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EFFECT OF HEAVY METAL TOXICITY STUDIES ON BIOCHEMICAL ATTRIBUTES IN *VIGNA UNGUICULATA* (COW PEA)

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ABSTRACT

Cow pea is valued primarily for its pod and seeds, used as food, the plants after harvesting, serve as valuable for livestock, green manure crop for soil improvement. Cow pea seeds were exposed to various heavy metals like Cadmium Oxalate, Nickel Sulphate, Copper Sulphate, Lead Nitrate, Silver Nitrate which was about 10grams dissolved in water along with control which was received only water. Biochemical attributes like Total reducing sugar, Total chlorophyll, Glycine, Protein and Reduced Glutathione were analysed in leaves of 6th day, 8th, 12th day Cow pea seedlings.

KEY WORDS: Cow pea, heavy metal, seeds, biochemical, manure.

INTRODUCTION

The seed is a miniature plant in a latent state, encomposing all the characteristisc of the type cultivate to which it belongs. All viable seeds germinate after a reasonable time, and normally develop into seedlings. The transition of the seed from the resting phase to one of the activity is known as Germination. Seed germination is the renewal of growth of dormant embryo generally caused by change in the environment. This is a natural process, which is governed by internal mechanisms within the seed and by external environment conditions.

The factors which control the germination are numerous. There are diverse type of stressors that may affect physiological patterns either in a positive or negative manner. The influence of particular stressor in the various physiological process of a plant is not always equally expressed. When the stress is unpredictable the appropriate responses will depend upon the severity of the stress and the amplitude of constitutive resistance.

Physiology of plants under stress condition is an important subject area for many disciplines which include ecology, plant physiology and plant biochemistry. The discovery process of stress induced physiological and biochemical changes can result in new tools for evaluating other physiological processes the plants employee to cope up with environmental stress.

Cowpea structure

Cowpea is a suberect; trailing or climbing, bushy annul with glabrous stems, found in the warmer parts throughout india; mostly under cultivation leaves pinnate; leaflets 7.5-15cm, long ovate; rhomboidal or broad or narrowly ovate; flowers in racemes; white; pale violet or purple in colour with an yellow eye turning yellow when faded; odds variable, upto 90cm long; 10-20 seeded; seeds varying in size, shape and colour.

Cultivation

In India it is grown as a vegetable garden plant in most parts of the country. As a field crop it is grown on a very limited scale in the high monsoon tracts of south west India. It is generally cultivated as a mixture with other cereals, mainly Kariff millets and ocasionally as a pure crop. Cowpea is a multipurpose legume and fits well in rotation with many crops. viz. wheat Barley, cotton, jowar, millets, rice etc.

Heavy Metals

Heavy metal are elements having a density greater than 5 in their elemental form and compromise some elements. However the term usually refers to 12 metals that are used and discharged by industries i.e. Cadmium, Chromium, Cobalt, Copper, Mercury, Manganese, Nickel, Lead, Tin, and Zinc.

Environmental processes are complex mixture of biological and abiotic reactions. The bulk of the elements are naturally bound as insoluble inorganic compounds in rocks and sediments. Heavy metals are among the first concern. When released in the environment by industrial activities or transportations, metals have a residence time in the soil and may continue to exert harmful effects on the biota.

Rise in the levels of heavy metals in the soil could be attributed to many factors such as soil properties of Agricultural Manufacturing, Mining, waste disposal activities applications of sewage sludge to agricultural land (Foy *et al*.,1978) polluted soils by heavy metals have ecological, biological and health significant.

Heavy metals are very toxic to both plants and animals. The toxicity of heavy metal is mainly attributed to their ability of binding to enzymes, resulting in the alteration of their catalytic functions and inactivation.

Accumulation of one metal can minimize the metabolism of another metal because certain metals, viz. Magnesium, Molybdenum and Iron are essential constituents of certain enzymes, pigments and aminoacids. The high concentration of non-essential metals may caused the toxic effect o plants.

Cadmium

Cadmium is used for metal plating for nickel cadmium and other batteries, for manufacturing pigments and plastic stabilizers, pesticides and alloys.

Nickel

Nickel is used to mark alloys permanent magnet materials and nickel-cadmium batteries and electroplating and ceramics.

Copper

Copper and many of its compounds are used in various industries. Copper metal is mainly used to make electrical wires and cables since it is an excellent electrical conductor.

Lead

Lead is used in the production of some types of batteries, electrical equipment, television glass, metal products such as lead sheet soldier and pipes ceramic glasses.

Biochemical Changes

Many biochemical changes is observed in plants by heavy metal toxicity. The levels of chloroplast pigments chl-a, chl-b, carotenes and xanthophylls decreased with the increasing concentration of Pb, Cd, Hg (Madhavi and Charyulu,1998).

Heavy metals caused the accumulation of aminoacids and Proline later during germination, particularly in the endosperm. Accumulation of Proline content in the endosperm is suggested as a biochemical marker for heavy metal stress (Nagoor and Vyas, 1998).

Heavy metal inhibits the synthesis of chlorophyll and alter the activities of catalase and peroxidase (Agarwala *et al*., 1977). Isoenzymes of peroxidase and amylase not present in untreated controls, were found in cotyledons and plumule radicles of heavy metals treated plants. (Ayaz and Kadioglu, 1996).

MATERIALS AND METHODS

Vigna unguiculata (Cowpea) seeds were obtained from Parry Mansanto Seeds Ltd, Kattur in Trichy. Healthy seeds were surface sterilized with 0.1% Mercuric chloride for 2mins, washed thoroughly with distilled water to remove traces of Mercuric chloride and then stored in polythene bags in dark .The seeds were subjected to various heavy metals stresses such as $AgNo_3$, $PbNo_3$, $CuSo_4$, $NiSo_4$, CdOx during germination. The treatments were given continuously for 18 days. During the treatment period germinating seedlings were collected on day 6, day 12, day 18.The collected seedlings were estimated for the following parameters like chlorophyll (Harborne,1973), Reducing sugar (Dubois *et al*,1956), Protein (Lowry *et al*.,1951), Glycine (Moore and Stein,1948), Reduced Glutathione (Ellman,1959).

Statistical Analysis

All quantitative measurements are expressed as mean values.

RESULTS AND DISCUSSION

The biochemical effect of various physical stress on Cowpea has been compared and enumerated in this study. After 6 days, 12 days, 18 days of invitro culture growth and biochemical attributes with reference to Reducing sugar, Chlorophyll, Glycine, Protein and Reduced Glutathione.

Table 1 shows that on day 12 chlorophyll level was decreased .The loss in chlorophyll corresponds to the pollutant heavy metals level(Dubey,1991). Aminolevulinic acid hydratase is affected by Pb invitro. Aminolevulinic acid dehydratase catalyses the conversion of 2 molecules of ALA into one molecule of porphobilinogen thus regulating chlorophyll biosynthesis.

TABLE.1. ESTIMATION OF CHLOROPHYLL (microgram/gram fresh weight).

	Std	$PbNo_3$	$CuSo_4$	$NiSo_4$	CdOx	$AgNo_3$
DAY 6	0.25	0.17	0.22	0.21	0.16	0.2
DAY 12	0.58	0.33	0.35	0.42	0.31	0.38
DAY 18	0.74	0.64	0.66	0.67	0.64	0.66

TABLE 2.ESTIMATION OF REDUCING SUGAR (mg/g fresh weight).

	Std	$PbNo_3$	$CuSo_4$	$NiSo_4$	CdOx	$AgNo_3$
DAY 6	0.06	0.01	0.0	0.02	0.01	0.01
DAY 12	0.15	0.02	0.06	0.05	0.01	0.05
DAY 18	0.21	0.08	0.09	0.06	0.03	0.08

Table 2 shows that the total reducing sugar was found to be reduced as the concentration of $AgNo_3$, $NiSo_4$, $CuSo_4$, $PbNo_3$, CdOx increases .This may be attributed to the high loss of energy by plants but survive in the hostile environment and also to the education in the chlorophyll -a which is needed for the photosynthesis apparatus similar results were observed by Ramadoss (1979).

TABLE 3.ESTIMATION OF PROTEIN (mg/g fresh weight).

	Std	$PbNo_3$	$CuSo_4$	$NiSo_4$	CdOx	$AgNo_3$
DAY 6	0.023	0.006	0.013	0.006	0.003	0.005
DAY 12	0.05	0.01	002	0.01	0.005	0.01
DAY 18	0.07	0.02	0.04	0.02	0.01	0.02

From Table 3 it is evident that the concentration of Protein in the stressor treated seedlings how remarkable variation when compared to the control. Seedling under the heavy metal stress show an increase in the protein concentration .The increase in the protein levels might be the reflection of gene induction in the response to various heavy metal stresses. Seedling treated with heavy metal like ($AgNo_3$) show marked decline in protein concentration. It leads to protein inhibiti0n of early seedling growth.

TABLE 4.ESTIMATION OF GLYCINE (mg/g fresh weight).

	Std	$PbNo_3$	$CuSo_4$	$NiSo_4$	CdOx	$AgNo_3$
DAY 6	0.025	0.02	003	0.01	0.02	0.01
DAY12	0.04	0.03	0.05	0.03	0.03	0.03
DAY 18	0.06	0.05	0.06	0.05	0.04	0.05

The variation in the concentration of Glycine has been tabulated in Table 4. The pattern of variation in different type of stress is found to be consistent. Heavy metal treated seedling was showed that decreased glycine content.

TABLE 5. ESTIMATION OF REDUCED GLUTATHIONE (microgram/mg fresh weight).

	Std	$PbNo_3$	$CuSo_4$	$NiSo_4$	CdOx	$AgNo_3$
DAY 6	0.02	0.01	0.01	0.02	0.02	0.02
DAY 12	0.05	0.03	0.03	0.03	0.03	0.03
DAY 18	0.07	0.05	0.06	0.06	0.04	0.04

From Table 5 Reduced Glutathione has increased with increase of the concentration of heavy metals like $AgNo_3$, $CuSo_4$, $NiSo_4$, CdOx, $PbNo_3$ is a plant protector which is synthesized during stress condition. Its increases and decreases as the concentration of $CuSo_4$, $NiSo_4$ becomes two much to resist. Heavy metals such as copper and cadmium induce Active Oxygen Species which include superoxide radicals, hydroxyl radicals and hydrogen peroxide through a series of redox reactions, leading to oxidative stress and lipid peroxidation (De Vos *et al.,* 1992, Halliwell and Gutteridge, 1984). These reactive electrophile have inhibitory effects on enzymes such as adenylate cyclase and inhibit DNA and Protein synthesis.

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