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Effects of Mercury and its Antagonistic Effect with Magnesium and Sucrose on Growth and NR Activity in *Hordium Vulgare (L.)*

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Abstract

The present investigation was designed to evaluate the effects of different doses of Mercury (0.02, 0.04, 0.06, 0.08, 0.10 mM) and combined effect of different doses of Mercury with Magnesium and Sucrose (0.02, 0.04, 0.06, 0.08, 0.10 mM) on growth and NR activity in two varieties of *Hordium vulgare* (L.) A concentration dependent decrease in growth parameters like seedling height, fresh weight of seedling chlorophyll content and carbohydrate content was observed under the influence of different doses of mercury in both the varieties. The lowest dose of mercury (0.02 mM) increased the NR activity and protein content while subsequent doses (0.02, 0.04, 0.06, 0.08, 0.10 mM) showed inhibitory effects. The lower doses of the combined treatment of mercury with magnesium and mercury with sucrose (0.02 & 0.04 mM) increased the growth parameters, NR activity and protein content of *Hordium vulgare* while subsequent concentrations (0.06, 0.08 & 0.10 mM) showed inhibitory effect. Thus the recovery of inhibitory effects of mercury on growth parameters, NR activity and protein content was noticed in combination treatments of mercury with magnesium and mercury with sucrose treatments.

Keywords: Antagonism, Magnesium, Mercury, Phytotoxicity, Sucrose.

Introduction

Most toxic ions are ubiquitous in the environment and many of them are essential for the growth of plant. However some of them (viz, Zn, Cu, Hg, Mn, Fe, Mo and Ni) proved to be acute toxic if present in higher concentration (Saxena & Saxena, 2002). Agarwal & Bhattacharya (1989) postulated that mercury (Hg^{2+}) binds with sulphydryl group (-SH) of protein and alter the molecular structure of protein. Parmar et al (2002) studied the effect of Hg^{2+} & Cr^{2+} in phaseolus seedling and found that both the heavy metals inhibited plant growth and peroxidase activity.

The inhibition of plant growth and development at higher concentration of mercury also observed by Sutter *et al*. (2003) and Nitika *et al*. (2006). Heavy metals in combination with sucrose also increase the NR activity in bean leaf segments (Puranik and Srivastava 1983).

Thus the present investigation has been undertaken to study the individual and combined effect of mercury with magnesium and sucrose on growth, NR activity and protein content in the two varities of *Hordium vulgare* (L.) foundation and BH-902.

Materials & Methods

The seeds of two cultivars of *Hordium vulgare* (L.), foundation and BH-902 were presoaked in distilled water and were sown in three separate plots of the field. In first, the irrigation of different doses of mercury (0.02, 0.04, 0.06, 0.08, 0.10 mM) and in second and third plot, the combined treatment of different doses of mercury with magnesium and mercury with sucrose (in ratio 1:1, 0.02, 0.04, 0.06, 0.08, 0.10 mM) was given at the interval of 15 days respectively. The effects of mercury alone and in combination with magnesium and sucrose was studied on seedling height, fresh weight of seedling, chlorophyll content, carbohydrate content, NR activity and protein content. The chlorophyll content was determined by the method of Arnon (1949), carbohydrate content was determined by the method of Morris (1948), Protein content by Lowry *et al*. (1951) and NR activity was determined by the method of Srivastava (1974).

Results and Discussion

Seedling Height and Fresh Weight of Seedling

A concentration dependent decrease was noticed in seedling height and fresh weight of seedling under the influence on different doses of mercury (0.02, 0.04, 0.06, 0.08, 0.10 mM) in both the varieties of *Hordium vulgare*. The maximum reduction in seedling height and fresh weight of seedling was obtained with the higher dose of mercury (0.10 mM) in both the varieties but the reduction was more pronounced in foundation 'rather BH-902'. In the combined treatment of mercury with magnesium, the lower doses (0.02 & 0.04) showed promotary effect on seedling height and fresh weight of seedling but the more pronounced increased was noticed with 0.02 mM concentration of mercury with magnesium as compared 0.04 mM concentration of mercury with magnesium. The subsequent concentration of the mercury with magnesium (0.06, 0.08, 0.10 mM) showed inhibitory effect on seedling height and fresh weight of seedling. (Table -1)

Similarly in case of the combination treatment of mercury with sucrose, the lower doses (0.02 & 0.04 mM) showed promotary effect on seedling height and fresh weight of seedling. An increase in seedling height and fresh weight of seedling was more with 0.02 mM concentration of mercury with sucrose treatment as compared to the 0.04 mM concentration of mercury with sucrose treatment. The increase in seedling height and fresh weight of seedling was more in mercury with magnesium treatment as compared to mercury with sucrose treatment. (Table-1)

Chlorophyll and Carbohydrate Content

Concentration dependent decrease was also noticed in chlorophyll and carbohydrate content where the maximum reduction in nitrogen content was observed with the higher dose of mercury (0.10 mM) in both the varieties. The combined treatments of mercury with magnesium and mercury with sucrose showed increase in chlorophyll and carbohydrate contents at lower concentrations (0.02 & 0.04 mM) but the more pronounced increase was noticed with 0.02 mM concentration as compared to 0.04 mM concentration of treatments. The subsequent concentrations of mercury with magnesium and mercury with sucrose treatments (0.06, 0.08, 0.10 mM) showed inhibitory effect on chlorophyll and carbohydrate contents as compared to control. The reduction was more in variety foundation as compared to variety BH-902. (Table-1)

NR Activity and Protein Content

The NR activity and protein contents were increased with the lowest concentration of mercury (0.02 mM) while the subsequent concentrations of mercury (0.04, 0.06, 0.08, 0.10 mM) inhibited the NR activity and protein content. In combined treatment of mercury with magnesium and mercury with sucrose the similar pattern of increase and decrease of NR activity and protein content was observed as noticed in case of chlorophyll and carbohydrate contents.(Table-2)

Heavy metals have been known to exert toxic influence at different levels on the tissues of higher organisms (Venugopal & Luckey,1978). At elevated levels in soil heavy metals generally becomes toxic and can ultimately cause the death of plants. In the present investigation the concentration dependent decrease was noticed in seedling height and fresh weight of seedling under the influenced different doses of mercury (0.02, 0.04, 0.06, 0.08, 0.10 mM) and the maximum decrease was noticed under the influence of higher dose of mercury (0.10 mM). Heavy metal toxicity at higher concentration is well known (Mhatre & Chaphekar 1982, Lata 1983, Sarkar and Aery 1990, Salgare *et al*. 2001, Sutter et al. 2002). The higher concentration of heavy metals have also been reported to retard cell division and differentiation and reduce their elongation and affect plant growth and development (Kastori *et al.* 1998, Tomar *et al.* 2000). Further the seedling height and fresh weight of seedling were increased with the lower concentration (0.02 & 0.04 mM) of combined treatments of mercury with magnesium and mercury with sucrose but at the higher concentration (0.06, 0.08, 0.10 mM) they were decreased. Thus mercury with magnesium and sucrose at lower concentration showed antagonistic effects. The similar trend of reduction of chlorophyll and carbohydrate content was also noticed under the influence of different doses of mercury (0.02, 0.04, 0.06, 0.08, 0.10 mM). Similarly, the reduction in chlorophyll and carbohydrate contents by mercury was also noticed by Vyas & Puranik (1993), Reeta *et al*. (1999) Mousumi *et al*. (2000) Baszinski *et al*. (1980) and Prasad & Prasad (1987) concluded that there are two enzymes i.e. δ Aminolaevulinic acid (ALA) dehydrates and Protochlorophyllide reducates which are involves in chlorophyll biosynthesis in higher plants and are inhibited by Hg^{2+} & Pb^{2+} because these metals binds with functional sulphydryl (-SH) group of the enzymes. The similar trend of increase and decrease was also noticed in case of chlorophyll and carbohydrate contents as obtained in seedling height and fresh weight of seedling under the influence of combined treatment of mercury with magnesium and sucrose. Arvind (2004) found that heavy metal protects *Ceratophyllum demersum* L. and Pedler *et al.* (2004) found that combined treatment of zinc with magnesium showed promotary effect in wheat and radish.

The enzyme nitrate reductase is substrate inducible enzyme which is found in cytosole and the reduction of nitrate to nitrte by the enzyme nitrate reductase, is rate limiting step in over all nitrate assimilation and its activity often controls the rate of protein synthesis in plants (Srivastava, 1980). In the present investigation the NR activity and protein content were increased with the lower concentration of heavy metal (0.02 mM) whereas the subsequent concentrations of (0.04, 0.06, 0.08 & 0.10 mM) of mercury showed inhibitory effect. Similar findings were also obtained by Veerappa & Samy (1999) where they found that lower concentration of mercury promote the NR activity whereas subsequent higher concentrations showed inhibitory effect in *Zea mays*. According to Singh *et al*. (1998), the inhibition of NR activity under the influences of heavy metals may be multifacial, eg. due to reduced supply of NADPH, disorganization of chloroplast, less NO_3 supply to the site of synthesis caused by water stress and direct effect of heavy metals on protein synthesis because it has a strong affinity for functional –SH group of the enzyme. Further the lower dose of combined treatments of mercury with

magnesium and mercury with sucrose (0.02 & 0.04 mM) were found to be promotary for NR and protein content whereas subsequent higher doses (0.06, 0.08 & 0.10 mM) showed inhibitory effect. Puranik and Srivastava (1983) and Vyas and Puranik (1993) observed that sucrose enhance the stability of NR and mobilization of endogenous nitrate pool. Bose and Mishra (1999) noticed that the magnesium salts increase the NR activity and protein content in *Brassica Juncea.* Kiss (1989) and Bose & Mishra (1992) concluded that application of magnesium to soil, increases the nitrogen status of plants and ultimately the protein content.

Thus the present investigation showed that all the concentrations of mercury exert toxic effect on all the parameters studied in *Hordium vulgare* (L.). NR activity and protein content which increases at lowest concentration (0.02 mM) while decrease at subsequent higher concentrations (0.04, 0.06, 0.08 & 0.10 mM) of mercury. The lower concentrations of combined treatments of mercury with magnesium and sucrose (0.02 & 0.04 mM) shows promotary effects on all the parameters studied while higher concentrations (0.06, 0.08 & 0.10 mM) shows inhibitory effect. Further, the increase in all the parameters in more clear in the combined treatment of mercury with magnesium as compared to mercury with sucrose. The variety foundation was found to be more susceptible to metal toxicity as compared to variety BH-902.

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Table -1 : Effects of Mercury and its Combined Treatments with Magnesium and Sucrose on Growth Parameters of *Hordium vulgare* (L.)

Concentration of treatment (mM)	Seedling height (c.m.)	Fresh weight of seedling (gm)	Chlorophyll content (mg g^{-1} fr.wt.)	Carbohydrate content (mg g^{4} fr.wt.)
Variety- foundation				
Control	20.25±0.35	21.65±0.22	1.89±0.45	80.25±0.85
Hg^{2+} 0.02	19.15±0.46	20.25±0.48	1.75*±0.92	80.15±0.69
Hg^{2+} 0.04	18.50*±0.55	19.45*±0.82	1.25*±1.25	78.96±0.95
Hg^{2+} 0.06	17.40*±1.25	18.80*±1.25	1.07*±2.45	66.19*±1.28
Hg^{2+} 0.08	14.95*±2.15	14.75*±3.16	0.92*±0.91	60.95*±3.19
Hg^{2+} 0.10	13.80*±4.25	13.45*±1.10	0.80*±1.13	55.18*±2.18
Hg^{2+} 0.02 + Mg^{2+} 0.02	24.25*±0.95	25.10*±0.86	1.87*±0.85	99.45*±0.92
Hg^{2+} 0.04 + Mg^{2+} 0.04	22.95*±2.11	22.95*±0.52	1.54*±0.81	95.16*±1.45
Hg^{2+} 0.06 + Mg^{2+} 0.06	19.65*±3.23	20.60*±1.25	1.38*±1.05	78.62*±2.39
Hg^{2+} 0.08 + Mg^{2+} 0.08	19.05*±1.15	18.45*±1.29	1.00*±2.93	75.16*±1.45
Hg^{2+} 0.10 + Mg^{2+} 0.10	17.12*±1.13	17.00*±1.15	0.88*±3.10	69.18*±2.15
Hg^{2+} 0.02 + sucrose 0.02	23.15*±1.14	24.35*±0.95	1.78*±0.95	95.15*±1.24
Hg^{2+} 0.04 + sucrose 0.04	21.44*±1.82	22.85*±1.25	1.59*±1.46	94.19*±1.45
Hg^{2+} 0.06 + sucrose 0.06	19.90*±3.16	21.10*±1.35	1.45*±2.32	76.65*±1.82
Hg^{2+} 0.08 + sucrose 0.08	18.35*±1.25	19.35*±2.15	0.95;*±1.85	72.16*±2.89
Hg^{2+} 0.10 + sucrose 0.10	16.95*±1.43	17.00*±1.32	0.80*±1.33	68.15*±1.87
Variety BH-902				
Control	20.45±0.85	22.00±1.45	2.00±0.95	78.29±0.85
Hg^{2+} 0.02	20.05±0.41	20.95±1.32	1.98*±1.29	78.19±1.27
Hg^{2+} 0.04	19.50*±0.92	20.05*±1.62	1.55*±3.13	64.25*±1.82
Hg^{2+} 0.06	18.40*±1.25	19.65*±1.89	1.15*±3.42	58.17*±1.11
Hg^{2+} 0.08	15.45*±1.43	15.10*±3.10	0.95*±1.05	54.22*±2.17
Hg^{2+} 0.10	14.15*±1.29	14.91*±1.33	0.85*±2.95	50.11*±2.22
Hg^{2+} 0.02 +Mg^{2+} 0.02	25.15*±1.12	25.95*±1.31	1.98*±1.35	98.16*±1.12
Hg^{2+} 0.04 +Mg^{2+} 0.04	23.65*±1.49	23.10*±2.11	1.78*±1.67	91.67*±3.16
Hg^{2+} 0.06 +Mg^{2+} 0.06	21.45*±1.36	21.24*±2.49	1.55*±1.98	75.16*±1.66
Hg^{2+} 0.08 +Mg^{2+} 0.08	20.95*±2.67	19.30*±1.17	1.10*±2.15	72.49*±2.11
Hg^{2+} 0.10 +Mg^{2+} 0.10	19.35*±1.85	17.00*±1.31	0.92*±1.65	65.92*±1.68
Hg^{2+} 0.02 +sucrose 0.02	24.85*±2.45	25.90*±0.89	1.85*±1.28	94.16*±1.15
Hg^{2+} 0.04 +sucrose 0.04	23.15*±1.35	24.45*±2.63	1.64*±1.42	92.13*±2.88
Hg^{2+} 0.06 +sucrose 0.06	20.75*±1.47	22.76*±1.68	1.50*±2.85	75.16*±2.12
Hg^{2+} 0.08 +sucrose 0.08	19.65*±1.62	20.10*±1.11	1.00*±1.37	69.42*±1.85
Hg^{2+} 0.10 +sucrose 0.10	18.00*±1.85	17.10*±3.21	0.88*±1.45	63.33*±1.43
***Significant at 5% level**				

Table -2 :Effects of Mercury and its Combined Treatments with Magnesium and Sucrose on Growth Parameters of *Hordium vulgare* (L.)

Concentration of treatment (mM)	NR activity (M $NO_2^{-1}hr^{-1}$fr,wt,)	Protein content (mgg^{-1} fr.wt.)
Variety- *foundation*		
Control	18.65±0.85	50.45±1.55
Hg^{2+} 0.02	19.45±1.62	53.15±1.32
Hg^{2+} 0.04	18.12*±2.63	51.45±1.66
Hg^{2+} 0.06	17.10*±1.25	43.39*±1.00
Hg^{2+} 0.08	14.79*±1.3	40.25*±1.95
Hg^{2+} 0.10	11.40*±1.24	36.57*±2.11
Hg^{2+} 0.02 + Mg^{2+} 0.02	25.10*±1.65	62.12*±1.05
Hg^{2+} 0.04 + Mg^{2+} 0.04	24.25*±1.82	59.13*±1.82
Hg^{2+} 0.06 + Mg^{2+} 0.06	17.92*±2.46	46.25*±1.25
Hg^{2+} 0.08 + Mg^{2+} 0.08	16.15*±1.80	41.89*±2.02
Hg^{2+} 0.10 + Mg^{2+} 0.10	13.25*±2.11	38.10*±0.92
Hg^{2+} 0.02 + sucrose 0.02	27.13*±1.13	61.85*±1.06
Hg^{2+} 0.04 + sucrose 0.04	22.14*±2.98	59.23*±2.86
Hg^{2+} 0.06 + sucrose 0.06	16.85*±1.3	44.19*±1.11
Hg^{2+} 0.08 + sucrose 0.08	16.27*±1.44	42.81*±1.05
Hg^{2+} 0.10 + sucrose 0.10	13.15*±1.00	37.54*±3.01
Variety- *BH-902*		
Control	19.25±0.93	52.10±1.32
Hg^{2+} 0.02	20.89±1.82	54.16±1.83
Hg^{2+} 0.04	20.10*±1.02	53.85±1.23
Hg^{2+} 0.06	17.55*±2.00	45.93*±1.02
Hg^{2+} 0.08	15.75*±1.31	42.95*±0.92
Hg^{2+} 0.10	13.45*±1.11	39.75*±1.26
Hg^{2+} 0.02 +Mg^{2+} 0.02	28.16*±2.05	63.99*±1.13
Hg^{2+} 0.04 +Mg^{2+} 0.04	26.00*±1.69	60.89*±1.18
Hg^{2+} 0.06 +Mg^{2+} 0.06	18.96*±1.01	48.76*±2.15
Hg^{2+} 0.08 +Mg^{2+} 0.08	16.95*±2.02	44.39*±1.16
Hg^{2+} 0.10 +Mg^{2+} 0.10	14.75*±1.83	39.92*±2.22
Hg^{2+} 0.02 +sucrose 0.02	27.77*±3.42	62.18*±1.88
Hg^{2+} 0.04 +sucrose 0.04	23.58*±1.25	60.37*±1.11
Hg^{2+} 0.06 +sucrose 0.06	17.95*±2.00	46.69*±1.32
Hg^{2+} 0.08 +sucrose 0.08	17.10*±1.19	42.15*±2.22
Hg^{2+} 0.10 +sucrose 0.10	14.10*±1.63	38.95*±3.15
***Significant at 5% level**		