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A STUDY ON INFLUENCE OF CADMIUM AND MERCURY ON GROWTH AND PROTEIN METABOLISM IN COWPEA SEEDLINGS

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Cowpea (*Vigna unguiculata* L. var Pusa Falguni) seeds were germinated in DW (control), $CdCl_2$ ($5x10^{-4}$ M) and $HgCl_2$ ($5x10^{-4}$ M) under laboratory conditions upto 96h. The embryo and cotyledons were studied for protease activity, protein content, amino acid content and proline content. The seedling growth in terms of elongation and weight was inhibited by cadmium and mercury. Root was more sensitive than shoot; mercury was most effective; protein metabolism was altered. Mercury caused more adverse effect. The intensity of the adverse effect may be correlated with alteration in protein metabolism.

Keywords: Cadmium; Cowpea; Growth; Heavy metals; Mercury; Protein metabolism.

Introduction

Presence of cadmium and mercury in the germination media during germination of the seeds is considered as toxic conditions and the toxicity is related with the concentration of heavy metal. Cadmium and mercury have been singled out as especially hazardous by the environmental protection agency[1]. Seedling growth was retarded by heavy metals[2,3]. Heavy metals decreased the metabolic processes of the cell including nucleic acid, protein content[4]. Cadmium and mercury were selected as a source of heavy metals and their effects on growth and metabolism in cowpea seedlings were studied, since cowpea is one of the extensively cultivated pulse crops in India.

Materials and Methods

Cowpea (*Vigna unguiculata* L. var Pusa Falguni) seeds were germinated in the petriplates lined with filter paper in laboratory conditions using distilled water (DW), $CdCl_2$ ($5x10^{-4}$ M) and $HgCl_2$ ($5x10^{-4}$ M) as media upto 96h. Seedlings were analyzed for their growth and biochemical changes at the intervals of 24h. Growth parameters include the elongation of root, hypocotyl, epicotyl and total seedling length. Fresh weight, dry weight and moisture percent of embryo and cotyledon were also recorded. Protein metabolism was studied from the embryo and cotyledon of seedlings in triplicates at the interval of 24h. Protein metabolism includes protease activity[5], protein content[6], total amino acid content[7] and proline content[8].

Results and Discussion

Table - 1 reprsents the data on seedling growth of cowpea seeldings grown in different media. The elongation of root, hypocotyl and epicotyl was increased with increase in growth period. The growth rate of root and hypocotyl in metal treated seedlings was less indicating adverse effect of heavy metals on seedling growth. Data supports the earlier findings[3,9,10]. The adverse effect of heavy metals was more on root than on shoot, indicating root is more sensitive to heavy metals than shoot. Breckle[11] reviewed the effects of heavy metal toxicity on root growth. Heavy metal accumulation is more in root than in aerial parts of th plant.

Data on fesh weight, dry weight and percent moisture of embryo and cotyledon was represented in Table-1. The fresh weight

Table 1. Effect of Cadmium and mercury of growth of cowpea seedlings.

Germination period (h)	Treatment	Root length (cm/seedling)	Hypocotyl length (cm/seedling)	Epicotyl length (cm/seedling)	Total seedling length (cm/seedling)	EMBRYO			COTYLEDON		
						Fr. Wt (g/seedling)	Dry Wt. (g/seedling)	% Moisture (%)	Fr. Wt. (g/seedling)	Dry Wt. (g/seedling)	% Moisture (%)
24	DW	0.88±0.03	1.11±0.02	0.14±0.02	2.13±0.04	0.018	0.004	77.78	0.160	0.058	63.75
	Cd	0.68±0.02	1.33±0.03	0.16±0.02	2.15±0.03	0.021	0.002	90.48	0.150	0.080	46.67
	Hg	0.22±0.02	0.51±0.03	0.11±0.01	0.83±0.04	0.004	0.002	50.00	0.160	0.110	31.25
48	DW	3.38±0.03	2.93±0.03	0.44±0.02	7.75±0.06	0.100	0.012	88.00	0.159	0.050	92.45
	Cd	0.82±0.04	2.72±0.04	0.34±0.03	3.88±0.07	0.070	0.009	87.14	0.152	0.065	57.24
	Hg	0.44±0.02	0.99±0.03	0.32±0.01	1.74±0.04	0.020	0.004	80.00	0.150	0.093	38.00
72	DW	3.98±0.04	6.07±0.03	0.69±0.03	10.72±0.08	0.227	0.032	85.90	0.140	0.045	67.86
	Cd	1.13±0.03	4.57±0.03	0.62±0.03	6.30±0.08	0.125	0.026	79.20	0.150	0.062	58.67
	Hg	0.51±0.02	1.49±0.03	0.35±0.04	2.35±0.06	0.042	0.010	76.19	0.149	0.075	49.66
96	DW	4.54±0.03	7.63±0.04	2.17±0.03	14.34±0.08	0.341	0.045	86.80	0.125	0.045	68.00
	Cd	1.74±0.03	5.18±0.05	0.93±0.04	7.85±0.07	0.229	0.033	85.59	0.150	0.060	60.00
	Hg	0.55±0.02	1.85±0.03	0.45±0.02	2.85±0.02	0.052	0.011	78.85	0.145	0.073	49.66

Mean ± SE

and dry weight of embryo axis was inhibited significantly by mercury. Such effect was seen in 96h old seedlings grown with cadmium. Nassbaum *et al.*[2] and Kalimuthu and Sivasubramanian[9] reported similar results in maize seedlings. The percent moisture is lowered especially in cotyledon of the heavy metal treated seedlings. The poor growth of the seedlings may be correlated with decrease in water uptake under heavy metal stress condition.

Table-2 represent the data on protein metabolism which include protease activity, protein, amino acids and proline contents in embryo and cotyledon of control and heavy metal treated cowpea seedlings. In embryo axis, protease activity was not much changed upto 72h in control seedlings, but increased in 96h old seedlings. In heavy metal treated seedlings and cotyledon, the activity was more when compared to that in control seedlings. Mercury was most effective at 24h. Generally hydrolyzing enzymes are stimulated in stress conditions to provide more nitrogenous substances to the growing axis. In the present study, cadmium and mercury caused stress condition to the cowpea seedlings. Similar results were reproted by Bhattacharyya and Choudhuri[12]

Protein content in embryo axis, was increased with increase in gemination period in control as well as in heavy metal treated seedlings. During 24h, the protein content in heavy metal treated embryo was almost double the amount that was present in control seedlings. In cotyledon, also, the protein content was more in heavy metal treated seedlings. Increase in the time of treatment increased the protein content upto 48h, then, there was a decline in protein content. The heavy metal treated seedlings had more protein content than the control seedlings. Generally, plants synthesize

Table 2. Effect of cadmium and mercury of protein metabolism in cowpea seedlings.

Germination period (h)	Teeatment	EMBRYO				COTYLEDON			
		Protease activity (*)	Protein content (µg/mg dry wt)	Amino acid content (µg/mg dry wt)	Proline content (µg/mg dry wt)	Protease activity (*)	Protein content (µg/mg dry wt)	Amino acid content (µg/mg dry wt)	Proline content (µg/mg dry wt)
	DW	0.941±0.007	43.296±0.525	44.658±0.649	1.951±0.063	1.06±60.032	83.230±0.570	16.508±0.289	0.503±0.009
24	Cd	3.040±0.004	92.405±0.651	50.242±0.424	2.681±0.063	1.573±0.071	9.491±1.125	24.082±0.354	0.974±0.012
	Hg	2.750±0.038	109.253±2.139	55.371±1.454	2.997±0.015	3.026±0.056	178.350±1.348	30.314±0.559	1.384±0.077
	DW	0.893±0.021	73.766±0.221	55.934±0.865	1.492±0.172	0.96±10.018	127.233±1.200	12.696±0.300	0.632±0.010
48	Cd	4.554±0.351	97.881±0.304	92.296±1.755	3.150±0.079	1.056±0.037	149.464±4.301	29.704±0.222	0.899±0.038
	Hg	3.855±0.033	94.878±0.990	98.669±0.807	3.014±0.024	1.496±0.047	155.115±2.963	28.869±0.336	0.955±0.016
	DW	0.906±0.009	79.960±0.657	66.144±1.240	2.122±0.033	0.761±0.020	109.438±0.502	30.124±0.458	1.336±0.51
72	Cd	3.546±0.039	91.111±0.625	106.470±2.229	3.916±0.046	1.024±0.057	160.909±3.200	62.945±2.033	2.934±0.067
	Hg	3.909±0.028	90.233±0.184	110.780±1.769	4.077±0.017	1.108±0.060	149.848±1.123	61.044±0.378	3.108±0.030
	DW	1.576±0.049	85.814±1.840	290.644±2.960	4.581±0.133	3.163±0.026	98.140±0.321	44.503±0.619	1.751±0.088
96	Cd	2.649±0.070	93.832±0.829	367.959±2.416	5.927±0.031	1.786±0.016	111.535±0.637	63.838+0.836	3.021±0.058
	Hg	3.890±0.019	95.645±1.218	396.603±0.464	7.051±0.035	1.593±0.038	110.584±0.879	65.749±0.368	3.431±0.058

Mean ± SE; * µg tyrosine lib/h/mg protein

some specific type of "stress proteins" in stress conditions. This may be one of the reasons for higher quantity of protein in cadmium and mereury treated seedlings. Cadmium binding protein was isolated from tomato roots[13]. Data supports the findings of Bhattacharya[14].

Amino acid content was less in the initial stage but increased with increase in germination period in both control and heavy metal treated cowpea seedlings (Table-2). The amino acid contents in embryo and cotyledons of the heavy metal treated seedlings was more compared to that in control seedlings. The accumulation of amino acids and proline was more in 96h old seelings, suggesting that the longer duration of the treatment results into accumulation of these substances. The amino acid and proline content was more in mercury treated seedlings, suggesting its more adverse effects in plants. Tolerance mechanism in plant is associated with accumulation of amino acids[15] and proline[16,17]. Above data supports the findings of Narwal and Singh[18] and Bhattacharyya and Choudhuri[12].

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