of both W and Mo on overall growth performance of wheat (Table 2 and 3).

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