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EFFECT OF ARSENIC (AS) TOXICITY ON THE PHYSIOLOGICAL ACTIVITY OF GROUNDNUT (*ARACHISHYPOGAEA*L.)

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ABSTRACT

Arsenic (As) contamination in environment, from both anthropogenic and natural sources occurs is a global problem. (As) is a metalloid having properties of both metals and non-metals, and can undergo different ranges of chemical interactions in plants. The present investigation was conducted to study the different treatment of Arsenic (As) on pigment content, biochemical parameters and enzymes activity of groundnut (Arachishypogaea L.). The Arsenic (As) at all concentrations (Control, 5, 10, 15, 20 and 25 mg L^{-1}) exhibited significant reduction in pigment content, total sugars, starch, amino acid and protein content of groundnut seedlings. Arsenic (As) induced increased in these enzymes activity proline, catalase, peroxidase and polyphenol oxidase. Arsenic (As) is known to induce oxidative stress in the seedlings, including readjustment of transport and metabolic processes and growth inhibition in the groundnut seedlings. Arsenic (As) a result, the electrontransport processes are impeded developing toxic systems. Several toxic Reactive Oxygen Species (ROS) aregenerated in the cell wall region as well as inside the cell during the process, which affects membrane permeability, enzyme activity, metabolic pool, plant biomass, leaf chlorosis and necrosis.

Key words: Arsenic (As), Toxic effect, Pigment content, Enzymes activity, *Arachishypogaea*L.

INTRODUCTION

A rapid industrialization and its use in agriculture had led to provincial and universal redistribution of metals with resulting environmental pollution. The role of environmental pollution to produce an assortment of types of deleterious effects on diverse living system has been well established. Heavy metals are the most hazardous pollutants as they are non-degradable and get accumulated and become toxic both to plants & animals [1]. Heavy metals constitute a very assorted group of elements widely varied in their chemical properties and biological functions. The term "heavy metals" defined as commonly held for those metals, which have specific weights more than 5g cm^{-3}[2]. Heavy metals are kept under environmental pollutant category due to their toxic effects in plants, human and food. Some of the heavy metals i.e. Arsenic (As), Cadmium (Cd), Lead (Pb), Mercury (Hg) are cumulative poison. These heavy metals are persistence, accumulate and not metabolized in other intermediate compounds and do not easily breakdown in environment. These metals are accumulating in food chain through up take at primary producer level and than through consumption at consumer level [3].

Arsenic (As) naturally occurs in over 200 different mineral forms, of which around 60% are arsenates, 20% are sulfides and sulfo salts and the rest 20% are arsenides, arsenites, oxides, silicates and elemental arsenic [4]. The source of arsenic is mainly geological, but anthropological actions like mining, burning of fossil fuels and uses of pesticides also cause arsenic contamination [5]. Arsenic (As) is an extensive natural element, which not a bioorganic element is to plants [6]. In terrestrial plants, both organic and inorganic Arsenic (As) species have been found [7 and 8], with the inorganic species (Arsenate [As (V)] and arsenite [As (III)]) being the most dominant. Arsenate is the predominant as species in aerobic soils, whereas arsenite dominates under anaerobic conditions [9]. Arsenic availability to plants is greatly influenced by its forms in soil. Agricultural application of arsenicals has introduced many different kinds of arsenic compounds to the soil environment, and the presence of Copper Chromated Arsenate-(CCA) treated wood. In this context, environmental investigators features the tricky challenge of determining whether arsenic detections at a site imitate the local soil type oranthropogenic inputs, particularly when detected in the upper range of arsenic concentrations thought to occur naturally. These arsenicals may influence arsenic mobility and plant uptake though they are subjected to oxidation-reduction transformation in soils [10].

However, little is known about the toxic effect of arsenic on photosynthesis, the basis of plant biochemical content. As almost all of the above mentioned adverse physiological and agronomical effects of Arsenic (As) are related to the basic photochemical reaction in groundnut seedlings, the photosynthesis, it is important to measure the chlorophyll a and b, the major photosynthetic pigments, biochemical contents in groundnut seedlings. The present study was undertaken to assess the toxic effects of arsenic concentrations on chlorophyll contents, biochemical parameters and enzymes activity of groundnut (*Arachishypogaea*L).

MATERIALS AND METHODS

Seed

The seeds of groundnut (*Arachishypogaea* (L.) var. VIRGn7) were obtained from Regional Research Institute, TNAU, Periyar Nagar at Virudhachalam taluk, Cuddalore district, Tamil Nadu, India. The seeds with uniform size, colour and weights were chosen for experimental purpose. Seeds were surface sterilized with 0.1 percent mercuric chloride solution and washed thoroughly with tap water and then with distilled water.

POT CULTURE EXPERIMENTS

The experiments were conducted on Jan - 2016.Groundnut (*Arachishypogaea* (L.) plants were grown in plastic pots in untreated soil (control) and in soil to which arsenic salts had been applied (Control, 5, 15, 15, 20 and 25mg L^{-1}). The inner surfaces of pots were lined with a polythene sheet. Each plastic pot contained 1kg of air dried soil. Ten seeds were sown in each pot. Then 250 ml solution of above concentrations was given in each pot kept in net house. Untreated pots were maintained as control (added with 250ml distilled water) to compares the results. The plants were irrigated with tap water as and when needed. Each treatment including the control was replicated five times. Plants were analyzed for all parameters after 15 days of sowing (DAS).

Biochemical analysis

Chlorophylls [11], Carotenoid [12], sugars [13], starch [14], amino acids [15], proline [16], protein [17] contents and enzyme assay like *viz.,* Catalase [18], Peroxidase and Polyphenol oxidase [19] were estimated for the sampled plants.

Results and discussion

Effect of Arsenic (As) on the chlorophyll 'a', 'b', total chlorophyll and carotenoid content of groundnut is represented in (Table 1). The highest value of pigment content was recorded at control plants (*viz.,* 3.593, 1.224, 4.817, and 1.946). 5 (mg L^{-1}) Arsenic (As) level showed a decrease in chlorophyll 'a', 'b', total chlorophyll and carotenoid content. The lowest value of pigment content was recorded at 25mg L^{-1} of Arsenic (As) level (*viz.,* 0.142, 0.1, 0.242 and 0.16) in 15th DAS of groundnut seedlings.

Heavy metals inhibit metabolic processes by inhibiting the action of enzymes, and this may be the most important cause of inhibition. Decreased chlorophyll content associated with Arsenic (As) stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis [20].Several reports show chlorophyll biosynthesis inhibition by metals in higher plants [21].

The decline in chlorophyll content in plants exposed to heavy metal stress is believed to be due to: Inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (ALAdehydratase) and protochlorophyllide reductase [22] associated with chlorophyll biosynthesis; impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls; Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase [22]; the replacement of Mg^{2+} ions associated with the tetra pyrrole ring of chlorophyll molecule. Our results of decrease in chlorophyll content corroborated with the findings of [23] who also found a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum*. The loss in chlorophyll content can consequently direct to disruption of photosynthetic machinery.

The other results of the effects on Arsenic (As) on the biochemical content such as total sugars, starch, amino acids, protein and proline content of groundnut is represented in (Table 2).

The highest value of biochemical content was recorded at control plants (*viz.*, 7.654, 6.945, 5.982, 10.45 and 0.98) in 15th DAS of groundnut seedlings. Added 5 (mg L^{-1}) Arsenic (As) level showed a decrease in total sugars, starch, aminoacids, protein and proline content when compared to control. The lowest value of biochemical content was recorded at 25mg L^{-1} of Arsenic (As) level (*viz.*, 2.002, 1.308, 1.59, 2.488 and 3.987) in 15th DAS of groundnut seedlings.

The decrease in total sugar content of stressed leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate. Higher starch accumulation in damaged leaves of the experimental seedlings may result both in the higher resistance of their photosynthetic apparatus [24] and low starch export from the mesophyll. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulosebisphosphate carboxylase [25]. The reduction in sugar contents may be attributed to reduction in chlorophyll contents of the leaf and also a decline in protein. This change might have already affected the photosynthetic activity of the plant and hence the reduction in contents [26 and 27]. The decrease in protein content in groundnut plants may be caused by enhanced protein degradation process as a result of increased protease activity [28] that is found to increase under Arsenic (As) stress conditions. It is also likely that these heavy metals may have induced lipid peroxidation in the seedlings and fragmentation of proteins due to toxic effects of reactive oxygen species led to reduced protein content [29].

The translocation of heavy metals in plants is dependent upon the carbohydrate partitioning, which is under control by the effects of heavy metals. From this point of view, it is quite clear that, plants under heavy metals treatment might be largely affected in terms of their soluble carbohydrate (starch and sugar) concentration [30].

It is also proposed that proline acts as a source of carbon and nitrogen for rapid recovery from the stress and acts as a stabilizer of plasma membrane and some macromolecules and free radical scavenger [31] thereby, protecting the plants under extreme stress conditions [32]. The enzyme assay like *viz.*, Catalase, Peroxidase, Polyphenol oxidaseit was found to be increased with the increasing concentration of Arsenic (As). Catalase is antioxidant and scavenging enzyme catalase is special type of peroxidase enzyme which catalase the degradation of H_2O_2, which is

natural metabolite and also toxic to plants [33]. AAS study reported that the increasing concentration of nickel caused an accumulation in nickel treated plants. Higher concentration was observed in 10mM nickel treated plant. This was in consonance with the reports of [34].

Below the metal-stress circumstances, including excess nickel exposure, an imbalance between generation and removal of ROS arise in plant tissues [35]. Peroxidase and catalase activities are essential components of plant antioxidant defence system. The enhance activity of Peroxidase in excess nickel treated plants might result moreover in peroxidative damage of the thylakoid membrane or lower auxin and protein contents in tissues [36]. The actions of catalase and Peroxidase protect the metabolism in plant cells [37]. The conclusion of the study illustrates the effect of arsenic on some plant physiological parameters. Arsenic (As) generates a considerable stress in groundnut seedlings, regardless of the forms of it, and as a result, the photosynthetic pigments, and proteins were suppressed. Application of Arsenic (As) recorded the lowest values of photosynthetic pigments, protein, starch and total sugars. The increasing Arsenic (As) concentration also enhanced increased the enzymes activities.

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Tbale.1 Effect of Arsenic (As) on change pigment content (mg/g fr. wt.) of groundnut (*Arachis hypogaea* L.) on 15th Days.

(As) added in the soil (mg L^{-1})	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Carotenoid
Control	3.593 ± 0.17965	1.224 ± 0.0612	4.817 ± 0.24085	1.946 ± 0.0973
5	2.703 ± 0.13515	0.914 ± 0.0457	3.617 ± 0.18085	1.002 ± 0.0501
10	1.513 ± 0.07565	0.524 ± 0.0262	2.037 ± 0.10185	0.738 ± 0.0369
15	0.383 ± 0.01915	0.286 ± 0.0143	0.669 ± 0.03345	0.535 ± 0.02675
20	0.218 ± 0.0109	0.205 ± 0.01025	0.423 ± 0.02115	0.21 ± 0.0105
25	0.142 ± 0.0071	0.1 ± 0.005	0.242 ± 0.0121	0.16 ± 0.008

± Standard deviation

Table 2. Effect of Arsenic (As) on the changes of biochemical and enzymes activity (mg/g fr. wt.) of groundnut (*Arachis hypogaea* L.) on 15th Day

(As) added in the soil (mg L^{-1})	Total sugar	Starch	Amino acid	Protein	Proline	Catalase	Peroxidase	Polyphenol oxidase
Control	7.654 ± 0.383	6.945 ± 0.347	5.982 ± 0.299	10.45 ± 0.523	0.98 ± 0.049	0.674 ± 0.034	0.487 ± 0.024	0.591 ± 0.0295
5	6.02 ± 0.301	5.52 ± 0.276	4.802 ± 0.240	8.492 ± 0.425	1.58 ± 0.079	0.98 ± 0.049	1.113 ± 0.055	1.42 ± 0.071
10	4.864 ± 0.243	3.866 ± 0.193	4.11 ± 0.206	7.212 ± 0.361	2.08 ± 0.105	1.662 ± 0.083	1.865 ± 0.093	2.63 ± 0.1315
15	3.201 ± 0.160	3.02 ± 0.151	3.215 ± 0.161	6.58 ± 0.329	2.90 ± 0.145	2.086 ± 0.104	2.844 ± 0.142	3.224 ± 0.1612
20	2.91 ± 0.145	2.224 ± 0.112	2.001 ± 0.101	3.785 ± 0.190	3.21 ± 0.160	2.979 ± 0.150	3.738 ± 0.186	3.988 ± 0.1994
25	2.002 ± 0.100	1.308 ± 0.065	1.59 ± 0.080	2.488 ± 0.124	3.206 ±0.160	3.002 ± 0.150	4.482 ± 0.224	5.002 ± 0.2501

± Standard deviation