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INFLUENCE OF ANTIMONY AND MERCURY ON PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF ELEUSINE CORACANA (L) GAERTN – RAGI. SEEDLINGS. 1. GERMINATION BEHAVIOUR, TOTAL PROTEIN AND PROLINE CONTENT AND PROTEASE ACTIVITY

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Heavy meals play a vital role in the growth and development of plants. These may act as cofactors of some enzymes of help in the formation of intermediate metabolities. When excess amount of metals are absorbed by plants toxic effects are produced resulting in the impairment of growth, inhibition of respiration and abnormalities in cell division (Stiles, 1980) and the extent of injury being dependent on the concentration of the metal present.

INTRODUCTION

It has been demonstrated that the toxic metals are capable of causing a reduction in the activity of hydrolases, viz., α-amylase, phosphatase, RNase and protease in germinating seedlings; whereas the activities of catalase, peroxidase, IAA oxidase and ascorbic acid oxidase undergo considerable stimulation. Work on germinating rice seeds revealed that activities of some enzymes like catalase, peroxidase and IAA oxidase increased at the toxic concentration of $HgCl_2$ and $PbNO_3$ which synchronized with the parallel rise in the level of soluble protein (Nag *et al.*, 1980). Excess copper in the germinating medium was shown to have similar effects on lettuce seedlings.

Root growth was inhibited in the presence of Lead in culture solutions (Maitra and Mukherji, 1979) and both intact plants and detached leaves supplied with lead exhibited reduced rates of photosynthesis and respiration. Cadmium, a non-essential toxic element, enters the environment through various industrial processes (Ernst, 1980 and Somashekaraiah *et al.*, 1992) and to lesser extent from natural weathering (Denise *et al.*, 1985). The presence of Hg^{++} in the environment has increased in some areas to levels which threatens the health of aquatic and terrestrial organisms. Hg^{++} was chosen as a probe metal ion because it is a wide-spread trace pollutant of high toxicity with a long biological half-life (Hilmy *et al.*, 1985).

The aim of the present investigation was to check the influence of salts of Sb and Hg on germination behaviour and growth rate as well as on some biochemical responses of germinating *Ragi* seeds. It was also a matter of interest to see whether Sb and Hg would respond in same or in different way.

MATERIALS AND METHODS

Seeds of Eleusin coracana L Gaertn were obtained from OUAT, Bhubaneswar. Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes and washed with distilled water (15' x 3 times), and finally imbibed in distilled water for 12 h. Water imbibed seeds were allowed to germinate in Petri plates on filter papers soaked with different concentration of $SbCl_2$, $HgCl_2$ (1 μM, 100 μM, 1000 μM) and this was considered as zero hour of the experiment.

Germination was allowed in darkness and at a temp. of 25°C $\pm$ 2°C and relative atmospheric humidity of 78% $\pm$ 2%. Germination was allowed to continue for 10 days as was necessary for the present investigation. Percent germination, root/shoot length and total protein measurement were done in an interval of 48 hours; whereas, proline measurement and assay of protease activity were done at the end of the experiment with 10 days old seedlings.

Proteins were extracted from the whole plant tissue as per the procedure described by Jayaraman, (1985) and estimated by Bradford's dye-binding method (Bradford, 1976). Quantitative estimation of proline was done following method of Sadasivan and Manikam, 1992 and assay of protease activity was according to method of Snell and Snell, (1971). The enzyme activity was determined according to Fick and Qualset, (1975).

Ragi is a tropical pulse crop of immense importance for their higher protein contents and for the last two decades it is facing a problem of soil and water pollution by heavy metals. As this is a very common and useful legume, it was selected as our experimental material.

Table 1a. Influence of $SbCl_2$ on seedling growth of *Ragi*

Concentration (M)	% decrease of roots/shoot length over control							
	24 h		72 h		120 h		240 h	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
1 μM	84.64	56.93	25.06	12.68	11.78	25.09	27.27	20.06
10 μM	90.39	80.06	80.09	68.26	83.35	70.60	81.89	70.90
100 μM	96.15	89.23	93.38	79.38	90.59	81.35	89.08	79.90

Table 1b. Influence of $HgCl_2$ on seedling growth of *Ragi*

Concentration (M)	% decrease of roots/shoot length over control							
	24 h		72 h		120 h		240 h	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
1 μM	23.08	36.76	39.00	30.00	5.80	6.26	8.99	10.06
10 μM	59.69	56.93	46.66	33.36	58.80	43.76	58.99	48.59
100 μM	71.15	73.30	76.00	68.29	69.58	71.26	68.28	55.09
1000 μM	100	100	93.36	84.13	88.25	75.09	90.98	75.08

RESULTS

The effect of various concentration of Sb and Hg on per cent germination and seedling growth (Table 1a and 1b) showed that the Sb at a concentration of 100 μM was absolute toxic in contrast to same concentration of Hg. Increasing concentration of both the metals decreased the germination rate. The most effective inhibitory concentration for Sb was 100 μM and for Hg, 1000 μM. At a later period (240 h), inhibitory effect was lesser in comparison with earlier periods. Comparatively, Sb was more inhibitory than Hg.

When the length of root and shoot of the seedlings was measured separately, increasing inhibition with increasing concentration of both the metals was evident. Inhibition of root growth was seen to be more than shoot growth in all the cases. The inhibitory effect of Sb was more pronounced than that of Hg.

There was a steady increase of total protein content upto 120 h and surprisingly enough, this amount decreased when seen at 240 h. When the increment of total protein in Sb treated plant was compared to that of Hg treated plant, Sb treatment seemed to be more effective in increasing total protein. There was also a steady increase with the increase in concentration upto 10 μM. No appreciable increase was noticed after that even though the concentration was increased upto 1000 μM. The decrease of protein at 240 h of treatment was more in Pb-treated plant than in Sb-treatment.

Activity of protease steadily decreased with the increase of concentration in case of both the metals and the rate was more in case of Sb treatment.

Proline accumulation was also more in case of Sb treatment with the increase of concentration and the response was highest in case of root and lowest in leaf (Table 2).

DISCUSSION

In case of germination and seedling growth, both Sb and Hg proved to be inhibitory and the effect of Sb was more pronounced. Photosynthesis and respiration in higher plants are highly sensitive to antimony and mercury and that Sb in particular inhibits chlorophyll biosynthesis by reacting with protochlorophyllide reductase and synthesis of 5-aminolevulinic acid (Bazzaz *et al*., 1974 and Stobart *et al*., 1985). Interaction of heavy metals with functional –SH groups was generally proposed as the mechanism of inhibition for several physiological reactions (Shio *et al.,* 1978 and Sadsmann and Boger, 1980). In our findings it was seen that at a later period (240 h), inhibitory effect of Sb and Hg was lesser in comparison with that of the earlier period. This might be due to initial metabolic impairment for the sudden shock and gradually the tissues triggered on their resistance mechanism inside and as a result, at the later period, the mobilization of protein from cotyledons to actively growing tissues might occur. While plant growth may be severely restricted by heavy metals, plants possess a unique ability to rapidly adapt and evolve tolerance to toxic or lethal levels of heavy metals (Steffens, 1990 and Woolhouse, 1983). Plant cells subjected to heavy metals rapidly synthesize a class of metal binding polypeptides whose function is to sequester and detoxify excess metal ions. Among the common metals, AS is by far the strongest inducer of Physochelatins (Huang *et al*., 1987).

Table 2. Effect of $SbCl_3$ and $HgCl_2$ on accumulation of praline (mg/g fresh wt.) in Ragi seedlings 10 days old).

Concentration(M)	Per cent increase over control					
	Root		Stem		Leaf	
	Sb	Hg	Sb	Hg	Sb	Hg
1 μM	76.69	70.1	60.28	69.29	60.38	60.1
10 μM	139.23	160.47	139.33	131.02	101.73	85.7
100 μM	184.33	175.83	149.93	149.33	120.00	101.2
1000 μM	Lethal	Lethal	Lethal	141.93	Lethal	Lethal

Study with the estimation of total protein also showed increasing pattern with prolonged duration of treatment and decreased at the later part (240 h). This might be due to the fact that with the longer duration the issues reached in a state of extreme toxicity when the protein synthesizing system failed to function or might be some protein degrading mechanism came into work.

Proline accumulation in plant issues can also be considered as a soluble nitrogen sink. Accumulation of praline upon dehydration due to water deficit or upon decreasing osmotic potential has been recorded in bacteria, algae and higher plants. More recently, some authors (Charest and Phan, 1990) have proposed that praline accumulation can play an important role in cellular pH control. Our results of praline accumulation in Ab-treated plants was more than that in Hg-treated plants and this might be due to some osmotic imbalance within the metal treated plants inhibiting the water transport system and leading to water deficit. As root is considered the tissue of primary response in comparison with shoot and leaf, the greater accumulation of praline in root might be due to that.

Fall in the protease activity of cotyledonary reserves as well as disruption in the rate of mobilization of precursors of protein production in the developing seeds causes an accumulation of proteins in the heavy metal treated seedlings. Our findings of decreasing protease activity with the increasing concentration of the metals and longer duration of treatment could be considered in the same perspective.

Sb was more toxic than Hg in the growth of *Ragi* seedlings and the effect was maximum in root than in shoot and leaf.

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