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Codium mediated anatomical improvement in *Trigonella foenum-graecum* shoot under mercury stress

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Abstract

The ability of green seaweed *Codium iyengrii* was analyzed to biosorb toxic metal mercury from Trigonella foenum-graecum. Plants were grown in randomized block design, supplied with varying concentrations of mercury (0, 5, 10, 15, 20, 25ppm Hg) in the soil separately and at the same time with seaweeds. Toxic effects of mercury and its biosorption by green seaweeds were examined on stress indicators such as phenol and proline contents. The anatomy of shoots was observed under scanning electron microscope (SEM). It was found that stress caused by Hg stimulate synthesis of phenolic substances and proline contents. Micrographs of shoots under mercury stress showed damaged internal structures, thick walled and blocked cell lumen. Application of *Codium iyengrii* powder in mercury contaminated soil enhanced the normal conditions of plant body by lowering the amount of stress indicators, and controlling thickening and blocking of cell wall in vascular tissues of shoot.

Keywords: *Codium iyengrii, mercury, Trigonella foenum-graecum. Phenol, Proline, electron microscopy*

Introduction

Agricultural lands are being devastated with numerous hazardous compounds, causing serious health problems (Patra *et al*., 2004; Askari *et al*., 2007; Askari and Azmat, 2013) [1, 2, 16]. The contaminants include microbes, metals or heavy metals of which heavy metals like mercury are the first and foremost concerns because they are persistent and non-biodegradable (Askari and Azmat, 2015) [19]. According to Ramachandra *et al*., (2003) [20] contaminants may be classified as, particulate contaminants, thin film contaminants, microbial or biological ones. Pakistan is facing serious environmental threats like water pollution from raw sewage, industrial waste, and agricultural runoff. A majority of the population does not have access to potable water, deforestation, as well as soil erosion are the current issues. Biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals or other types of molecules or ions from even very dilute aqueous solutions (Volesky,1986) [28]. It is the ability of biological material to accumulate heavy metals from waste water through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992) [9]. This process is characterized as less disruptive and can be often carried out on sites, eliminating the need to transport the toxic material to treatment sites (Gavrilescu, 2004) [11]. The biosorbents should be low cost, highly efficient, contain minimum amount of chemical and biological sludge (Kratochvil and Volesky, 1998a) [14]. Present study was based on question that whether *Codium iyengrii* could alleviate Hg stress by improving anatomical structure in Trigonella foenum-graecum.

Materials and Methods

Three sets of soil bags were prepared, each with 1 kg of garden soil. First set named control plants was provided with distilled water only, second set named experimental plants was provided with different concentrations of mercury (0,5,10,15,20 & 25ppm Hg) and third set of soil bags was provided with different concentrations of mercury along with 3gm of seaweeds/kg soil (Askari and Azmat, 2013 & 2015) [1, 19]. Seeds of Trigonella foenum-graecum were surface sterilized with bleach followed by repeated washing with distilled water. The soaked seeds were carefully sown. The plants were harvested after 15 days of germination for analysis of Proline contents by Bates *et al.* (1973) [4] method and Phenols by Swain and Hillis (1959) [25] method. Anatomical analysis of vascular tissues was done under scanning electron microscope (SEM). Transverse sections of fresh plant material were gold coated in an auto coater and were observed under SEM ,their images were saved

The aim of present work was to develop a better understanding about the capabilities of green seaweed, the *Codium iyengarii* to biosorb toxic levels of mercury in plants. Stress indicators such as phenol and proline contents of experimental and treated plants of *Trigonella foenum-graecum* were determined and compared for anatomical features of shoot after its interaction with mercury and seaweeds.

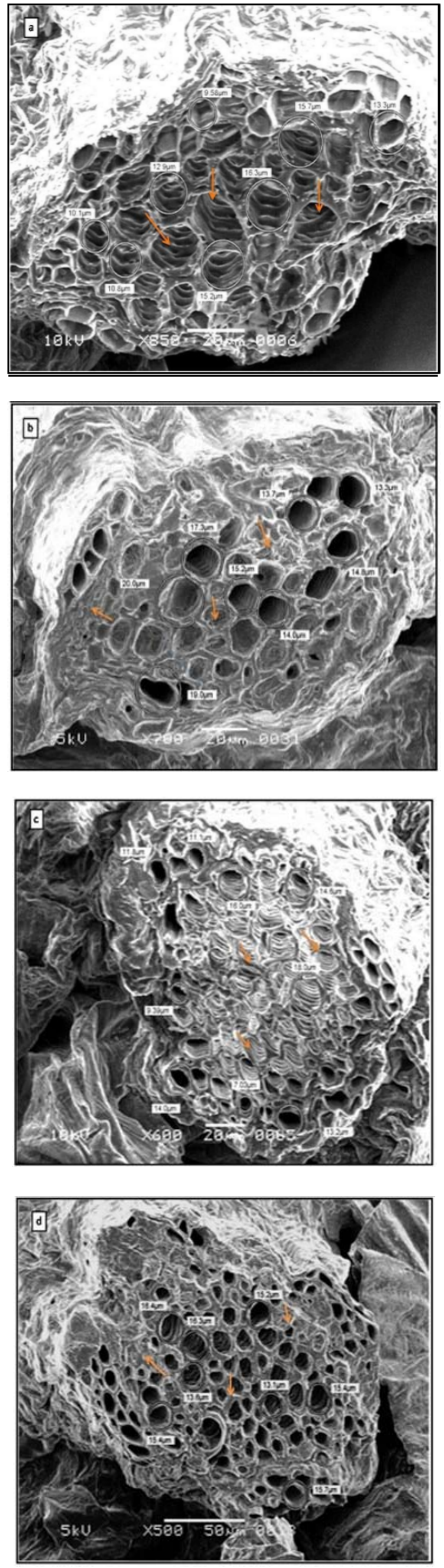
Result

Changes in Phenol and Proline Contents

Results obtained from biochemical analysis of *Trigonella foenum-graecum* indicated a proportional increment in Phenol contents of *Trigonella foenum-graecum* increased significantly up to5.436±0.0145, 5.553±0.0233, 6.540±0.0152, 6.550±0.023 and 6.753±0.0088µmol/gm f.wt respectively at all applied levels of mercury over control which was 5.126±0.012µmol/gm f.wt. The toxic effects of mercury were significantly improved by the application of green seaweeds as 5.286±0.0032, 5.423±0.0088, 6.216±0.0088, 6.460±0.01 and 6.716±0.0259µmol/gm f.wt respectively. Results given in table revealed that proline contents increased upto 1.826±0.012, 2.133±0.0881, 2.160±0.0208, 2.416±0.0176 and 2.510±0.01527µmol/gm f.wt respectively at all applied concentrations of mercury whereas control was 1.453±0.0202µmol/gm f.wt. Toxicity of mercury was significantly adsorbed in *Trigonella foenum-graecum* by the application of green seaweedsupto1.543±0.0088, 1.546±0.0088, 1.736±0.0066, 2.066±0.0185 and 2.413±0.0145 respectively (Azmat *et al*., 2006) [3].

Histomorphology of *Trigonella foenum-graecum* shoot under scanning electron microscope (SEM)

Trigonella foenum-graecum is a dicot plant belongs to family Fabaceae. Collateral, conjoint and open vascular bundles and large pith is the characteristic feature of dicot stem. The micrograph of *Trigonella foenum-graecum* stem at 0ppm Hg under scanning electron microscope (SEM) exhibited normal conditions (Figure, a) like thin walled alive cortical cells, prominent vascular tissues especially xylem and large pith with thin walled and opened cell lumen. Plants grown in mercury contaminated soil exhibited various anatomical anomalies such as disintegrated and disorganized cortical cells, collapsed and blocked vascular tissues (Choudhry and Khan,2007) [6] deformed and thick walled pith cells (Figure, b, d, f, h & j). These abnormalities in internal tissues may be due to shortage of water, oxidative stress, and off course due to synthesis of stress indicator lignin which is obvious in micrographs of all experimental plants. Lignin is a phenolic compound (Table) which increase in stress and provide mechanical support to the cells (Roy and Bera, 2002b; Pedersen, 1984) [5, 18]. Almost all micrograph of *Trigonella foem graecum* indicated a pronounced progress by green seaweed treatment (Figure, c, e, g, i & k). Cortical cells are thin walled, lively and organized. Vascular tissues especially xylem is prominent with open lumen. Parenchymatous cells of pith are thin walled and loosely arranged with large cell lumen. Seaweed have a potential to biosorb on its surface a heavy amount of toxic metal mercury and makes the soil free of contaminations (Patra and Sharma, 2000; Staden *et al*., 2002; Askari *et al*., 2007; Azmat *et al*., 2006; Igwe *et al*., 2008; Kumar *et al*, 2009; Askari and Azmat 2013; Askari and Azmat, 2015) [17, 24, 2, 3, 12, 15, 1, 19].



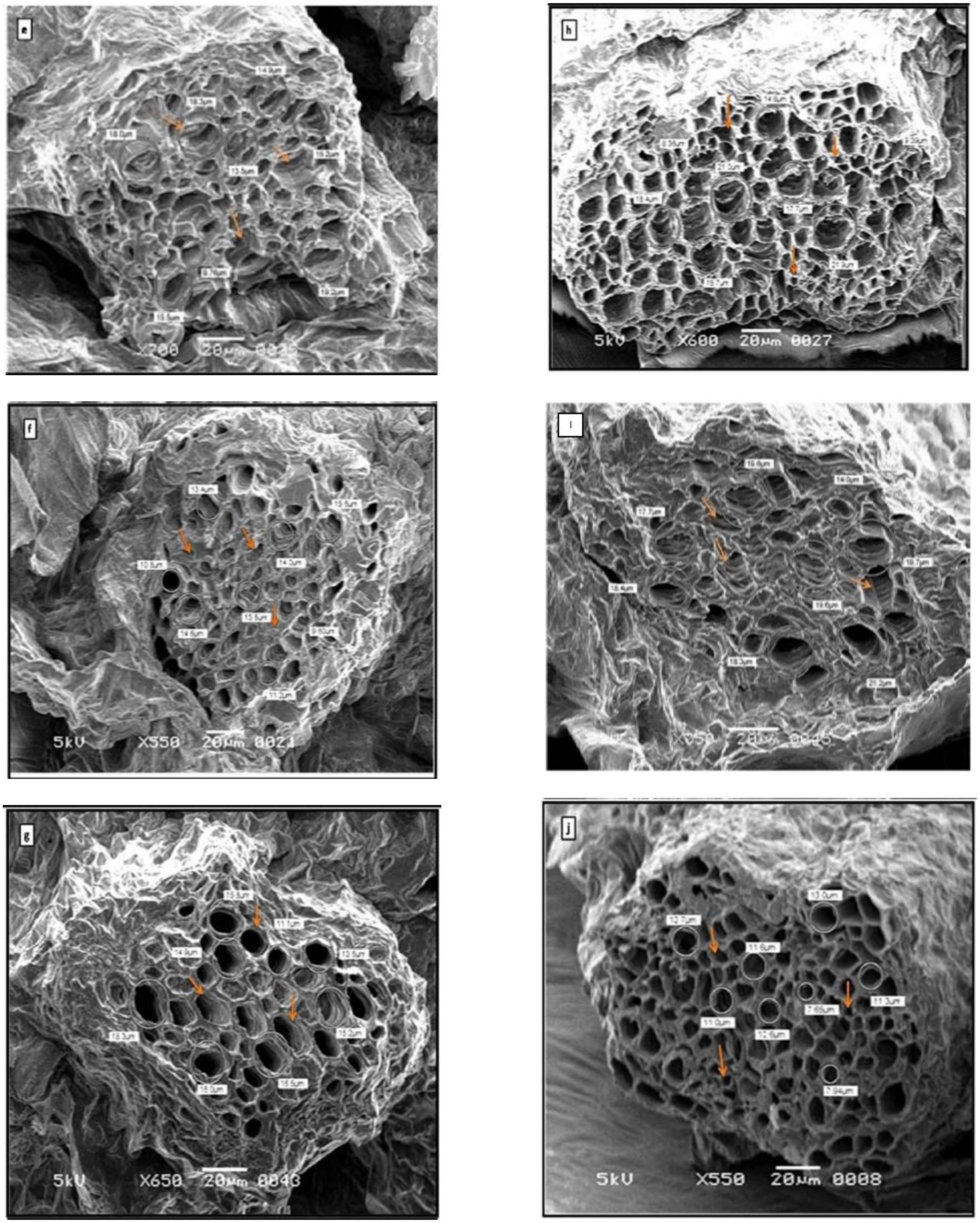


Fig: a (Control plant) Showing thin walled parenchymatous pith with large cell lumen. Figures: b, d, f, h, j (Experimental plant supplied with Hg) showing most of the parenchyma cells especially in pith region became thick walled or blocked and deformed with reduced cell lumen. Figures: c, e, g, i, k (Treated plant supplied with Hg+SW) showing thin walled loosely arranged parenchyma cells in pith region with large cell lumen.

Table: effect of Hg and seaweed on phenol and proline (µmol/gm f.wt)

Hg.[ppm]	Experimental Plants		Treated Plants	
	Proline	Phenol	Proline	Phenol
0	1.453±0.0202	5.126±0.012	1.453±0.0202	5.126±0.012
5	1.826*±0.012	5.436*±0.0145	1.543**±0.0088	5.286**±0.0032
10	2.133*±0.0881	5.553*±0.0233	1.546**±0.0088	5.423**±0.0088
15	2.160*±0.0208	6.540*±0.0152	1.736**±0.0066	6.216**±0.0088
20	2.416*±0.0176	6.550*±0.023	2.066**±0.0185	6.460**±0.01
25	2.510*±0.01527	6.753*±0.0088	2.413**±0.0145	6.716±0.0259

Result express as Mean ± SEM of triplicate. Marked effects are significant at $p < 0.05000$ (*indicated significant values of experimental plants over control ones, whereas ** shows significant values of treated plants over experimental). n=3

Discussion

Plants produce phenolic compounds in response to environmental stress such as contaminated soil due to salts and heavy metals, microbes and insect attack (Klepacka *et al*., 2011) [13]. They are antioxidant compounds which protect plants from oxidative damages. The increment in phenol was proportional to mercury concentrations. Accumulation of phenol in response to mercury is observed by many researchers (Roy and Bera, 2002; Esteban *et al*., 2008; Umadevi *et al*., 2009) [22, 8, 27]. Increased synthesis in phenols minimizes the oxidative stress of Hg. Here one striking point is to be noted that reduction in phenol content by seaweed at 25 ppm Hg is not significant showing drastic effects of mercury toxicity. Proline is an osmo protectant imino acid. It is a major constituent of cell wall structural proteins in plants. Our results coincide with various authors who also reported that proline synthesis is directly proportional to the mercury concentration (Zengin and Munzuroglu, 2005; Gauba *et al*., 2007; Ravikumar et.al, 2007; Esteban *et al*., 2008; Umadevi *et al.* 2009; Tantrey and Agnihotri, 2010; Sharma and Subhadra, 2010) [29, 21, 8, 27, 26, 23]. Proline protects membranes and proteins against the adverse effects of high concentrations of inorganic ions like Hg and temperature extremes also. Proline may also function as a protein-compatible hydrotrope and as a hydroxyl radical scavenger. Heavy accumulation of proline protects plant against damage by binding with metal ions. Accretion of free proline may attribute as a mechanism designed to alleviate mercury toxicity (Ciobanu and Cristina, 2006) [7]. Biosorption by green seaweeds at 5ppm Hg (1.543±0.0088) was very close to control (1.453±0.0202). According to Zerouala, *et al*., 2003; [30] Askari *et al*., 2007; [2] Kumar *et al*., 2009; [15] Askari and Azmat, 2015 [19] etc 'green seaweeds are excellent, ecofriendly and cost-effective biosorbent material for the removal of heavy metals'.

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