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Research Article

BIOCHEMICAL STUDIES OF HORSE GRAM (*Dolichos biflorous linn.*) USING LEAD ACETATE

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ABSTRACT

The present investigation has been carried to find out the different concentration of lead on biochemical changes of horse gram (Dolichos biflorous Linn.) seedlings.the seeds were procured from tamilnadu agricultural university (TNAU) coimbatore.Three varieties of horse gram seeds were used for this experiment.various concentration of lead acetate solutions were prepared .(5,10,25,50,75,100,250,500 and 1000 ppm by using distilled water.the seedlings under 500 and 1000 ppm lead acetate concentration were found to be leathal.The biochemical studies such as chloriphyll 'a"chlorophyll 'b' total chlorophyll,carotenoid,sugars,starch,amino acids,protien and proline have been studied.

Keywords: Lead acetate, biochemical, aminoacids, Dolichos biflorous.

INTRODUCTION

The term heavy metal refers to all metals and metalloids with the exception of alkali and alkaline earth elements having a density more than five times higher than that of water,specific heat greatr than 4 or 5 atomic weight greater than 20.the metal pollutant released in to the environment due to increased industrialization,mi ning,smelting,combustion of fuel.the heavy metal cause soil pollution are contributed from natural sources,surface runoff,dumping of domestic sewage,metal smelting and industrial effluents.the practice of using sewage sludge and industria effluents as manure which enriching the sioil with heavy metals and has detrimental effects on plants.the main source vof the lead pollution in the environment are petrol, particulates and exhaust fumes from petrol,combustion,fossil fuel combustion in the soil.

MATERIALS AND METHODS

The work has been carried out in laboratory condition to find the effect of different concentrations(control,5,10,25,50,75,100,250,500 and 1000 ppm) of lead acetate on seeds growth ,biochemical changes of(Dolichos biflorous Linn.)

Seed material

The horse gram (Dolichos biflorous Linn.) was purchased from tamilnadu agricultural university (TNAU) at Coimbatore.

Metal solution

1 gm of lead acetate was dissolved in 1000 ml of dissitilled water.(1000 ppm)the various concentrations of lead acetate solutions were prepard from standard solutions using disstilled water. the prepared solutions were more important for biochemical studies.

Biochemical studies

The 9thdayseedlings were seperated in to leaf,shoot and root.they were used for biochemical analysis like chlorophyll'a"chlorophyll 'b' total chlorophyll,carotenoid,sugars,starch,amino acids,protien and proline have been studied by following methods.

Estimation of chlorophyll (Arnon,1949)
0.5 gm of fresh leaf material was ground with pestle and mortar using 10 ml of 80 % acetone.the homogenate was centrifuged at 3000 rpm for 10 minutes.the supernatant was collected and then pellet was re extracted with 5 ml of 80% acetone.All the supernatant were collected and utilized for chlorophyll determination.the absorbance was measured at 645 nm and 663 nm using a spectrophotometer.the chlorophyll content was determined by using the formula ,

$$chlorophyll - a = \frac{12.7 \times A663 - 2.69 \times A.645}{a \times 1000 \times sample\ weight} \times V$$

$$chlorophyll - b = \frac{22.9 \times A645 - 4.68 \times A.663}{a \times 1000 \times sample\ weight} \times V$$

$$chlorophyll - c = \frac{20.2A \times 645 + 8.02A \times A.663}{a \times 1000 \times sample\ weight} \times V$$

Estimation of carotenoid :(Kirk and Allen, 1965)
The procedure for the measurement of carotenoid was same as that of chlorophyll estimation. The extract was measured at 480nm in spectrophotometer. The amount of carotenoid present in the extract was calculated by using the formula.

Carotenoid (mg.g -1) =A480- 0.114 x A663-0.638 x A645

A = length of light path in cm
V = total volume of the extract

Estimation of total sugars (Nelson, 1944)
The samples were treated with 80% boiling ethanol for taking extractions. To 1 ml of extract added with I ml of 1N sulphuric acid and heated in a water bath for 15 minutes. One ml of methyl red and one ml of 0.1 N NaOH was added and heated in a water bath for 10 minutes..1 ml of reagent "C" was added and heated with water bath for 30 minutes. The excess amount of ethanol evaporated. Now 1 ml of arsenomolybdate reagent was added and made up to 25 ml. The solution was measured at 495 nm in spectrophotometer.The sugar of unknown samples were calculated from glucose sstandards.

Estimation of total free amino acids (Moore and stein, 1948)
0.5 g of plant material was ground with 10 ml of 80% ethanol with the help of pestle and mortar.The extract was centrifuged at 800 rpm for 10 minutes. The supernatant was collected and make up to 10 ml with 80 % ethanol.1 ml of extract was taken and added a drop of methyl red,1 ml of 0.1 N NaOH, and 1 ml of ninhydrin reagent and heated in a water bath for 30 minutes. Then the solution was made up to 20 ml with distilled water .The absorbance was measured at 570 nm in a spectrophotometer.

Estimation of protein (Lowry et al., 1951)
0.5 g of plant material was ground with 10 ml of 20 % TCA. The homogenate was centrifuged for 10 minutes at 800 rpm. The supernatant was collected and pellet was re-extracted with 5 ml of 0.1 N NaOH. The supernatant was saved to 1 ml of extract, added 5 ml of reagent C and 0.5 ml of folin phenol reagent was added. The absorbance was calculated at 600 nm in a spectrophotometer.

RESULTS AND DISCUSSION
The various bio chemical changes like chlorophyll,aminoacids,protein content,starch and sugar contents have been recorded on 9th day seedlings.The biochemical changes is response to lead treatment the tolerant variety (Co-1) and susceptible variety (P-2) of horse gram were found to be in the same trend.Chlorophyll estimation is one of the important plant parameters which is used as an index of production capacity of the plants.the chlorophyll content is positively correlated to net photosynthetic rate and hence its rate a major role in controlling grain growth and grain filling process(Liu 1980).
The leaves of chlorophyll 'a', b' and total chlorophyll and carotenoid contents decline with lead treatment in the horse gram. (Co-1 and P-1) varieties.the pigment Contents were gradual decline with the increase of lead concentration.The lowest chlorophyll and carotenoid contents were recorded at 250 ppm concentration in both varieties.An

excess of lead is known to disturb the carbohydrate metabolism.The total sugar and starch content in root and shoot of the horse gram (Co-1,P-2) crop plants decreased with the increasing of lead concentration.The maximum amount of sugar and starch content have been found at control and decreased up to 250 .

The Co-1 variety performs better than the P-1 and P-2 varieties under lead treatment. Hence it is recommended to cultivate the C0-1 variety of horse gram in the near by areas of the lead discharging industries. It will minimize the harmful effect of lead on the growth of the crop to a great extent.

Table shows photosynthetic pigment (mg g^{-1} fr.wt) contents of horse gram [dolichos bi florus(Linn.)] Var, Co-1 under lead treatment

Concentration (ppm)	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	carotenoid
Control	0.865	0.698	1.563	0.658
5	0.844	0.671	1.515	0.627
10	0.764	0.548	1.312	0.558
25	0.685	0.592	1.277	0.508
50	0.628	0.495	1.123	0.454
75	0.549	0.428	0.977	0.387
100	0.478	0.364	0.842	0.354
250	0.388	0.291	0.679	0.317

Table shows photosynthetic amino acid & protein (mg g^{-1} fr.wt) contents of horse gram [dolichos bi florus(Linn.)] Var,Co-1 under lead treatment

Concentration (ppm)	Amino acid		Protein	
	Root	Shoot	Root	Shoot
Control	1.553	2.409	1.532	1.965
5	1.483	2.210	1.454	1.826
10	1.402	2.148	1.380	1.682
25	1.360	2.061	1.285	1.593
50	1.282	1.893	1.212	1.460
75	1.209	1.798	1.158	1.365
100	1.148	1.623	1.104	1.310
250	1.006	1.386	0.943	1.186

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