Effect of Mercury and Triadimefon on the Growth and Development in *Centella asiatica* (L.) Urb.

Thesis submitted to the University of Calicut
in partial fulfillment of the requirement for the Degree of

DOCTOR OF PHILOSOPHY

By

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CERTIFICATE

This is to certify that the thesis entitled **"Effect of Mercury and Triadimefon on the Growth and Development in *Centella asiatica* (L.) Urb."** submitted by **Vijayalakshmi, K.M.** in partial fulfillment of the requirement for the degree of **Doctor of Philosophy** in Botany, University of Calicut, is a bonafide record of research work undertaken by her in this department under my supervision during the period 2002-2009 and that no part there of has been presented before for any other degree or diploma.

K. M. Jayaram

DECLARATION

I hereby declare that the thesis entitled **"Effect of Mercury and Triadimefon on the Growth and Development in *Centella asiatica* (L.) Urb."** Submitted by me for the award of the degree of **Doctor of Philosophy** in Botany of the University of Calicut is an original research work carried out by me in the department of Botany, University of Calicut. No part of the work has formed the basis for the award of any other degree or diploma.

C. U. Campus **Vijayalakshmi, K. M.**
29.06.2009

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Dedicated to

My Beloved Father & Mother

CONTENTS

INTRODUCTION

Large areas of land are contaminated with heavy metals like lead, cadmium, chromium and mercury releasing from urban activities, agricultural and industrial practices. Mercury has already been reported to be more toxic to plants compared to other heavy metals (Kneer and Zenk, 1992; Gadallah, 1994; Garg *et al*., 1994; De Grado *et al*., 1999; Xiong, 1999). Generally mercury binds strongly to the organic compounds of soil, so that mobility by leaching is minimal and contamination of ground water is unlikely. Most important form of mercury to which living organisms exposed is elemental mercury in liquid form, which is of considerable importance toxicologically due to the high vapour pressure and slight solubility in water and lipid (Berlin, 1986). Another form of mercury is ionic as Hg^{2+} (mercuric) and Hg^{+} (mercurous). These ions, particularly Hg^{2+} form complexes with organic compounds notably sulfhydryl groups. The third type is organic form, which binds with carbon producing methyl or ethyl mercury. The organic forms are readily react with biologically important ligands mostly S-H groups (Jain and Puranik, 1993).

Even though mercury is phytotoxic, absorption and translocation of organic and inorganic forms from soil by plant is low (Lodenius, 1990). However, excessive concentration of this metal in the soil leads to growth

retardation and impaired metabolism of plants. Given the phytotoxicity of mercury, some plants show slight tolerance by adopting different strategies such as avoidance or exclusion (Baker and Walker, 1989; Turner, 1994; Salt *et al*., 1998; Patra and Sharma, 2000; Pilon-Smits, 2005) and internal detoxification by biochemical elimination (Berry, 1986; Orcutt and Nilsen, 2000).

Mercury enters plants as inorganic form from the soil and/or volatile form from the atmosphere through stomata. Phytotoxic effects of mercury have been investigated in many plants and parameters adopted for the elucidation of physiological and biochemical aspects include growth retardation exhibited in morphology, anatomy and dry matter productivity (Beauford *et al*., 1977; Wilkins, 1978; Wong and Bradshaw, 1982; Kumar *et al*., 1995; Linger *et al*., 2005; Velasco -Alinsug *et al*., 2005). Due to the high affinity of Hg^{2+} ions to sulfhydryl groups, protein and enzymes get precipitated or inactivated (De Filippis, 1979; Woolhouse, 1983; Reed and Gaad, 1990; Vyas and Puranik, 1993; Keshan and Mukherji, 1995).

Exposure of plants to mercury is reported to effect photosynthesis (Bernier *et al.*, 1993; Bernier and Carpentier, 1995; You *et al.*, 1999). Chlorophyll synthesis (Krupa and Baszynski, 1995; Shaw and Rout, 1998; Zengin and Munzuroglu, 2005), water relation and membrane potential (Ma, 1998; Zhang and Tyerman, 1999), lipid peroxidation and production of

reactive oxygen species (ROS) (Van Assche *et al*., 1988; Yi Xian *et al*., 2000) and induced synthesis of antioxidant enzymes like peroxidase and super oxide dismutase(SOD) (Ma, 1998; Zhang *et al*., 2007). Eventhough angiosperms are not hyper accumulators of mercury, bioaccumulation studies also have been conducted in few plants (Lenka *et al*., 1993; Velasco- Alinsug *et al*., 2005).

Although exposure of plants to mercury is known to elicit a range of important symptoms, reports dealing with the physiological and biochemical aspects related to mercury treatment in medicinal and /or wetland plants are scanty. Because of the medicinal use of *Centalla asiatica* it is highly critical to monitor and understand the bioaccumulation potential of the plant when exposed to a range of concentration of mercury. Therefore, one of the important objectives of this study is to analyse the growth pattern of rooted propagules cultivated in Hoagland's nutrient medium artificially contaminated with known quantities of mercuric chloride. In addition, morphological and anatomical changes are indeed responsive to added $HgCl_2$ the present author identified specific concentration that elicit marked responses.

Triadimefon, a triazole compound is reported to function as growth regulators (Fletcher and Hofstra, 1985), as well as the protective role of this compound against stresses due to drought, chilling, desiccation, heavy metals etc. also have been reported (Asare- Boamah *et al*., 1986). Fletcher *et al*.

(2000) suggested that at biochemical level, triadimefon enhances the activities of antioxidant system to scavenge free radicals enabling the plants to cope up with environmental constrains. So the first and foremost objective of the present study is to elucidate the role of triadimefon as a growth regulator and to test the antagonistic effect of triadimefon on mercury toxicity in *Centella asiatica* plants.

Centella asiaitca (synonym: *Hydrocotyl asiatica)* is a prostrate, faintly aromatic, stoloniform perennial herb with glabrous stem and long petiolated fleshy leaves rooting at the nodes. This plant is a member of Apiaceae family, commonly known as “Indian penny wort” or “Mandooka parni”. *C. asiatica* grows in India up to an altitude of 600 m above sea level on moist sandy or clayey - soil forming a dense green carpet (Anonymous, 1992).

The plant enjoys considerable reputation in Indian system of medicine as diuretic, alterative and tonnic. Analytical studies have shown that *C. asiatica* contain triterpenoids, essential oils, amino acids and vellarin (Dutta and Basu, 1968; Singh and Rastogi, 1969). This plant shows good therapeutic effect on peptic ulcers and it is one of the components of the drug ‘Geriforte’ (Anonymous, 1992). Ahamad (1993) stated that annual requirements of *C. asiatica* as medicine come around 12700 tonnes of dry matter.

The medicinal use of *C. asiatica* vary wide and it is used for treating asthma, kidney troubles, leprosy etc. (Kakkar, 1988) and this plant shows anti

bacterial, anti stress and anti tuberculosis activities (Chakraborty *et al*., 1996; Srivastava *et al*., 1997).

Generally wetlands are used for disposal of waste and industrial effluents, which are sources of various metal contaminants. Natural habitat of *C. asiatica* plants include mainly areas of wetlands, and the plants grow profusely in such areas, hence the chance of exposure to heavy metal pollution is more. Recently a characteristic study on the distribution of metals in the sediments of tropical wetland ecosystem showed the presence of toxic heavy metals such as cadmium, mercury, lead and chromium along with considerable amount of iron, manganese, copper and zinc (Harikumar *et al.*, 2009).

Although phytochemical investigation have been conducted in *C. asiatica* (Dutta and Basu, 1968; Solet *et al.*, 1993; Inamdar *et al.*, 1996; Gupta *et al.*, 1999), physiological and biochemical aspects have not yet investigated. More over as mentioned earlier, *C. asiatica* plants get exposed to soil/ water pollution since these plants thrive well in marshy areas or wetlands.

Taking all these factors in to consideration the objectives of the present investigation include; check the sensitivity of *C. asiatica* plants to various concentrations of mercury levels applied in Hoagland's nutrient culture medium and analyses of morphological, anatomical, physiological/

biochemical aspects by estimating metabolites in the samples taken at several intervals of growth from the plants treated with optimal concentrations of $HgCl_2$. Another objective of the present study is the localization of mercury by histochemical method and bioaccumulation of mercury in the plant body such as root, stem/runner and leaves.

As mentioned earlier triadimefon is well known to antagonize many type of stress including that induced by heavy metals. Hence, the effect of triadimefon on growth and development of *C. asiatica* also is propsed to include in this study. The application of triadimefon alone and a combination of $HgCl_2$ + triadimefon were done for a comparative study. By this approach, the synergistic and / or antagonistic effect of this triazole compound on the stress induced by mercury could be elucidated. The elucidation of the mode of bioaccumulation of mercury in the plant body, particularly in shoot is most important because bioaccumulation potential is paradoxical to medicinal use.

REVIEW OF LITERATURE

Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, electro plating, energy and fuel production, power transmission, intensive agricultural practices, sludge and industrial effluent dumping and military operations (Foy *et al*., 1978; Salt *et al*., 1998; Orcut and Nilsen, 2000; Cseh, 2002; Pilon-Smith 2005). Non essential heavy metals such as lead, cadmium, chromium, mercury etc. are potentially toxic to plants and cause many phytotoxic effects.

According to Patra and Sharma (2000), mercury poisoning has become problem of current interest as a result of environmental pollution on a global scale. Considerable amounts of mercury are getting added to agricultural land with sludge, fertilizers, lime and manure. The most important sources of contaminating agricultural soil have been the use of organic mercurials as a seed coat-dressing to prevent fungal diseases in seeds.

The first major incident involving mercury poisoning in human was reported from Minamata bay, Japan. Eating contaminated fish caused the disease of the central nervous system, now known as 'minamata disease'. The methyl mercury contamination was a result of water discharges from an acetaldehyde plant. Fish and shellfish concentrated mercury to the levels of 10 mg kg^{-1} (Anonymous, 1972).

Annual production of mercury in the world is estimated to be about 9000 tons and about 50% of it is lost to the environment as pollutant (Goldberg, 1976). Reports are available on the deposition of atmospheric mercury vapour especially in specific geographic areas (Lindberg, 1987; Pacyna, 1987) and by a lesser extend for the entire globe (Galloway *et al*., 1982), but they are often excluded from global estimate of mercury cycling (Friedland, 1990).

It is estimated that approximately 50% of global mercury cycling is anthropogenic in origin (Miller and Buchanan, 1979). Moore and Ramamoorthy (1984) reported that natural weathering has contributed approximately 1.6×10^{10} metric tons of total mercury to the environment throughout geological times. Total production of mercury during this century has been about 4.36×10^{5} metric tons. According to those authors mercury released in this century through human activities is almost ten times the calculated amount released due to natural weathering.

Orcutt and Nilsen (2000) reported that total annual global emission of mercury from natural sources estimates to 0.16×10^{6} kg. Various agricultural amendments have been reported to contain mercury in the range: sewage sludge - 0.1-55 $\mu g g^{-1}$ composted refuse - 0.09-21 $\mu g g^{-1}$, farmyard manure -0.01 - 0.36 $\mu g g^{-1}$, phosphate fertilizers - 0.01 - 2.0 $\mu g g^{-1}$, nitrate fertilizers - 0.3 -2.9$\mu g g^{-1}$, lime 0.05 $\mu g g^{-1}$ and pesticides - 0.6 – 6.0 $\mu g g^{-1}$. The ratio of

anthropogenic to natural sources of particulate atmospheric emission of mercury is 0.44 and the toxic level of mercury in plant leaf tissues and soil surface are 1 to 3 ppm dry weight and 0.3 to 5 ppm dry weight respectively.

A wide variety of physical, chemical, biological and radiological mercury pollutants have been identified in the environment consequent to urbanization, industrialization and new technological developments (Lagerwerff, 1972; Pillai, 1989). According to those authors, sources of pollution of heavy metals such as Hg, Pb, Cd, As, Cr, Zn, Cu, Mn, and Fe are mainly aquatic releases from industrial operations, atmospheric releases from fossil fuel burning, domestic sewage discharges and land run-off. Several-fold increase in the concentrations of Zn, Cd, Hg etc. have been observed in some rivers in India (Pillai, 1989; Bhattacharyya *et al*., 1999; Lokhande and Kelkar, 1999).

According to Goldberg (1976), maximum consumption of mercury is in the chlor-alkali plants. The products of chlor-alkali industry can also be source of long term mercury pollution. House-hold bleaching solutions contain 17-24 ppb of mercury (Siegel and Eshleman, 1975). Mercuric chloride is used as catalysts in the manufacture of plastics and acetaldehyde, and effluents from such plants contribute mercury into the aquatic environment. Mercury is also used in the production of batteries, street lamps, fluorescent tubes, circuit breakers etc., all of which are finally discarded as

waste. Mercury compounds are used in anti-fouling paints, pulp and paper industry and are lost in the waste water effluents. Phenyl mercuric acetate and ethyl mercuric chloride have been used as fungicides.

Fossil fuel burning and cement manufacturing cause emission of mercury into the atmosphere (Syamala and Rao, 1999). Sewage disposal contributes to mercury contamination of aquatic environment. Mercury may get remobilized by burning of these sledges or when used as fertilizers (Moore and Ramamoorthy, 1984).

Mercury may be present in industrial wastes in the form of elemental mercury, inorganic or organic mercury compounds. Methylation of mercury to CH_3Hg^+ and $(CH_3)_2$ Hg takes place in the aquatic environment as a result of activities of bacteria, fungi or enzyme systems. Bacteria also convert phenyl, ethyl and methyl mercury, phenyl mercuric acetate and diphenyl mercury as well as Hg^{2+} ions to elemental mercury (Pillai, 1989). According to the author other possible reactions in the environment are conversion of methyl mercury into dimethyl mercury in alkaline condition, production of insoluble HgS in anaerobic conditions and oxidation of HgS to sulphate in aerobic conditions and conversion to methyl mercury.

Mercury is discharged into the aquatic environment mainly in the form of elemental mercury (Hg), divalent mercuric ions (Hg^{2+}) and phenyl mercuric acetate [C_6H_5Hg (CH_3COO)] (Moore and Ramamoorthy, 1984; Driscoll *et al*.,

1994). According to those authors, mercury in natural waters exist in three oxidation states: elemental mercury (0) mercurous (+1) and mercuric (+2) states.

Mercury enters plants as inorganic forms from the soil or water or from the process of methylation that occurs in plants. According to Woolhouse (1983), mercury after being absorbed from the soil remains deposited mostly in the root tissues. In higher plants accumulation of mercury is normally quite low even in heavily contaminated soils and content of mercury varies among plant groups (Foy *et al*., 1978). Moore *et al*. (1995) reported that the consequences of mercury distribution is in the order, herbs < trees and shrubs <aquatic macrophytes < sphagnum < mosses < lichens < fungi. According to those authors, grasses and herbs contain 20 μg kg^{-1}, trees and shrubs 29 μg kg^{-1}, aquatic mesophytes 40 μg kg^{-1}, sphagnum moss 69 mg kg^{-1}, lichen 170 μg kg^{-1} and fungi 280 μg kg^{-1} of mercury. Considerable amount of mercury and methyl mercury is reported to be present in vegetables and the range of mercury is 3-139 μg kg^{-1} and that of methyl mercury is 0.3 - 30 μg kg^{-1} (Cappon, 1987). According to Orcutt and Nilsen (2000) critical toxic level of mercury in most plants is considered to be 1-8 ppm.

Mercury has always been reported to be more toxic compared to other heavy metals like cadmium (Nordberg, 1976; Rai *et al*., 1981; Fergusson, 1990; Kneer and Zenk, 1992; Gadallah, 1994; Shaw, 1995), chromium

(Chandra and Garg, 1992; Garg *et al*., 1994) and lead (Huang *et al*., 1987; De Grado *et al*., 1999; Xiong, 1999; Jindal and Kaur, 2000; Orcutt and Nilsen, 2000).

Toxicity of mercury has been reported in many plants and very low concentrations of mercury causes hazards to plant growth (Vallee and Ulmer, 1972; Sandmann and Boger, 1983; Kagi and Hapke, 1984; Baker *et al*., 1985; De *et al*., 1985). Those authors also suggested that mercury is phytotoxic even in small quantity and the toxicity has bearing on its strong affinity to acidic and thiol groups of protein and nucleotides, thus interfering with the functions of the compounds/organelles. In addition, mercury competes with other metals such as Cu or Zn within the cell (Marschner, 1983).

Much earlier work by Hitchcock and Zimmerman (1957) had shown that higher plants were sensitive to the presence of vapors of mercury and its compounds under certain conditions. Ross and Stewart (1960; 1962) have shown that mercury compounds such as phenyl mercuric acetate (PMA) can be translocated and redistributed in plants when applied as fungicides to the foliage of trees. Siegel *et al*. (1974) reported that certain plants can accumulate mercury from soils and release a volatile form of the element through their leaves.

Beauford *et al*. (1977) studied the uptake and distribution of mercury in higher plants such as *Pisum sativum* and *Mentha spicata* and reported that

mercury inhibited the synthesis of leaf protein in the plants. Those authors further noticed reduction in shoot and root length in plants treated with mercury. Roots act as a significant absorption site for mercury and consequently create a barrier to further transport of this element to the foliage, but when the concentration was raised the root barrier becomes less effective and Mercury level raised in the shoots. At critical level the element exerts its toxic effect on the biochemical and physiological activities of the cell.

The effect of mercury on the movement of stomata has been investigated by various authors (Pallas, 1962; Zelitch, 1963; Siegenthaler and Packer, 1965; Mansfield, 1967; Davenport *et al*., 1971). According to Pallas (1962) mercury inhibited amylase activity, which prevented the conversion of starch to osmotically active compounds and there by inhibited stomatal opening. Zelitch (1963) suggested that phenyl mercuric acetate (PMA) effects stomatal movement by reacting with sulfhydryl groups associated with guard cell membranes there by altering membrane permeability and the ability of the guard cells to maintain turgor. Siegenthaler and Packer (1965) proposed that PMA may influence stomatal movement via an effect on light induced volume changes in guard cell chloroplast. Mansfield (1967) believed that PMA may influence stomatal opening by inhibiting the photosynthetic CO_2 uptake there by increasing the CO_2 concentration in the intercellular spaces and in the guard cells. But Davenport *et al*. (1971) reported that in *Nerium oleander* stomatal closure was retarded by the activity of phenyl mercuric

acetate (PMA), i.e, PMA may conceivably decrease the permeability of guard cell membranes to solutes, there by retarding all stomatal movements that are osmotically induced.

Pathak *et al*. (1987) observed 100% inhibition in seed germination by treatment with 1000 mgl^{-1} concentration of mercury. But low percent inhibition was observed in case of mercury in combination with manganese of concentrations 1, 10 and 100 mgl^{-1} as compared to individual concentration of mercury from 1-1000 mgl^{-1}. The authors further observed that increasing concentration of mercury also reduced the average length of root and shoot. Studies on the effect of heavy metals like Zn and Hg on growth and biochemical constituents of *Vigna radiata* seedlings (Pratima *et al*., 1989) showed a decline in the respiratory rate. The levels of total nitrogen, total sugars and malic acids declined in the embryo with a concomitant accumulation in cotyledons. According to Kalimuthu and Sivasubramanian (1990), the percentage of germination, root and shoot length of maize seedlings decreased with increasing concentration of lead and mercury.

Puerner and Siegel (1972) studied the toxic effects of mercury compounds in the growth and orientation of cucumber seedlings and noticed that mercury with fluorescein affected not only the orientation of cucumber seedlings but also the toxicity of the Hg ions itself. Mercuric chloride was

found to be more toxic than fluorescein indicating that the combination of mercury with fluorescein can mitigate metal toxicity.

Several researchers (Iqbal *et al*., 1991; Shaukat *et al*., 1999; Al-Yemeni and Al-Helal, 2000) have reported that inhibition of seed germination at higher concentration of heavy metals is mainly caused by ion toxicity. Ion toxicity is associated to changes in cellular permeability, inhibition of protein activity or direct toxicity to the embryo and seedling (Dubey and Dwivedi, 1987). Al-Yemeni (2001) studied the effect of cadmium, mercury and lead on seed germination and early seedling growth of *Vigna ambacensis* and reported that all the three heavy metals caused significant reduction in germination of the seeds with the increase in their concentration. According to those authors the radicle and shoot length of *V. ambacensis* seedlings were significantly inhibited by mercury, cadmium and lead where the radicle length was more adversely affected compared to the shoot. For all the three heavy metals, the radicle and shoot dry weight accumulation was significantly reduced with the increase in heavy metal concentration of mercury, cadmium and lead solutions compared with the control.

Mor *et al*. (2002) studied the effect of mercury toxicity on hypocotyl elongation and cell wall loosening in *Phaseolus* seedlings and suggested that mercury effects and inhibits cell elongation.

Various forms of growth retardation and physiological changes have been reported in wheat by mercury toxicity (Nag *et al*., 1980). In *Cyperus* and *Chloris* root growth inhibition has been reported due to mercury treatment and the rate of inhibition increased with the increase in concentration of mercury (Lenka *et al*., 1993). Maitani *et al*. (1996) observed a reduction in the relative root elongation of *Rubia tinctorum* in root cultures when treated with 10 μM Hg^{2+}.

Mercury is highly toxic to vascular plants and the toxic effect of mercury had been observed in young barley plants at levels of 3 ppm dry weight while volatile forms of mercury had been reported to inhibit growth in sensitive plants at 0.5-1 ppm and earlier investigators suggested that growth inhibition by mercuric chloride may occur at concentrations of 0.002 - 0.25 mgl^{-1} (Hollibaugh *et al*., 1980).

Chaphekar and Kulkarni (1979) reported that mercury destructs the wall of chloroplast in some cryptogams. Due to mercury treatment, reduction in photosynthetic pigment had observed in *Amaranthus* sps. (Mukhiya *et al*., 1983). The chlorophyll levels of primary needles and cotyledons of the spruce seedlings (*Picea abies*) were markedly influenced by heavy metals like Cd, Zn and Hg, where the decrease in chlorophyll concentration was enhanced with increasing the concentrations of these heavy metals (Schlegel *et al*., 1987). Similarly, in cotyledons of plants exposed to methyl mercury, a

significantly greater decrease in chlorophyll level was observed than in plants exposed to $HgCl_2$. The author further noticed that the heavy metal influence gas exchange and water relationships through a number of sequential effects.

Schlegel *et al.* (1987) explained the inhibition of CO_2 uptake induced by zinc, cadmium and mercury in spruce seedling by lowered chlorophyll concentration and the inhibition at 0.01 μM methyl mercury and 5 μM cadmium were by stomatal closure, indicated by decreased availability of water in the needles of spruce.

Prasad and Prasad (1987), Jain and Puranik (1993) and Prasad (1997) have reported decline in chlorophyll content in many plants due to mercury toxicity and according to those authors, the decline is linked to the photosynthetic productivity. Photosynthesis, transpiration, water uptake and chlorophyll synthesis are reported to be adversely affected by mercury treatment in spruce seedlings (Godbold and Huttermann, 1986).

Cargnelutti *et al.* (2006) suggested that mercury causes oxidative stresses in cucumber seedlings cultured under different concentrations of $HgCl_2$. According to those authors growth reduction in cucumber seedlings is related to decreased chlorophyll content with consequent reduction in the rate of photosynthesis. An increase in the membrane damage which could account for higher level of lipid peroxidation and protein degradation also are reported, due to mercury stress.

Bernier *et al*. (1993) investigated the effect of mercury on photosystem II (PS II) sub membrane fraction of barley leaves (*Hordeum vulgare*) and reported that mercury inhibits photosynthetic electron transport, photosystem II being the most sensitive target.

The molecular mode of action of mercury in the oxygen evolving complex of PSII is described by Bernier and Carpentier (1995). Incubation of thylakoid membranes in the presence of mercury cause depletion of an extrinsic polypeptide of molecular weight 33 kDa, without affecting other two closely related extrinsic polypeptides of 16 and 23 kDa. This indicates the existence of an intrinsic binding site for EP 23. According to those authors, the mercury inhibition of PSII might be due to the release of EP 33 and the Cl^- reduction in mercury toxicity might be due to reduction in the extend of EP 33 depletion.

Murthy and Mohanthy (1995) reported that mercury inhibit photosynthetic electron transport at various sites in *Spirulina platensis* and the photosystem II is more susceptible to heavy metal ions compared to photosystem I(PS I).

Mercury has a strong affinity for sulfhydryl or thiol groups involved in enzyme reactions (Passow *et al*., 1961). According to Kuramitsu (1968) mercury affects metabolic activities by producing structural changes in enzymes following mercaptide formation with –SH groups. Golle and Luttge,

(1983) noticed the inhibition of glucose and aminoacid carriers of *Lemna gibba* with pretreatment with 10^{-3} to 10^{-2} mol m^{-3} $HgCl_2$ which was due to irreversible binding of mercury to essential -SH groups of the sugar and aminoacid co-transport carriers, thus uptake of these solutes is specially inhibited while the H^+ extrusion pump remains rather unimpaired by these low $HgCl_2$ concentrations.

Mercury (II) has a tremendously high affinity for sulfur and hence it can coordinate to essential functional groups of proteins and render them inactive. It is well established that under various stress conditions, transport of nitrogenous compounds is altered and specific amino acids such as proline and γ-amino-butyric acid (GABA) accumulate (Heineke *et al.*, 1992; Breitkreuz and Shelp, 1995).

Mercury forms stable complexes with a variety of organic ligands and has exceptional affinity for sulfhydryl groups of proteins (Falchuk *et al*., 1977; Nath *et al*., 1993). The strongest covalent complexes are formed with S-containing ligands such as cysteine, the next strongest with aminoacids and hydroxy carboxylic acids.

According to Jain and Puranik (1993) one of the mechanisms by which mercury exerts its toxic effects is by interaction with essential -SH group of enzymes and structural proteins. Those authors reported that supply of 0.01 to 0.1 mM reduced glutathione (GSH) to excised greening maize leaf segments

prevented the inhibitory effect of mercury on chlorophyll biosynthesis and the supply of other thiols such as dithiothreitol (DTT), cysteine and mercaptoethanol also reduced the inhibition of chlorophyll formation by mercury. The authors further suggested that the accumulation and thereby the toxicity of the mercury may be reduced by the supply of thiols. Reduced glutathione (GSH) is the predominant, free thiol present in plants. The concentration of GSH in plant cell is modified by development and environmental factors such as heavy metals, and cell culture studies have indicated that GSH is a substrate for the synthesis of the heavy metal binding phytochelatins (Rauser, 1987; Scheller *et al*., 1987; Obata *et al*., 1994). According to Tukendorf and Rauser (1990) and De Vos *et al*. (1992) accumulation of phytochelatins is associated with decline in GSH.

Phytochelatins are unique family of thiol containing metal binding polypeptide derived from glutathione (Rauser, 1990). According to Maitani *et al*. (1996) phytochelatin (PC_2) is the most abundant class III metallothioneins produced in higher plants due to Hg^{2+} exposure. The authors suggested that, since mercury (II) has a linear configuration in coordination compounds, PC_2 can effectively protect plants against the Hg^{2+} toxicity. Those authors used root culture of *Rubia tinctorum* and confirmed that mercury induced phytochelatin synthesis. The analysis of phytochelatin-deficient mutant of *Arabidopsis* showed a detoxifying role of phytochelatins against mercury (Howden and Cobbett, 1992).

Metal binding polypeptides produced by higher plants are abundant in both sulfhydryl and carboxyl groups and could have affinity for a wide range of metal ions. These metal binding polypeptides chelate metal in the cytoplasm and thereby reduce the concentration of cytotoxic free metal ions (Gekeler *et al.,* 1989; Grill, 1989; Robinson, 1990; De Knecht *et al*., 1994).

Satoh *et al*. (2002) determined the cellular levels of non-protein thiols in phytoplanktons and their correlations with susceptibility to mercury and indicated that the intracellular concentrations of the non-protein thiols reflect antioxidant activity and susceptibility to heavy metals. Here the intracellular concentrations of cysteine and glutathione are related to cellular oxidation levels and demonstrate that intracellular GSH concentrations are related to mercury susceptibilities.

Inhibition of enzyme activity is an important effect of mercury toxicity in plants (Keshan and Mukherji, 1995). There are two predominant mechanisms of enzyme inhibition; (1) Binding of the metals to the sulfhydryl groups, involved in catalytic action or structural integrity of enzymes and (2) Deficiency of an essential metal in metal-protein complexes eventually resulting in substitution of the toxic metal for the deficient element (Reddy and Prasad, 1992 a)

Mercury is an enzyme- and protein- inhibitor in biological systems and all mercurial compounds are highly toxic to plants in general and aquatic

plants in particular (De Filippis, 1979; Baker and Walker, 1989; Reed and Gadd, 1990).

Peroxidase induction by trace metal was observed in *Phaseolus vulgaris* (Van Assche *et al*., 1988). According to Mishra and Choudhuri (1996) mercury and lead cause membrane damage and an increased activity of lipogenase and malondialdehyde content occur in rice plants. Mercury increased the activity of peroxidase and the level of H_2O_2 while super oxide dismutase (SOD) and catalase activity was decreased. Those authors opined that $Hg^{2+}$and Pb^{2+}caused membrane damage in *Oryza sativa* and the damage was mediated by reactive oxygen species and hydrogen peroxide induced by these metals. Peroxide induction is a general response of higher plants to the uptake of toxic amount of metals such as Cd, Cu, Pb, Hg, Zn and Ni (Yi Xian *et al*., 2000). In addition to peroxidase the activities of malic enzyme, glucose-6-P-dehydrogenase, isocitrate dehydrogenase and glutamate dehydrogenase are also increased in *Phaseolus vulgaris* when treated with zinc and cadmium (Van Assche *et al*., 1988).

According to Shaw (1995), mercury and cadmium significantly inhibited seed germination and seedling growth in *Phaseolus aureu*s but had little primary damaging effect on membranes. According to the author, lipid peroxidation occurs in *Phaseolus* by mercury treatment and it is indicated that guaiacol and ascorbate peroxidases and catalases are actively involved in

scavenging cellular H_2O_2 and other free radicals. The lipid peroxidation induced by these metals is a consequence rather than the primary cause of toxicity.

In *Bacopa monnieri*, increased activity of antioxidant enzymes - ascorbate oxidase has been reported as an impact of mercury stress (Sinha *et al*., 1996). Zengin and Munzuroglu (2005) opined that a significant increase of ascorbic acid content was occurred in *Phaseolus vulgaris* grown in Hoagland solution supplied with various concentrations of mercury.

Increased lipid peroxidation is an important adverse effect of mercury in tomato seedlings (Cho and Park, 2000). According to Qureshi *et al*. (2005) generation of reactive oxygen species has been proven to be one of the agents causing tissue injury after the exposure of plants to heavy metal stresses and UV radiation. Those authors further suggested that enzymes such as catalase, ascorbate peroxidase and superoxide dismutase are active in neutralizing and scavenging reactive oxygen species in order to protect cellular membranes and organelles from the damaging effect of reactive oxygen species.

The inhibitory role of mercury on nitrate reductase activity was studied by Vyas and Puranik (1993). Those authors suggested that the supply of 0.01 and 1.0 mM $HgCl_2$ substantially inhibited *in vivo* as well as *in vitro* the total nitrate reductase activity and endogenous nitrate pool in excised bean leaf segments and the *in vitro* specific activity remain unchanged. The authors

further noticed that percent inhibition in nitrate reductase activity by metal increase with incubation period and the toxic effect of mercury was overcome completely by the supply of glutathione, cysteine and sucrose and partially by molybdic acid and NADH.

Proline accumulation has been reported in response to heavy metal stresses in plants (Alia and Saradhi, 1991; Bassi and Sharma, 1993). Khanna and Rai (1995) investigated mercury-induced inhibition of *Raphanus sativus* seedling growth and this could be reversed in the presence of L. proline, L. histidine and L. methionine, but L. phenyl alanine, L. alanine and L. aspartic acid had no effect on mercury toxicity. The reduced level of mercury in *R. sativus* tissues, when supplemented with exogenous proline indicate that effects of proline on amelioration of mercury toxicity were related more to its inhibitory effects on mercury uptake rather than on mercury toxicity itself. These authors further suggested that stress induced accumulation of amino acids within the plant have significant effect on the ion uptake or ionic balances of plants, which in turn would help the plant in mitigating the stress.

Shieh and Barber (1973) reported that mercury caused a breakdown in the permeability of cell membranes in *Chlorella* resulting in a net efflux of internal K^+. Mishra and Choudhuri (1996) studied the membrane damage caused by Pb^{2+} and Hg^{2+} in two rice cultivars and observed an increase in the activity of lipogenase and malondialdehyde content due to treatment with

both the heavy metals. As a result of mercury treatment increased activity of peroxidase and the level of H_2O_2 and decreased levels of of SOD and catalase activities occurred in rice plants. The authors concluded that, Hg^{2+} and Pb^{2+} caused membrane damage in *Oryza sativa* was mediated by reactive oxygen species and hydrogen peroxide induced by these metals.

Enhanced lipid peroxidation and activities of antioxidative enzymes like catalase, peroxidase and ascorbate oxidase exhibits increasing trends in the seedlings of *Phaseolus aureus* subjected to $HgCl_2$ treatment (Shaw, 1995). Cakmak and Horst (1991) and Hendry *et al.* (1992) reported that active participation of these enzymes in scavenging H_2O_2 formed as a result of mercury injury on lipids. Antioxidative systems play vital roles in the maintenance of plants that grow under heavy metal stress. Mercury stressed plant cells showed increased activities of antioxidant enzymes like superoxide dismutase and catalase in various degrees and showed positive endogenous protection effects (Ma, 1998). However, the protection effect is found to be disappeared at higher concentration levels of mercury.

Parmar *et al*. (2002) reported that effect of mercury on *Phgaseolus vulgaris* seedlings inhibited root and hypocotyl elongation. According to Parmar and Chanda (2005) peroxidase and IAA oxidase activities are increased in *P. vulgaris* due to the treatment with mercury and these enzyme activities are considered as a defensive mechanism against metal toxicity.

Free radical generation as one of the initial responses of plants exposed to lead copper, cadmium and mercury also have been reported in *Phaseolus vulgaris* (Zengin and Munzuroglu, 2005). Those authors suggested that a significant increase of non enzymatic antioxidants like ∞ tocopherol and ascorbic acid content was detected in the leaves after exposure to these metals and the strongest effect was induced by mercury. According to Zhang *et al*. (2007) heavy metals inclusive of mercury induce stress in plants revealing an enhanced lipid peroxidation and the anti oxidant enzymes like SOD, CAT and POD show enhancement in their activities and the dynamics of these reactions vary from plant to plant.

De and Mukherjee (1996) used the suspension cultures of tomato cells to study the membrane injury by the toxic concentration of mercuric chloride and the assessment of electroyte leakage, UV-absorbance of the tissue leachate, relative leakage ratio, injury index, membrane lipid peroxidation, lipoxygenase activity, α–amino nitrogen and total soluble carbohydrate contents showed the extent of membrane damage as a function of the increasing concentration of mercuric chloride.

Inhibitory effect of $HgCl_2$ and some organomercurials on protein molecules is widely used in molecular biology for the elucidation of proteinaceous nature of biomolecules such as a membrane protein inclusive of aquaporins (Borgnia *et al*., 1999).

Studies on the genotoxicity of heavy metals in plants have been conducted to determine the impacts of these metals in the genetic system. Clastogenic and mutagenic potency effects of mercury on chromosomal aberration and polyploidy are reported in the meristematic roots of *Hydrilla verticellata* (Pal and Nandi, 1989; 1990), *Lathyrus sativus* (Gupta and Ghosh, 1992), and *Allium cepa* (Agar and Vysal, 1997). Formation of micronuclei due to mercury toxicity in the meristematic roots of *Hordeum vulgure* has been reported by Panda *et al.,* (1992) and Patra *et al., (*2000).

In an excellent review of genotoxicity of metal on plants, Patra *et al*. (2004) opined that the most noticeable and consistent effect of mercurials is the induction of mitosis resulting in the formation of polyploidy and aneupliod cells.

In general the availability of soil mercury to plants is low, and there is a tendency for mercury to accumulate in roots, indicating that the roots serve as a barrier to mercury uptake (Gracey and Stewart, 1974). The fraction of mercury retained in the roots is about 20 times that observed in the shoots (Linderberg *et al*., 1979). According to those authors, the mercury concentration in above-ground parts of plants appears to depend largely on foliar uptake of volatilized mercury from the soil.

Mercury accumulation takes place in lichens, mushrooms, aquatic and terrestrial plants (Ferrara *et al*., 1989; Merian, 1991). Uptake and

accumulation of mercury depend on the form of mercury available in the soil/ water varies from species to species as well as period of exposure. In *Phaseolus vulgaris* cultivated in different levels of mercury contamination, translocation to grain was very low (Semu *et al*., 1985). Zhou and Zhao (1992) reported that in *Lycopersium esculentum* uptake of mercury is directly related to the level of mercury pollution.

Analysis of about 2,500 vegetable samples and 650 soil samples showed that considerable part of the vegetables, especially those with edible roots, contained mercury usually in the order of thousand of milligram per kilogram (Zawadzka *et al*., 1990).

The uptake and excretion of mercury have been studied in seedlings of woody plants (Kotov, 1983), in shoots of lettuce (Staiger, 1983), in *Zostera marina* (Lyngby and Brix, 1982) and in *Picea abies* (Godbold and Huttermann, 1985). Significant correlation exists between the mercury concentration of the soil and the plant tissues (Lenka *et al.*, 1992). Mercury content of plant tissues of *Lolium* sp. (Singh and Jeng, 1993) and *Rosmarium officianalis* (Barghigiani and Ristori, 1995) are related to its concentration in the soil.

Among aquatic plants, in natural sediments enriched with CH_3HgCl (4mg Hg kg^{-1} fresh weight) *Elodea densa* showed a high accumulation of mercury in the leaves, stem and roots. In long term experiments, root

absorption was the more dominating factor of mercury accumulation, the leaves being the principal organ of stress (Maury-Brachet *et al.*, 1990).

According to Ribeyre and Boudou (1994) bioaccumulation capacity of wetland plants (*Elodea densa, Ludwigia natans*, *Lysimachia numularia*) varied significantly. The accumulation after 18-21 days of exposure was more in shoot when the metal was introduced in organic form and bioaccumulation from the water source was about 10 times greater than that from the sediments. *Eichhornia crassipes*, *Typha latifolia, Sparganium minimum* and *Menyanthes trifoliate* roots readily absorb mercury ions from aqueous solutions. The hydrophilic parts of the roots accumulated significantly more mercury than did the hydrophobic parts (Robichaud *et al*., 1995).

Plants that can accumulate higher amounts of mercury are used for phytoremediation technology. For removal of mercury bioaccumulator plants such as *Typha* (Krishnan *et al*., 1988) and *Eichhornia crassipes* (James *et al*., 1992) are commonly employed. These species are used to assess bioaccumulation and genotoxicity of aquatic mercury in water contaminated with mercury salts/ compounds.

Genetically engineered plants have been developed for phytoremediation of mercury and the transgenic plants clear mercury ions from methyl mercury complex, reduce mercury ions to metallic form, take up metallic mercury through their roots and evolve less toxic elemental mercury

(Rugh *et al*., 1998). These genetically modified plants contain modified forms of bacterial genes that breakdown methyl mercury and reduce mercury ions (Bizily *et al*., 1999; Patra and Sharma, 2000).

Natural elements inclusive of heavy metals are getting accumulated in plant tissues at levels several fold higher than those found in the soil. Hence this plants become hyper accumulators and the concentration of these elements may be higher than that are actually required for normal growth and development. Some plants are known as heavy metal hyper accumulators that can accumulate unusually high content of heavy metals from the environment via root system and translocate to the above-ground parts (Baker and Brooks, 1989; Brown *et al*., 1994; Kumar *et al*., 1995; Xiong, 1998).

Plants adapted to metal contaminated ecosystem can be used as indicators for exploration of metals (Brooks and Mallaisse, 1985; Baker and Proctor, 1990; Dickinson *et al*; 1991; Kumar *et al*., 1995). Phytoremediation is a technology using plants for environmental clean up (Pilon-Smits, 2005). During the past 10 years phytoremediation has been accepted as a cost-effective, non invasive alternative of complimentary technology for remediation of heavy metal pollution (Raskin *et al*., 1994; Salt *et al*., 1995; 1998).

Tolerance of plant population to mercury is comparatively less known (Chaney and Strickland, 1984; Godbold and Huttermann, 1985). Deshkar *et*

al. (1990) reported that modified *Hardwickia binata* bark can adsorb Hg(II) from wastewater upto a capacity of 21 mg g^{-1} and thus it can be used for purifying wastewater. In a biomonitoring study by Lenka *et al*. (1993) two mercury tolerant grasses, *Chloris barbata* and *Cyperus rotundus* were isolated from a mercury polluted locality near a chlor-alkali plant, where mercury contamination was as high as 557 mg kg^{-1} soil and according to them tolerance to mercury was more in *C. barbata* than *C. rotundus.* It appears to be easily mobilized in plants; hence it can be accumulated in plant parts that could be consumed by humans or other animals. Consequently, like other heavy metals that can be accumulated in plants, it is a concern relative to animal and human health (Orcutt and Nilsen, 2000).

Mishra *et al*. (1999) reported that *Azolla* accumulated a substantial amount of mercury from the contaminated waste soil which increased with increasing concentrations of the treatment and inoculation period. So *Azolla* have been reported to accumulate mercury from the contaminated medium.

Induced accumulation of mercury by plants was investigated for the species *Phaseolus vulgaris*, *Brassica juncea* and *Vicia villosa* by (Moreno *et al*., 2005a) in the presence of sulfur containing ligands and humic acids (HA). According to those authors the mercury availability and mercury-plant uptake are interrelated process that appears to be controlled by plant species. Accumulation of mercury by plants has been investigated in *Phaseolous*

vulgaris, the results revealed that mercury got translocated to the shoot parts of the plant up to 25 times the concentration in the growth medium, similarly *Brassica juncea* also was reported as an accumulator of mercury (Moreno *et al*., 2004, 2005 b) But those authors suggested that volatalization is a dominant pathway for mercury removal for the growth medium. Recently, Moreno *et al*., (2008) reported that in *Brassica juncea* most mercury volatalization occurred form the roots and the volatalization process took place in the roots by converting it in to vapour form and the production of the vapour in the medium may result from the acting of root-associated algae or mercury-resistant bacteria. This phytoremediation designated as phytofiltration effectively remove up to 95% mercury from the contaminated growth medium or solution by both volatalization and plant accumulation.

Mercury binding peptides have been isolated and characterized using reverse phase high performance liquid chromatography (RP-HPLC) from the root, stem and leaf tissues of *Chromolaena odorata* and revealed that the activity of this plant to accumulate and regenerate mercury ions was primarily attributed to the production of mercury-binding proteins (Velasco-Alinsug *et al*., 2005).

Sarma *et al.* (2006) studied the impact of toxic heavy metals like lead, mercury and cadmium in paddy and showed that due to treatment with these metals there was a significant decline in harvest index (percentage of grain

yield/ total biological yield) compared to control. Those authors also reported that the highest accumulation of mercury was in root followed by stem and leaf sheath.

Many plant species possesses the ability to tolerate and accumulate heavy metals inclusive of mercury. In order to achieve successful phytoremediation of soil and water contaminated with heavy metals plants are used us phytoremedients. Samecka-Cymerman and Kempers (1996) stated that *Scapania undulata* has very high mercury concentration in the tissue and hence can be used to remove mercury from sewage collected from pesticide producing factories. According to those authors the mercury concentration in the plant tissue reached 2-4 mg kg^{-1} dry weight after 14 days.

Kamal *et al*. (2004) demonstrated the bioaccumulation potential of the plant *Myreophyllum aquaticum, Ludwegina palustris* and *Mentha aquatica* by exposing to nutrient medium contaminated with mercury salt and reported that *M. aquatica* showed more bioaccumulation potential.

Fletcher *et al*. (2000) in their excellent review on triazole compound suggested that several compounds of triazoles are very active fungicides and their fungicidal activity is due to the interference with the biosynthesis of fungal steroids. Of the various triazoles, paclobutrazol (PBZ) and uniconazole (UNI) have been found to be the most active in involving plant growth. According to those authors certain triazole compounds interfere with the

biosynthesis of plant growth regulators such as gibberellins and influence morphogenesis of plant and hence function as plant growth hormones. Fletcher and Hofstra (1988) suggested that traizoles are potential plant protectants since these compounds interact with GA and the hormonal and protective effects are a consequence of their primary action as inhibitors of GA biosynthesis. Triazoles like paclobutrazol also have been reported to effect photosynthetic rate, transpiration and stomatal resistance in *Panicum miliaceum* (Bisht *et al.*, 2000) and in *Setaria italica* (Bisht *et al*., 2007).

The triazole derivatives have both fungal toxic and plant growth regulating properties and triadimefon (a triazole compound) is reported to change the balance of important plant growth regulators including gibberellins, ABA, cytokinins etc. (Fletcher and Hofstra, 1985). Those authors further reported that in addition to its fungicidal properties, triadimefon protects the plant from drought, desiccation, chilling and ozone stress. The protective role of triazoles have been reported in bean plants treated with triazole fungicide, which induced transient rise in abscisic acid levels, reduced transpiration and protected the plant from drought (Asare-Boamah *et al*., 1986).

Triadimefon is a fungicide which is highly active against several fungal diseases. Besides this compound protect plant from injury due to biotic and abiotic stresses and hence triazoles are referred to as plant

multiprotectants (Fletcher and Hofstra, 1985). According to Fletcher *et al*. (2000) at biochemical level triazoles enhances activity of antioxidant system to effectively scavenge free radicals and thus enable the plants to better cope with environmental constraints.

Fletcher and Nath (1984) have reported that triadimefon treatment caused decrease in fresh and dry mass of the shoot in radish and an increase in the root/shoot ratio. According to Sairam *et al*. (1989) triadimefon treatment increased stomatal diffusive resistance and decreased transpiration in stressed wheat plants and increased chlorophyll a and b content under moisture stress as well as irrigated conditions. Those authors further observed that the application of triadimefon enhanced photosynthesis and nitrate reductase activity under moisture stress in tolerant type of wheat compared to susceptible ones.

During 1990s a number of investigations have been conducted to elucidate the role of protection of triadimefon on heavy metal induced stress in wheat (Babu and Singh, 1992) and salinity stress in *Raphanus sativus* (Panneerselvam *et al.*, 1997), *Glycine max* (Panneerselvam *et al*., 1998), *Vigna unguiculata* (Gopi *et al*., 1998; 1999), *Cajanus cajan* (Karikalan *et al.*, 1999) and Bhendi (*Abelmoschus esculentus*) (Sujatha *et al*., 1999 a; 1999 b). Babu and Singh (1992) studied the toxicity of cadmium in wheat leaves and suggested that the plants treated with cadmium showed chlorotic and necrotic

lesions after 15 to 20 days of treatment, while triadimefon treated plants did not show any toxic symptoms. Chlorophyll contents, nitrate reductase activity, total nitrogen and dry weight were significantly less in Cd treated plants and decreased further with increasing concentrations of Cd. In case of triadimefon+cadmium treated plants reduction in chlorophyll content, nitrate reductase activity, total nitrogen and dry weight were comparatively low.

Chouhan *et al*. (2007) evaluated the performance of *Lablab perpurius* seedlings under UV- B stress and the role of triadimefon in improving the tolerance to UV- B stress. According to those authors the treatment of triadimefon alone showed negative impact on soluble protein, proline content, alanine amino transferase and nitrite reductase. However, increased activities of superoxide dismutase and peroxidase participated in enhancing the tolerance to oxidative damage under UV stress and so the triadimefon can alleviate the effect of UV- B stress by enhancing the activities of antioxidative enzymes in *Lablab perpurius*.

Panneerselvam *et al*. (1997) studied the effect of NaCl and triadimefon on the chlorophyll content, net photosynthetic rate, rate of transpiration and intercellular CO_2 concentration in *Raphanus sativus* and the effect of NaCl salinity was partially ameliorated by triadimefon, which caused increase in chlorophyll content, net photosynthetic rate and intercellular CO_2

concentration. Triadimefon also increased root dry matter production, decreased rate of transpiration and increased water use efficiency.

Muthukumarasamy and Panneerselvam (1997a) reported the amelioration of NaCl stress by triadimefon in peanut seedlings. According to Gopi *et al*. (1998) Triadimefon treatment to the NaCl stressed seedlings of *Vigna unguiculata* significantly increased root growth, stem length and leaf area when compared to the stressed seedlings. Triadimefon treatment to the NaCl stressed seedlings showed increased dry weight of *Raphanus sativus* seedlings (Muthukumarasamy and Panneerselvam, 1997 b) and in *Vigna unguiculata* (Gopi *et al*., 1998).

NaCl stressed seedlings of *V. ungiculata* treated with triadimefon resulted in increased root growth, stem length, leaf area, and protein content compared to NaCl stressed plants without triadimefon, which showed significant reduction of all the above characters (Gopi *et al*., 1998). Those authors further suggested that the NaCl stress increased amino acid, proline and glycine betaine contents in all parts of the seedlings, but the triadimefon treatment to NaCl stressed seedling lowered the NaCl induced increase of these metabolites.

According to Panneerselvam *et al.* (1998) NaCl stress caused a reduction in root length, shoot length, root dry mass and shoot dry mass of *Glycine max*. Accumulation of proline and other aminoacids, decreased

protein and nucleic acid also were the effect of NaCl stress in this plant. But the dry weight of root and shoot was higher than stressed seedlings of *G. max* when treated with triadimefon.

Inhibitory effect of NaCl treatment in *Cajanus cajan* expressed as reduced germination percentage, root and shoot growth, leaf area, dry weight and increased sugar content, α and β amylase and ATPase (Karikalan *et al.*, 1999) All these stress effects are reversed by treatment with triadimefon along with NaCl.

According to Gopi *et al*. (1999) the NaCl stress decreased root and shoot length and dry weight; the triadimefon treatment increased the shoot and root growth and dry matter of *Vigna unguiculata* seedlings. The activity of peroxidase was inhibited by NaCl stress in all parts of the seedlings but triadimefon treatment to the NaCl stressed seedlings increased the activity of peroxidase.

Sujatha *et al.* (1999a; 1999b) investigated the effect of NaCl stress on *Abelmoschus esculentus* and found that in stressed plants reduction of root length, shoot length, dry weight, chlorophyll content and carotenoides, protein content, peroxidase and superoxide dismutase activity were occurred. Treatment with triadimefon resulted in the reversal of all the inhibitory effect of NaCl such as growth retardation, reduction of pigments, proteins, peroxidase and SOD activities. On the other hand, NaCl stressed seedlings

exhibited increase of reducing and non reducing sugar content, activity of amylase (both α and β), protease, ATPase, total free aminoacids and proline. All these physiological effects of NaCl is found to be increased in *Abelmoschus esculentus* seedlings treated with triadimefon after NaCl stress. Those authors opined that triazole compounds like triadimefon could protect plants against many stresses as these compounds have been reported to act as "plant multi protectants" by Fletcher and Hofstra (1988).

Triazoles group of compounds have been used as plant growth regulators or fungicides and also as plant protectants against stresses inclusive of heavy metal toxicity (Singh, 1993; Thomas and Singh, 1996). According to Purohit and Singh (1999) in *Abelmoschus esculentus* inhibition of growth and increased membrane permeability occurred due to heavy metal toxicity (cadmium and lead). Treatment with uniconazole which is a derivative of triazole resulted in the reduction of growth inhibition and membrane permeability, there by ameliorating the toxic effect of these metals to considerable extent.

Toxic effect of cadmium on wheat seedlings treated with cadmium chloride showed reduction of chlorophyll, nitrate reductase activity, total nitrogen and dry weight. But triadimefon treatment resulted in an increase of nitrate reductase activity and chlorophyll pigment synthesis and total nitrogen content (Babu and Singh, 1992).

Muthukumarasamy *et al*. (2000) reported that activities of peroxidases, polyphenyl oxidases and superoxide dismutase (SOD) were decreased by NaCl stress in *Raphanus sativus* and addition of triadimefon to the NaCl stressed plants showed increased activities of these enzymes and thereby ameliorated the negative effect of NaCl stress.

Triadimefon helps to maintain membrane integrity, there by reducing electrolyte leakage (Fletcher and Hofstra, 1988; Sailerova and Zwiazek, 1993). According to Paul and Ezekiel (2004) triadimefon treatment reduced ion leakage in potato tubers and that the decrease in ion leakage due to triadimefon treatment is related to the physiological age of the tubers.

MATERIALS AND METHODS

1. PLANT MATERIAL

Centella asiatica (L.) Urb. is a common plant belonging to the family Umbelliferae (Apiaceae). Healthy Plants were collected from the paddy fields in Malappuram and Kozhikode Districts for the present study. Multiplication of the plants were done by planting the cuttings of runner collected from the healthy plants grown in garden pots filled with garden soil, sand and dried powdered cow dung mixed in the ratio 2:1:1. The pots with plants were watered regularly and allowed to establish and maintained in the net /poly house of the Department of Botany, University of Calicut, throughout the course of the investigation.

2. NUTRIENT CULTURE STUDIES

Cuttings of plants growing in garden pots were collected from healthy plants and rooting was done by planting in trays filled with soil. When the cuttings develop roots from the nodes the plants were carefully removed from the soil, which was used for the nutrient culture studies.

2. 1 Preparation of Nutrient Solution

The modified Hoagland's solution after Epstein (1972) as described by Taiz and Zeiger (2002) was prepared and used for the present study. Composition of the modified Hoagland's solution is shown in Table 1.

The stock solution of each nutrient was prepared separately and appropriate volumes were mixed to make up nutrient solution of final volume and concentration. The pH of the final solution was adjusted to 6.8 using 0.1 N HCl or NaOH.

2.2 Preparation of Mercuric Chloride and Triadimefon Solutions

One molar solution of mercuric chloride ($HgCl_2$) was prepared as the stock solution. Trial experiment was conducted by planting rooted propagules of *C.asiatica* plants in nutrient solution containing different concentrations of mercuric chloride such as 5, 10, 15 and 20µM in order to determine the optimum concentration in which the experimental plants survived and exhibited about 50 percent growth retardation. Out of the various concentrations tried 15µM was found to be optimum for the present study. Appropriate volumes of $HgCl_2$ solution were mixed with required volume of nutrient stock solution and final volume was made up to 1000ml with 15µM $HgCl_2$.

Triadimefon stock solution was prepared by dissolving 1gm of triadimefon in one litre of Hoagland's solution. Rooted propagules of *Centella asiatica* were planted in nutrient solution to which different concentration of triadimefon was added. From the trials, plants grown in $15mgl^{-1}$ triadimefon solution were selected for further experiments. In order to determine the antagonistic effect of triadimefon, the selected concentrations of both $HgCl_2$ and triadimefon solution were mixed together and experimental plants were also allowed to grow in it. So a combination of mercuric chloride and triadimefon solution was prepared by adding 15 mg of traidimefon to 1000 ml of 15 µM $HgCl_2$ solution prepared in Hoagland nutrient medium and the present investigation includes the following sets:

1. Control plants growing in Hoagland's medium (Represented as C),

2. Plants grown in 15µM $HgCl_2$ solution (Represented as Hg),

3. Plants grown in $15mgl^{-1}$ triadimefon solution and (Represented as Tri), and

4. Plants grown in a combination of 15µM $HgCl_2$ + $15mgl^{-1}$ triadimefon solution (Represented as Hg + Tri).

Table 1: Composition of a modified Hoagland nutrient solution employed in the present study.

Compound	Molecular weight	Concentration of stock solution	Concentration of stock solution	Volume of stock solution per litre of final solution	Final concentration of element	
	$g\ mol^{-1}$	mM	$g\ l^{-1}$	ml	μM	ppm
Macronutrients						
KNO_3	101.10	1,000	101.10	6.0	16,000	224
$Ca(NO_3).\ 4H_2O$	236.16	1,000	236.16	4.0	6,000	235
$NH_4H_2PO_4$	115.08	1,000	115.08	2.0	4,000	160
$MgSO_4.\ 7H_2O$	246.48	1,000	246.49	1.0	2,000	62
					1,000	32
					1,000	24
Micronutrient						
KCl	74.55	25.0	1.864		50.0	1.77
H_3BO_3	61.83	12.5	0.773		25.0	0.27
$MnSO_4.\ H_2O$	169.01	1.0	0.169		2.0	0.11
$ZnSO_4.\ 7H_2O$	287.54	1.0	0.288	2.0	2.0	0.13
$CuSO_4.5H_2O$	249.68	0.25	0.062		0.5	0.03
H_2MoO_4 (85% MoO_3)	161.97	0.25	0.040		0.5	0.05
NaFeEDTA (10% Fe)	558.50	53.7	30.00	0.3-1.0	16.1-53-7	1.00-3.00

Adopted from Taiz and Zeiger (2002)

Good quality of plastic containers with size 20×15×5cm were selected to take nutrient solution. Slightly large sized plastic net trays of size 30×20×5 cm having holes of 5×5 mm were placed above the plastic container. The roots of the propagules were inserted through the holes of the tray in to the nutrient solution taken in the container. Plastic twine of diameter with 1mm was tied interweavingly length-wise and breadth-wise on the trays forming a mesh to provide mechanical support to the propagules. The trays were placed in such a way that only the roots become in touch with the nutrient solution. Ten trays for each treatment were taken and 1000 ml of Hoagland's solution was added to one tray, which served as the control. Three trays each were filled separately with 1000 l of nutrient + $HgCl_2$, nutrient + triadimefon and combination of both $HgCl_2$ and triadimefon. Additional nutrient solution was added to replenish the nutrient medium at an interval of 4 days as and when required.

3. SAMPLING

Random sampling was followed by collecting a minimum of six plants from the trays of each treatment and control at intervals of 4 days from the day of treatment up to 24^{th} day there by constituting seven samples of each treatment. The collected plants were thoroughly washed in tap water, blotted to remove water adhered on it and separated into root, runner and leaves. All these samples were used separately for various studies.

4. MORPHOLOGICAL STUDIES

Growth of the treated as well as control plants was assessed in terms of root length, runner (stem) length, petiolar length, leaf area, tolerance index percentage, stomatal index and total biomass. The fresh weight and dry weight of individual plant parts like roots, runner and leaves were also determined.

The length of root, runner and petiole was measured using a graduated scale and was expressed in centimetres. The runner length was measured from the basal part of the runner to the extreme tip of the plant. In *C. asiatica* the root system appear as a tuft of "fibrous like" and so the length of the longest root in each tuft is taken in to consideration for measuring the length of the roots.

The leaf area of the treated and control plants were measured using graph paper method and was expressed in cm^2. All these data were taken from randomly selected six plants each and the mean values were calculated.

4.1 Fresh Weight

For fresh weight determination the plants were carefully removed from the nutrient solution, washed thoroughly in water and blotted to dryness. The plant parts such as root, runner, and leaves were separated. Fresh weight of individual plant parts was determined using a Shimadzu electronic balance.

4.2 Tolerance Index Percentage

Tolerance index (TI) percentage was calculated according to the method of Turner (1994).

$$TI\% = \frac{\text{Observed value in solution with metal}}{\text{Observed value in solution without metal}} X\ 100$$

For calculating Tolerance Index root length, runner length and leaf area were taken as parameters.

4.3 Studies on Stomata

To study stomatal density, number of stomata on abaxial and adaxial sides of the leaf were counted under a light microscope, preparing impressions using a colourless nail-polish. Stomatal index was calculated according to the following method of Meidner and Mansfield (1968).

$$\text{Stomatal index} = \frac{\text{Number of stomata per unit area}}{\text{Number of stomata per unit area} + \text{number of epidermal cells per unit area}} \times 100$$

4.4 Biomass Determination

Total biomass content of the control and experimental plants were calculated on per plant basis. For biomass determination each plant from treated and control were selected and the root, stem (runner) and leaf tissues were taken separately for dry weight determination. The separated root, stem and leaf tissues were oven dried initially at 100^0C for one hour and then at

60^0C till the weight becomes constant. Total biomass per plant was calculated from the sum of the dry weight of root, stem (runner) and leaf tissues.

5. ANATOMICAL / HISTOCHEMICAL STUDIES

Free-hand sections of the root, stem and leaf tissues of control and treated plants of *C. asiatica* samples on 4^{th}, 12^{th}, and 24^{th} days were taken and stained with safranin and semi permanent slides were prepared. Sections were observed using Nikon microscope (Model Nikon ECLIPSE E400) and photomicrographs were taken using Nikon Digital Camera (Model D x M 1200F) and image analyser.

6. PHYSIOLOGICAL/ BIOCHEMICAL STUDIES

6.1 Chlorophyll

Chlorophyll estimation of leaves of treated and control plants was done according to the method of Arnon (1949). Two hundred milligram of fresh leaf tissues of each sample were homogenized using chilled acetone in a pre-chilled clean glass mortar and pestle. The homogenate was centrifuged for 10 minutes and the supernatant was collected. The residue was again extracted with 80% acetone and centrifuged. The supernatant was pooled together and the extraction process was repeated until the residue become colourless. Volume of the combined supernatant was noted. The absorbance of the solution was measured at 645 nm and 663 nm against the solvent (80% acetone) as blank.

The amount of chlorophyll present in the extract was calculated in mg chlorophyll per gram tissue according to the following equation (Arnon, 1949).

$$\text{mg chlorophyll a/}_{\text{g tissue}} = 12.7\ (A_{663}) - 2.69\ (A_{645})\ \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll b/}_{\text{g tissue}} = 22.9\ (A_{645}) - 4.68\ (A_{663})\ \frac{V}{1000 \times W}$$

$$\left.\begin{array}{l}\text{mg total}\\ \text{chlorophyll /}_{\text{g tissue}}\end{array}\right\} = 20.2\ (A_{645}) + 8.02\ (A_{663})\ \frac{V}{1000 \times W}$$

Where A = Absorbance at specific wave lengths

V = Final volume of chlorophyll extract in 80% acetone

and W = Fresh weight of tissue extracted

6.2 Protein

Protein content of individual plant parts sampled at each interval was estimated using Folin – Ciocalteau reagent according to the method of Lowry *et al.* (1951).

6.2.1 Extraction

Five Hundred mg of fresh tissues of treated as well as control plant parts, such as root, runner and leaves were homogenized in 5ml of 50 mM Phosphate buffer (pH 7.5) containing 100 mM 2-mercapto ethanol using pre-chilled glass mortar and pestle. Volume of the homogenate was measured. One millilitre of the homogenate in duplicate was taken in clean dry test tubes for the estimation of total protein. The remaining part of the homogenate was transferred to centrifuge tubes and centrifuged for 10 minutes. The supernatant was collected and used for the estimation of soluble proteins. One millilitre each of the homogenate as well as supernatant was taken in duplicate and equal volume of 10% (w/v) trichloroaceticacid was added and kept in a refrigerator for one hour for flocculation. The protein precipitated was collected by centrifugation for 5 minutes, the supernatant was decanted off. The residue was washed twice with cold 2% trichloroaceticacid (w/v) followed by washing with anhydrous acetone and then by 80% (v/v) acetone.

6.2.2 Estimation

The pellet obtained after centrifugation was digested in 5 ml of 0.1 N sodium hydroxide by heating in a bath of boiling water for 5 minutes. The suspension was clarified by centrifugation and supernatant was collected. Suitable aliquots were taken in triplicate and the volume was made up to 1 ml with double distilled water. To the aliquots 5ml of alkaline copper reagent

was added and shaken well. After 10 minutes 0.5 ml of IN Folin- Ciocalteau reagent was added and shaken well immediately. The tubes were kept for 30 minutes for colour development. The optical density of the solution was read at 700 nm using a GENESIS 20 Spectrophotometer. Bovine Serum Albumin fraction V powder was used as standard.

6.3 PAGE Studies of Proteins

SDS polyacrylamide gel electrophoresis was carried out according to the method of Gaal *et al*. (1980). Five hundred milligrams of the root, stem and leaf tissues of the plants of control as well as the treatments were homogenized using a chilled mortar and pestle in 50mM phosphate buffer in the presence of polyvinyl polypyrrolidone as phenolic binder and 10% sodium dodecyl sulphate. The 10% homogenate was centrifuged at 16,000Xg for 20 minutes using a Plastocraft model ROTA R4 Rv/Fm refrigerated centrifuge at 4^0C and the supernatant was collected.

6.3.1 Preparation of Gels

The resolving gel was prepared by mixing 3.3ml of acrylamide/bissacrylamide (30% T and 2.67 % C), 5ml of 1.0M resolving gel buffer (pH 8.8), 50µl of 10% ammonium persulphate, 50µl of 10% SDS and 5.0µl TEMED. The mixture was made upto 10ml with deionized water.

The stacking gel was prepared by mixing 0.99ml of acrylamide/ bisacrylamide (30% T and 2.67% C), 3ml of 0.5M resolving gel buffer (pH – 6.8), 30µl of 10% ammonium persulphate, 30µl of 10% SDS and 5µl TEMED. The mixture was made upto 6ml with deionized water.

6.3.2 Gel Casting

The gel was cast in a Genei Mini vertical gel casting unit. The glass plates, the comb and the spacers of the casting unit were wiped clean with alcohol using tissue paper. The glass plates were then wiped with acetone. The dried glass plates were clamped on the casting unit with the spacers placed in between them. The resolving gel was poured into the casting unit and the top was layered with a small volume of deionized water to avoid contact with air. After completion of polymerization, the water was removed with strips of filter paper. Then the comb was placed and the stacking gel was poured carefully. The gel was topped with deionized water. After polymerization, the comb was removed carefully and the well were cleaned thoroughly.

6.3.3 Electrophoresis

Fourty millilitre of the extract containing 20% sucrose was added to each well. Bromophenol blue was used as the tracking dye. Medium range molecular weight Marker (Genei) was loaded in one of the wells.

Electrophoresis was carried out using the electrophoretic reservoir buffer, Tris-glycine, pH-8.4. Initially the gels were maintained at a voltage of 80v. Once the stacking has taken place, the voltage was raised to 120v and was maintained there till the electrophoretic run was completed. At the end of the run, the gel was carefully removed and was stained with 0.2% (w/v) coomassie brilliant blue R 250 in methanol acetic acid mixture. After 3 hours of staining, the gels were destained in methanol acetic acid mixture and stored in 7% (v/v) acetic acid.

The gels were analysed in a Broad Geldoc and molecular weight of the bands was determined using the software, Quantity-One.

6.4 Total Free Amino Acids

Total free amino acids of plant parts such as root, runner and leaves of treated and control plants of *C. asiatica* were estimated according to the method of Lee and Takahashi (1966).

6.4.1 Extraction

One gram of the fresh tissue was homogenized in 80% (v/v) alcohol using a clean glass mortar and pestle. The homogenate was transferred to a round-bottomed flask fitted with vertical condenser and refluxed on a boiling water bath for 2 hours. Then the suspension was centrifuged and the supernatant was collected. The residue was re-extracted with 80% alcohol and

after each centrifugation the supernatant was combined with the original supernatant. The combined supernatant was then evaporated to dryness over a boiling water bath, eluted using a known quantity of 10% iso-propanol. This extract was used for the determination of total free amino acids using ninhydrin.

6.4.1.1 Preparation of ninhydrin – citrate – glycerol reagent

One ml of 1% (w/v) ninhydrin solution in 0.5M citrate buffer (pH 5.5) was mixed thoroughly with 2.4 ml of glycerol and 0.4 ml of 0.5 M citrate buffer (pH5.5)

6.4.2 Estimation

To 0.2 ml of the sample, 3.8 ml of ninhydrin – citrate – glycerol mixture was added. After shaking well, the mixture was heated in a boiling water bath for 12 minutes and cooled to room temperature, by keeping in tap water. Within one hour the optical density of the resultant solution was measured at 570nm using a GENESIS 20 Spectrophotometer. The reagent blank was prepared by mixing 0.2 ml water and 3.8 ml ninhydrin – citrate – glycerol. Glycine was used as the standard.

6.5 Proline

Proline content in the plant parts was estimated according to the method of Bates *et al*. (1973).

6.5.1 Extraction

One gram fresh tissue of plant parts such as root, runner and leaves each of the experimental and control plants was homogenised in 10 ml of 3% (w/v) aqueous sulfosalicylic acid using a clean glass mortar and pestle. The homogenate was transferred to centrifuge tubes and centrifuged for 10 minutes, and the supernatant was collected and estimation of proline was done using acid ninhydrin.

6.5.1.1 Preparation of acid ninhydrin

Acid Ninhydrin was prepared by dissolving 1.25 gm of Ninhydrin in a mixture of 30 ml of glacial acetic acid and 20 ml of 6 M ortho phosphoricacid.

6.5.2 Estimation

From the supernatant 2 ml was taken in test tubes in triplicate and equal volumes of glacial acetic acid and acid ninhydrin were added to it. The tubes were then heated in a bath of boiling water for one hour and then the reaction was terminated by placing the tubes in an ice bath. For colour development 4 ml of toluene was added to the reaction mixture and stirred well for 20-30 seconds. Then the toluene layer was separated carefully and brought to room temperature. The colour intensity of the solution was

measured at a wave length of 520 nm using PHOTOCHEM colorimeter. L-Proline was used as the standard.

6.6 Phenolics

Total phenolics was estimated using Folin-Denis Reagent (Folin and Denis, 1915). Tannic acid was used as the standard.

6.6.1 Extraction

One gram of fresh tissue of treated and control plant parts was homogenized in 10 ml of 80% (v/v) alcohol using a clean glass mortar and pestle. The homogenate was quantitatively transferred to a round bottomed flask fitted with vertical condenser, and refluxed for two hours over a boiling water bath. The suspension was then cooled, centrifuged for 10 minutes and the supernatant was collected. The residue was re-extracted with 80% alcohol and refluxed for one hour. The supernatant was collected, and combined with the original. The total volume of the supernatant was noted.

6.6.1.1 Preparation of Folin-Denis reagent

To 750 ml of distilled water, 100 g sodium tungstate, 20 g phosphomolybdic acid and 50 ml ortho phosphoric acid were added. This mixture was refluxed for 2 hrs, cooled and diluted to 1 litre. Normality of the reagent was determined by titrating it against an alkali NaOH with phenolphthalein as an indicator.

6.6.2 Estimation

Aliquots of 2ml in triplicate were pipetted out and equal volume of Folin – Denis reagent was added. The contents were thoroughly mixed and after 3 minutes 2 ml of IN sodium carbonate was added. This mixture was kept for one hour, after thorough mixing, for colour development. The optical density of the resultant solution was measured at 700 nm using a GENESIS - 20 Spectrophotometer.

6.7 Peroxidase Assay

Peroxidase activity of plant parts such as root, runner and leaf was measured according to the method of Abeles and Biles (1991).

6.7.1 Extraction of Enzymes

From the randomised tissue samples1.0 g each of root, stem (runner) and leaf of control as well as treatments, were weighed and a 10% homogenate was prepared by grinding in 50 mM Tris-HCl buffer (pH 7.5) and 50 mg of polyvinyl poly pyrrolidone using a pre-chilled mortar and pestle. The extract was filtered through two layers of muslin cloth and was made up to 10 ml. The filtrate was transferred to centrifuge tubes and centrifuged in a cold centrifuge at 15,000 rpm for 15 minutes at 0°C using Plastocraft cold centrifuge model (ROTA R4 R_V/F_M). The supernatant was

transferred to a clean test tube and stored in ice bath. One part was used for enzyme assay and the rest for protein estimation.

6.7.2 Enzyme Assay

Assay system consisted of 1.5ml of 100 mM phosphate buffer, 0.3ml of 10 mM guaiacol, 0.3ml enzyme extract and 0.6ml distilled water except hydrogen peroxide. The hydrogen peroxide 10 mM (0.3ml) was added to initiate the enzyme activity. Immediately after the addition of hydrogen peroxide the mixture was transferred to a Spectrophotometer cuvette and the activity was measured at 470 nm by direct measurement of optical density using a Shimadzu UV-visible Spectrophotometer UV- (1601) for 3 minutes at intervals of 30 seconds. The blank was prepared by adding 0.3ml water to the reaction mixture instead of enzyme extract. The optical density was determined by subtracting the initial absorbance from the optical density at 180^{th} second, and the unit activity was calculated on per gram tissue fresh weight basis. The unit activity of peroxidase enzyme was expressed as the change in absorbance per minute per gram fresh weight of tissue.

The rate of formation of guaiacol dehydrogenation product is a measure of the peroxidase activity. The enzyme activity was expressed in terms of specific activity, which is the activity per mg protein.

7. BIOACCUMULATION STUDIES

Bioaccumulation studies were conducted by estimating mercury content present in the tissue components such as root, stem (runner) and leaf samples. Mercury content retained in the residual solution also was analysed.

Different plant parts – root, stem and leaf tissues were sampled at each interval upto 24 days of growth in the culture medium artificially contaminated with mercury, a combination of mercury + triadimefon and triadimefon along with control. Accumulation of Hg in the samples collected at each interval like 4, 8, 12, 16, 20 and 24 days were recorded.

Mercury content was analyzed using Atomic Absorption spectrophotometer (AAS). Samples were prepared according to the method of Allan (1969). Oven dried plant materials were used for the analysis. Known weight (500mg) of each sample was wet digested by refluxing in 10ml of concentrated nitric acid and perchloric acid mixed in the ratio of 10:4 until the solution became colourless in Kjeldahl's flasks and made upto 50ml. Atomic Absorption Spectrophotometer (PERKIN ELMER A Analyst 300) available at Cashew Export and Promotion Council (CEPC), Kollam, Kerala, was used for estimating mercury in all the samples.

8. STATISTICAL ANALYSIS

All experiments were repeated a minimum of six times and the mean values are given in tables and figures. Standard deviation and standard error were also calculated. The values in tables were mean value ± standard error. Test of significance was done using Fisher's 't' test.

RESULTS

1. MORPHOLOGICAL STUDIES

1.1 Root Length

Root length of control plants showed a gradual increase during the period of investigation (Table 2; Fig. 1). In plants treated with mercury, there was only a slight increase in root length during 24 days of growth. Plants treated with triadimefon showed the same trend as that of control plants. These plants exhibited gradual increase in root length throughout the period of investigation. But in plants treated with a combination of Hg and triadimefon, a slight increase was noted during the entire period. Among treated plants, triadimefon treated plants showed maximum increase in root length.

1.2 Runner Length

Runner length of control plants was 20.28cms (Table 3). The control plants showed a slight increase during 4 days. A sharp and significant ($p < 0.05$) increase was noted on the 8^{th} day. After 8 days the runner length of control plants increased throughout the period of experiment.

A gradual but slight increase in runner length was exhibited by the plants treated with mercury (Fig. 2). The plants treated with triadimefon

showed a regular increase in runner length throughout the period of study.

The plants treated with the combination of mercury and triadimefon also showed only insignificant increase in runner length during the experimental period.

During 24 days of growth, the control plants showed maximum increase in runner length than the treated plants. Among treated plants the maximum increase in runner length was exhibited by the triadimefon treated plants. Mercury treated plants showed minimum increase in runner length.

1.3 Petiolar Length

Control plants showed a significant increase in petiolar length ($p < 0.02$) on 4^{th} day (Table 4; Fig. 3). There after it increased regularly during the period of investigation. Petiolar length remained almost same during the entire period, in plants treated with mercury. Plants treated with triadimefon maintained almost same pattern of increase in petiolar length as that of the control plants. But the plants treated with a combination of mercury and triadimefon showed a gradual and regular increase in petiolar length. The rate of increase in this treatment was less than that of control plants and the plants treated with triadimefon.

1.4 Leaf Area

The leaf area of control plants showed about a two-fold increase on the

4th day of investigation. Thereafter it increased regularly. On 24th day leaf area was about six times as that of the zero day (Table 5; Fig. 4)

In mercury treated plants, leaf area showed a slight increase on 4th day. It increased regularly in subsequent intervals. On 24th day leaf area was only more than two times of the zero day.

Leaf area in plants treated with triadimefon showed a significant increase ($p < 0.01$) during the 4th day. Then it increased regularly. On 24th day of treatment the leaf area showed more than 5 times increase than that of initial day.

In plants treated with a combination of mercury and triadimefon, leaf area showed a gradual increase throughout the entire period of investigation. Final leaf area was only more than three times compared to the initial area.

1.5 Tolerance Index Percentage

Tolerance index percentage with respect to root length showed a significant decrease ($p < 0.01$) in plants treated with mercury (Table 6; Fig. 5A). It decreased regularly upto 20th day. Plants treated with triadimefon maintained almost same pattern as that of control plants throughout the period of study. In plants treated with a combination of mercury and triadimefon, tolerance index percentage with respect to root length showed a slight decrease during initial days. It decreased gradually up to 16 days and thereafter it remained almost same upto 24 days.

In plants treated with mercury the tolerance index percentage with respect to runner length showed a significant reduction during 4th and 8th days ($p < 0.02$). Then it reduced gradually. Plants treated with triadimefon showed almost same value as that of control plants on 4th day (Fig. 5B). Thereafter it showed a gradual reduction up to 24 days. The plants treated with a combination of mercury + triadimefon showed a significant reduction on 4th day and 8th day. Then it remained almost same up to 24th day of sampling. The mercury treated plants showed more reduction in tolerance index percentage with respect to runner length than other treatments.

Plants treated with mercury showed a sharp significant reduction ($p < 0.02$) in tolerance index percentage with respect to leaf area on 4th and 8th days of sampling (Fig. 5C). It was reduced to almost half on 8th day compared

to the zero day. After 12th day the reduction was gradual. In plants treated with triadimefon a significant reduction in tolerance index percentage ($p < 0.02$) was observed on 4th day and the value was remained the same upto 24th day of sampling.

A sharp and significant ($p < 0.01$) reduction was observed in plants treated with a combination of mercury + triadimefon on 4th day. Tolerance index percentage with respect to leaf area again decreased in these plants and remained almost same on 24th day of sampling.

Among treated plants, the maximum reduction in tolerance index percentage with respect to leaf area was observed in mercury treated plants.

1. 6 Stomatal Index

Control plants showed a gradual increase in stomatal index of upper and lower epidermis, during the period of investigation (Table 7; Fig. 6A & 6B)). The stomatal index on lower epidermis was doubled on 24th day. Stomatal index on upper epidermis remained same on 16th, 20th and 24th day of experiment.

A rapid and significant increase ($p < 0.02$) was noted in stomatal index of lower epidermis on the 4th day of mercury treated plants. Stomatal index in upper and lower epidermis showed an increase throughout the period of investigation.

A very gradual increase in stomatal index was noted in plants treated with triadimefon. In plants treated with a combination of mercury and triadimefon a significant increase ($p < 0.01$) in stomatal index on the upper and lower epidermis was observed on the 4^{th} day. These increase continued throughout the period of investigation.

Maximum increase in stomatal index was exhibited by plants treated with mercury than other treatments.

2. ANATOMICAL / HISTOCHEMICAL STUDIES

2.1 Root

Transverse sections of root of control plants showed single layered epidermis, broad cortex consisting of airspace and a central stele (Fig. 7, 8 & 9). The cortical cells were thin walled and small. Pith cells were also thin walled but slightly larger than cortical cells. In plants, treated with mercury the outer layers of cortex and xylem vessels were found filled with many orange red spots, which were absent in triadimefon treated plants. In plants treated with a combination of mercury+ triadimefon also some stained spots were observed.

2.2 Stem

Cross section of stem of control plants stained with safranin showed typical anatomical features of a dicot stem consisting of single layered

epidermis, collenchymatous hypodermis, chlorenchymatous broad cortex and stele (Fig. 10, 11 & 12). The stele consisted of limited number (6-7) of vascular bundles with xylem and phloem with cambium in between them. Pith cells are large, parenchymatous and thin walled. In the stem of mercury treated plants there were orange coloured spots in the xylem and cortical cells upto 12th days of treatments. Some spots were observed in the stem of plants treated with a combination of mercury and triadimefon during these intervals. On the 24th day such spots were not observed in the stem tissues of mercury and mercury + triadimefon treated plants. The pith cells showed deformities on 12th and 24th days of treatment.

Mercury treatment resulted in the development of sclerenchymatous bundle cap consisting of thick and compact cell. But the bundle cap showed distorted arrangement on the same day in mercury + triadimefon treated plants. In the stem of mercury treated plants, certain stained spots were localized in some of the cortical cells, which was not seen in the stem of control and triadimefon treated plants. In the plants treated with a combination of mercury and triadimefon also the stem tissues contained such stained spots in the cortical region.

2.3 Leaf

The cross section of leaves of control plant showed single layered epidermis with palisade and spongy tissue. There is a vascular region at the

centre. The section of leaves treated with mercury and mercury + triadimefon showed thick xylem vessels compared to triadimefon treated and control plants (Fig 13, 14 & 15).

3. BIOMASS DETERMINATION

The control plants showed gradual but insignificant increase in the biomass content of root (Table 8; Fig 16A, 16B &16C). In mercury and combination of mercury + triadimefon treated plants the increase was not significant. In triadimefon treated plants the root biomass showed a significant increase in their value. The roots of triadimefon treated plants showed maximum biomass content compared to other treatments and control.

In the case of stem tissue there was a significant increase in biomass content of control plants as well as plants treated with triadimefon. In mercury treated plants as well as the plants treated with mercury and triadimefon there was an increase in the biomass content during the experimental period, but it was not significant. In the case of leaf tissues, the control as well as triadimefon treated plants showed an increase in the biomass content, which was significant. But the leaf tissue of plants treated with mercury as well as the combination of mercury + triadimefon showed insignificant increase in the biomass content.

In the case of total biomass, it was maximum in the plants treated with triadimefon during the growth (Table 9; Fig. 17). The biomass was minimum in the plants treated with mercury. In plants treated with a combination of mercury and triadimefon, the biomass content showed an increase over plant treated with mercury, but it was lesser than that of control and plants treated with triadimefon.

3.1 Root/ Shoot Ratio

Root/shoot ratio, based on biomass content showed that in control and plants treated with mercury and combination of Hg + triadimefon there was no significant change during the growth (Table 10; Fig. 18). In these plants it was more or less uniform throughout the experimental period. But in plants treated with triadimefon there was an increase in the root/ shoot ratio upto 24th day of treatment.

4. PHYSIOLOGICAL/ BIOCHEMICAL STUDIES

4.1 Chlorophyll

Control plants contained 3.33mg chlorophyll a content (Table 11). The chlorophyll b content of control plant was 1.25mg, and was increased throughout the experimental period. More than four times increase was observed on 24th day (Fig. 19A).

In mercury treated plant, only slight increase in chlorophyll a and

chlorophyll b content was observed on 4th day (Fig. 19B). Maximum chlorophyll content was observed on 16th day, which was about 3 times as that of initial content. On 20th day and 24th day the chlorophyll content was decreased. A significant decrease ($p < 0.01$) was observed on 20th day.

Plants treated with a combination of mercury and triadimefon showed double the amount of chlorophyll content on 4th day (Fig. 19C). Then a slight increase was observed throughout the experimental period. On 24th day more than four times increase in chlorophyll content was observed.

In triadimefon treated plants, a sharp and significant ($p <0.01$) increase was observed on 4th day of experiment (Fig. 19D). Thereafter, a slight increase was observed throughout the experimental period. More than five times increase was observed on 24th day.

Among all the treatments, the maximum increase in chlorophyll content was observed in triadimefon treated plants and minimum content was in mercury treated plants.

4.1.1 Chlorophyll a/b ratio

The chlorophyll a/b ratio of control plant was 2.66 (Table 12; Fig. 20). It remained almost same throughout the experimental period.

In mercury treated plants, slight fluctuations were observed in chlorophyll a/b ratio. On 24th day the ratio was almost same as that of control

plants.

Triadimefon treated plants also showed slight fluctuations in chlorophyll a/b ratio during the initial days. On 24th day, the ratio was almost same as that of the initial value.

In plants treated with a combination of mercury and triadimefon, the ratio was significantly ($p < 0.02$) reduced on 4th day. Thereafter it showed slight fluctuations.

4.2 Total Protein

4.2.1 Root

Root of control plants showed about 12% total protein content during early days of sampling, and showed gradual increase during growth (Table 13; Fig. 21A). It showed a significant increase ($p < 0.02$) on 12th day, and at this stage it was about more than double as that of initial day. During 16th and 20th day of sampling total protein content was almost same. i.e., about 30%. After 20th day the protein content showed a slight decrease.

In plants treated with mercury, the total protein content showed a slight decrease on 4th day, maintained almost same content on 8th day. The total protein content of mercury treated plant increased significantly on 12th and 16th day ($p < 0.01$, $p < 0.02$). Total protein content on 16th and 20th day remained almost same. Thereafter it showed a significant reduction ($p < 0.02$).

In triadimefon treated plants, total protein content of roots showed an increase on 4th day. It increased significantly ($p < 0.01$) on 8th day and it was almost doubled on 8th day. A sharp increase was observed on 16th day. During subsequent days of sampling the total protein content of root showed a gradual decrease.

In the roots of plants treated in a combination of mercury and triadimefon the total protein content was almost same on 4th day. A sharp and significant ($p < 0.02$) increase in total protein content on 12th day of sampling was observed. A slight increase was observed on 16th day. Thereafter it maintained the same value upto 24th day of sampling.

Among different treatments, the total protein content of root was maximum in plants treated with triadimefon on 16th day of sampling.

4.2.2 Stem

The total protein content in the stem of control plant was only 2%. (Table 13; Fig. 21 B). The total protein content was doubled on 8th day and it showed a gradual increase upto 16th day. On 16th day it was more than 4 times as that of the initial protein content. After 16th day it showed a very gradual reduction up to 24th day of sampling.

In plants treated with mercury, the total protein content of stem showed a gradual increase up to 8th day. On 12th day there was a sharp and significant

($p < 0.01$) increase in total protein content. On 16th and 20th day it was about 4 times as that of the initial content. After 20th day there was a significant ($p < 0.02$) reduction in total protein content.

Total protein content in the stem of plants treated with triadimefon a sharp and significant increase was observed on 8th ($p < 0.01$), 12th ($p < 0.02$) and 16th ($p < 0.01$) days of sampling. It was maximum on the 16th day, and it was almost five times as that of the initial content. The 20th day sample maintained almost same protein content as that of 16th day sample. After 20th day there was a slight reduction in total protein content.

In plants treated with a combination of mercury and triadimefon the total protein content increased up to 16th day and almost the same protein content was observed on 20th day. After 20 days, the protein content showed a significant ($p < 0.01$) reduction.

4.2.3 Leaf

Leaves of control plant showed 12% total protein content (Table 13; Fig. 21C) at the initial days of sampling. On 4th day and 8th day, the total protein content of leaves showed a regular increase. On 12th and 16th day the total protein content increased significantly ($p < 0.01$, $p < 0.02$). The control leaves showed maximum protein content on 16th day i.e., about 29% and on 20th day also almost same protein content was observed as that of 16th day sample. After 20th day the total protein content of control leaves showed a

significant reduction ($p < 0.01$).

In plants treated with mercury, protein content of leaves remained almost same on 4th day. Total protein content of leaves of these plants showed a regular increase up to 16th day. A sharp and significant increase in total protein content was observed on 16th day. After 16th day, there was a sharp reduction in leaf total protein content, which continued up to 24th day of experiment.

In triadimefon treated plants, the total protein content of leaf tissue increased regularly upto 16th day. Maximum total protein content of leaves was shown on 16th day i.e., about 33%. After 16th day, total protein content of leaves declined sharply, and it continued upto 24th day.

Plants treated with a combination of mercury and triadimefon exhibited a slight increase in protein content of leaves on 4th day. Thereafter it showed a regular increase upto 16th day of sampling. The samples of 16th and 20th days showed almost same total protein content i.e., about 28%. After 20th day total protein content of leaves declined. The plants treated with triadimefon on 16th day of sampling showed the maximum value of total protein content of leaves.

4.3 Soluble Protein

4.3.1 Root

Roots of control plants showed only 2.7% of soluble protein content (Table 14; Fig. 22 A). It increases slightly on 4th day, but on 8th day the increase was sharp and significant ($p < 0.01$). Thereafter the soluble protein content of root increased regularly up to 16th day. The sample on 16th day showed maximum soluble protein content i.e., 7%. After 16th day the soluble protein content of control roots showed a sharp and significant reduction ($p < 0.02$) on 20th day and the same was maintained on 24th day.

Soluble protein content in the roots of mercury treated plants showed a slight reduction on 4th day. On 8th day it showed a sharp and significant increase ($p < 0.01$). It increased up to 16th day and after that it was slightly reduced.

In plants treated with triadimefon, the soluble protein content of roots showed an increase on 4th day and it increased in subsiquent days upto 16th day. Maximum total protein content in roots was observed on 16th day, which was about 3 times as that of initial content. After 16th day, it declined sharply and significantly ($p < 0.02$). The 24th day sample showed only half soluble protein content as that of 16th day sample.

In plants treated with a combination of mercury and triadimefon, the soluble protein content of roots remained almost same on 4th day as that of initial day. Then it was increased regularly upto 16th day and there after it decreased slowly.

4.3.2 Stem

The soluble protein content of stem of control plants was very low i.e., only 1%. (Table 14; Fig. 22B). It showed a slight increase on 4th and 8th days. Maximum soluble protein content in control stem was observed on 16th day. It remained almost same on 20th day of sampling and on 24th day it slightly decreased.

Plants treated with mercury exhibited more or less same values of soluble protein content in stem at the initial stage and on 4th day. It showed a significant increase on 8th day ($p < 0.01$). The samples on 12th, 16th and 20th days showed almost same soluble protein content. But the 24th day sample showed a reduction and was almost same soluble protein content as that of the 8th day.

In triadimefon treated plants, the soluble protein content of stem increased regularly upto 16th day of sampling. Thereafter it reduced slightly.

In the plants treated with a combination of mercury and triadimefon, the soluble protein content of stem increased significantly on 8th day. The samples on 12th, 16th and 20th days showed more or less same amount of soluble protein content in the stem tissues, and this decreased gradually on 24th day of investigation.

4.3.3 Leaf

Leaves of control plants showed about 3.7% of soluble protein content. It was almost double on 12th day (Table 14; Fig. 22C). Maximum soluble protein content in leaves was observed on 16th day. During subsequent days the soluble protein content decreased.

In the mercury treated plants, the leaves maintained almost same soluble protein content as that of initial day upto 8 days. It increased significantly ($p < 0.01$) on 12th day and the same was maintained upto 20 days. On 24th day, it showed a significant ($p < 0.02$) reduction.

Plants treated with triadimefon exhibited a significant increase ($p < 0.01$) in soluble protein content on 4th day. The same trend continued up to 16days. Maximum soluble protein content of leaves was observed on 16th day. It was more than three times as that of initial protein content. On 20th day, it decreased significantly, which continued upto 24th day of experiment.

In plants treated with a combination of mercury and triadimefon, the soluble protein content of leaves increased slightly upto 8 days. A significant increase occurred on 12th day. Maximum soluble protein content in leaves of these plants was observed on 16th day and it remained same on 20th day. There was a significant reduction ($p < 0.02$) on 24th day.

Among treated plants the maximum soluble protein content of root,

stem and leaves was shown by the 16th days samples of triadimefon treated plants.

4.4 Electrophoeretic Separation of Protein

One dimensional SDS polyacrylamide gel electrophoresis showed certain extra bands in the protein profile of the root, stem and leaf tissues of different treatments from that of the control.

The gel of the roots of control plants exhibited 5 bands ranging from 13.07kDa to 60.54kDa on 4th day of treatment (Fig. 23A). At this interval there was no much difference in other treatments, except the appearance of a band of 38.05kDa in mercury + triadimefon treatment.

The gel of the stem of control plants on 4th day of treatment showed 7 bands ranging from 31.14 kDa to 111.62 kDa (Fig. 24A). In mercury treated plants the bands around 73.32 kDa and 66.98 kDa was disappeared and in mercury + triadimefon and triadimefon treated plants, this band was very clear. The band around 49.37kDa and 43.67 kDa was less stained in mercury treated plants and deeply stained in mercury + triadimefon and triadimefon treated and control plants. The band around 35kDa was not observed in mercury treated plants.

On 24th day of treatment a thick band at 50.31kDa and 20.42 to 26.51 kDa region in the stem of mercury treated plants was observed (Fig. 24B). It

was less deeply stained in the same region in mercury + triadimefon and triadimefon treated plants.

The leaf of the control plant on 4th day of treatment showed 14 bands ranging from 14.33kDa to 116.26 kDa. There was no much difference in the treatments in the arrangement of bands (Fig. 25A). The bands around 117.02 kDa was not seen in mercury + triadimefon and triadimefon treatment. On 24th day of treatment the bands around 51.38kDa was more deeply stained and thick in mercury treated plants and it was less stained in other treatments and control (Fig. 25B). A band with 73.25kDa was very clear in mercury treatment.

4.5 Amino Acid

4.5.1 Root

Total amino acid content of control plants increased regularly throughout the experimental period. It was doubled on 12th day (Table 15; Fig. 26 A).

On 20th day of treatment total amino acid content was about three times as that of initial value. Samples of 20th day and 24th day showed same amino acid content.

Mercury treated plants showed doubled the value of the total amino acid content on 4th day than at the initial stage. During subsequent days of the

experiment, the amino acid content of roots increased regularly. Maximum amino acid content was observed on 24th day and was about five times as that of initial content.

Triadimefon treated plants and plants treated with a combination of mercury and triadimefon, showed the same trends in the increase of amino acid content in roots as that of control plants.

4.5.2 Stem

Initial amino acid content in stem of control plant was 20% (Table 15, Fig. 26 B). It increased throughout the experimental period. More than two fold increase was observed on 12th day and 16th day ($p < 0.01$). It increased during subsequent days of experiment.

In plants treated with mercury, the amino acid content of stem increased more than two times on 4th day and it remained almost same on 8th day. Then it showed a regular increase throughout the experimental period.

Plants treated with triadimefon showed almost same trend in amino acid content change in stem as that of control.

In plants treated with a combination of mercury and triadimefon, a two-fold increase in amino acid content was observed on 4th day. Then it increased throughout the period of experiment.

4.5.3 Leaf

The amino acid content of leaves of control plants was 3%. It increased slightly on 4th day. It remained almost same upto 8 days and it remained almost same upto 16th day and thereafter increased slightly (Table 15; Fig. 26 C).

In mercury treated plants, amino acid content of leaves increased on 4th day and remained same on 8th day. Thereafter it showed a regular increase. After 20th day, a sharp and significant increase ($p < 0.01$) in total amino acid content was observed. Maximum amino acid content in these plants was observed on 24th day.

A slight increase in amino acid content was observed on 4th day in the leaves of plants treated with triadimefon and it remained almost same upto 16th day. Thereafter it increased slightly upto 24th day.

In plants treated with a combination of mercury and triadimefon, the change in amino acid content was almost same as that of triadimefon treated plants upto 12th day. Thereafter it showed a slight increase upto 20th day and it remained same on 24th day.

Among the treated plants, the mercury treated plants showed maximum amino acid content in root, stem and leaves on 24th day of sampling.

4.6 Proline

4.6.1 Root

Proline content in roots increased throughout the experimental period (Table 16; Fig. 27 A). It increased regularly up to 24 days. Maximum proline content was observed in roots on 24th day.

In mercury treated plants, a sharp and significant increase ($p < 0.01$) in proline content in root was noticed on 4th day. Then a regular increase in proline content was observed upto 24 days.

The initial proline content remained almost same upto 12 days in the plants treated with triadimefon. Thereafter it increased regularly upto 24 days.

A sharp increase in proline content was noticed in roots of plants treated with a combination of mercury and triadimefon on 4th day. It increased regularly upto 24 days.

4.6.2 Stem

Proline content of control stem also increased regularly during the entire experimental period (Table 16; Fig. 27 B). Maximum proline content was observed on 24th day and is about three times as that of the initial value.

In plants treated with mercury, only a slight increase was noticed on 4th days. After 12th day a significant ($p < 0.02$) increase was observed on 20th day. On 24th day also the proline content increased significantly ($p < 0.01$). On 24th

day the proline content of stem was more than 5 times than that of initial value.

Proline content in stem of plants treated with triadimefon remained same as that on 4th day and thereafter increased regularly upto 24 days.

In the plants, treated with a combination of mercury and triadimefon, the proline content of stem increased regularly upto 16 days. A significant increase ($p < 0.01$) was observed on 20th day and 24th days. On 24th day the proline content was about more than 4 times as that of initial content.

4.6.3 Leaf

The proline content of leaves of control plants remained almost same as that at the initial stage upto 8 days (Table 16; Fig. 27 C). Thereafter it showed a regular increase upto 24 days.

In mercury treated plants, a sharp increase in proline content was noticed on 4th day. It remained almost same on 8th day. Then it increased regularly upto 20 days. On 24th day a sharp and significant increase in proline content was observed.

The initial proline content of leaves of triadimefon treated plants remained as such upto 8 days. Then it increases slightly upto 24 days. On 24th day these plants registered only two-fold increase than initial content.

In plants treated with a combination of mercury and triadimefon, the

proline content of leaves increased on 24th day, but remain without any significant change upto 8th day. Then it showed a regular increase upto 24 days. On 24th day the proline content was more than 3 times as that of initial content.

Among control and treated plants, the root, stem and leaves of mercury treated plants showed the maximum proline content.

4.7 Phenolics

4.7.1 Root

The phenolics content of control plants remained almost same upto 4 days (Table 17; Fig. 28 A). On 8th day the phenolic content increased significantly ($p < 0.01$). Thereafter it increased regularly upto 24th day. On 24th day the phenolics content was increased more than 3 times as that of initial content.

In mercury treated plants also, a significant increase was observed on 8th day and thereafter regularly increased upto 24 days. A more than threefold increase in phenolic content was observed on 24th day.

In plants treated with triadimefon, initial phenolic content of roots remained unchanged on 4th day and thereafter increased regularly. Only more than two fold increase was observed on 24th day.

Phenolic content in the roots of plants treated with a combination of

mercury and triadimefon showed an increase on 4th day. But on 8th day a significant increase ($p < 0.02$) was noticed. Then it increased regularly. On 24th day more than threefold increase was noticed in the phenolic content.

4.7.2 Stem

The phenolic content in stem of control plants remained almost same upto 4 days (Table 17; Fig. 28 B). Then a gradual increase was observed.

In the case of plants treated with mercury, an increase in phenolic content was observed on 4th day, which was followed by a slight increase throughout the experimental period.

In plants treated with triadimefon, the phenolic content change of stem was same as that of the control plants. But the plants treated with a combination of mercury and triadimefon, the phenolic content showed the same pattern of change as that of mercury treated plants.

4.7.3 Leaf

The phenolic content of leaves of control plants remained same up to 4th day. There after it increased significantly ($p < 0.01$) on 8th day, which remained the same upto 16th day (Table 17; Fig. 28 C). Then the phenolic content increased slightly on 20th and 24th days. In mercury treated plants, a significant increase in phenolics was observed on 4th day. On 8th day, the phenolic content was more than double as that of initial content. It was

increased to three times on 12th day. It remained almost same on 16th day and then increased. A significant increase ($p < 0.01$) in pehnolics content was observed on 24th day and it was about five times as that of initial value.

In plants treated with triadimefon there was no much increase in phenolic content of leaf upto 8th day. Thereafter it increased slightly. On 16th day the phenolic content was doubled. It was increased to about 3 times on 24th day.

Leaves of plants treated with a combination of mercury + triadimefon registered a significant increase on 4th day of experiment. On 8th day the phenolic content was increased to two fold. It was increased to more than three times on 16th day. More than four times increase in phenolic content was observed on 24th day. The phenolic content in leaves of plants treated with a combination of mercury and triadimefon showed the same pattern as that of control plants on 24th day.

4.8 Peroxidase Activity – Unit Activity

4.8.1 Root

Roots of control plants showed 7.38 unit of peroxidase activity. On 4th day it maintained the same activity (Table 18; Fig. 29A). More or less same unit activity was observed on 8th, 12th and 16th days. Then the peroxidase activity showed a slight increase up to 24th day.

In mercury treated plants, peroxidase activity showed a significant ($p < 0.01$) increase on 4th day. It was about twice as that of the initial activity. Then the activity increased slightly throughout the experimental period. On 24th day, the activity was more than two times as that of initial activity.

Plants treated with triadimefon, showed a significant decrease ($p < 0.02$) in peroxidase activity on 4th day. The activity on 8th day was almost same as the initial activity. Thereafter, the activity showed slight increase throughout the period.

The peroxidase activity in roots of plants treated with a combination of mercury + triadimefon showed a significant increase ($p < 0.01$) on 4th day. Then it showed a slight increase in peroxidase activity.

Among treated plants, peroxidase activity in root was maximum in mercury treated plants and minimum in triadimefon treated plants.

4.8.2 Stem

Stem of control plants showed 2 units of peroxidase activity. It showed a significant increase on 8th and 12th days (Table 18; Fig. 29B). Then the activity was slightly increased throughout the experimental period. More than two fold increase in peroxidase activity was observed on 24th day as that on 4th day.

A significant increase ($p < 0.01$) in unit activity of peroxidase was

observed on 4th day in the stem of mercury treated plants. Then the activity showed slight increase upto 24 days. On 24th day these plants showed about three times increase as that of initial activity.

Stems of plants treated with triadimefon showed a slight increase on 4th day. The plants on 24th day showed only two times increase as that on 4th day.

In plants treated with a combination of mercury + triadimefon a significant increase in peroxidase activity was observed on 4th day. Thereafter it showed an increase throughout the experimental period. On 24th day more than 2.5 times increase in the unit activity was observed as that of initial value.

4.8.3 Leaf

At the initial stage the unit activity of peroxidase in control leaf was 1.40 and then showed a slight increase throughout the experimental period (Table 18; Fig. 29C). On 24th day a two fold increase was observed.

In mercury treated plants, the activity showed a significant ($p < 0.02$) increase on 4th day. Another significant hike in the activity was observed on 12th day and it was more than three times as that of initial activity. Thereafter the unit activity showed a regular increase. On 24th day the activity was about more than four times as that of initial activity.

In plants treated with triadimefon, there was a slight decrease in

peroxidase activity on 4th day. But it showed a significant increase ($p < 0.01$) on 8th day. Thereafter the activity showed a slight increase and on 24th day the activity was about two times as that of initial activity.

Unit activity of peroxidase enzyme in the leaves of plants treated with a combination of mercury and triadimefon registered about two fold increase on 4th day. It remained almost same on 8th day. Another significant increase in the activity was observed on 12th day and 24th day. On 24th day more than three times increase in the activity was observed as that of initial activity.

4.9 Specific Activity

4.9.1 Root

The specific activity of peroxidase in control roots was 2.88 (Table19; Fig. 30 A). The activity was increased significantly on 4th day. But it was significantly lowered on 8th day and it remained almost same on 12th and 16th days. On 20th day, there was a significant increase in specific activity. It showed a slight increase on 24th day.

The roots of mercury treated plants registered a rapid and significant increase in the specific activity of peroxidase enzyme on 4th day. On 8th day the specific activity was decreased significantly and it remained the same on 12th day. Thereafter it increased. Specific activity was increased significantly on 16th, 20th and 24th day. On 24th day about 4 times increase in specific

activity was observed.

In triadimefon treated plants, the specific activity was decreased significantly on 4th day. The decrease was continued upto 16 days. On 20th day there was a sharp increase in the specific activity, and it was continued up to 24th day. On 24th day, there was only two fold increase in specific activity.

Plants treated with a combination of mercury + triadimefon exhibited more than two fold increase in specific activity on 4th day. It was reduced to half on 8th day and it remained almost same on 12th day of experiment. Thereafter it showed a slight increase initially but significant increase was observed on 20th and 24th days. On 24th day the specific activity was about 3 times as that of initial activity.

4.9.2 Stem

The specific activity of peroxidase in the stem of control plants almost remained unchanged upto 16th day with slight initial fluctuations (Table 19; Fig. 30B). There after it increased up to 24 days.

A sudden and sharp increase in specific activity was observed in the stems of mercury treated plants on 4th day. Then it decreased to half on 8th day and maintained the same activity upto 20 days. A significant increase in activity was observed on 24th day.

In plants treated with triadimefon the initial specific activity remained

almost same upto 20 days with slight fluctuations. A significant increase in the activity was observed on 24th day.

The specific activity of peroxidase in the stem of plants treated with a combination of mercury and triadimefon showed a sharp increase on 4th day. The activity was decreased on 8th day and maintained the same activity upto 20 days. A significant increase in the activity was observed on 24th day.

The maximum specific activity in stem was shown by mercury treated plants and the least was shown by control and triadimefon treated plants.

4.9.3 Leaf

Initially the specific activity of peroxidase in control leaf was 0.23 (Table 19; Fig. 30C). It was increased only on 8th day and maintained the same up to 20th day with slight fluctuations. The specific activity was increased more than 2 times on 24th day than at the initial stage.

In mercury treated plants the specific activity of peroxidase in leaves increased two times on 4th day. A sharp increase in the activity was observed on 8th day and was maintained unchanged upto 16th day. Thereafter the specific activity was increased sharply. On 24th day, the specific activity was about six times as that of the initial activity.

No change in specific activity of peroxidase enzyme was observed in triadimefon treated plants upto 4 days and remained almost same activity upto

16th day with slight fluctuations. On 20th day and 24th day there occurred a sharp increase in specific activity of enzyme. On 24th day these plants registered a threefold increase in enzyme activity.

In plants treated with a combination of mercury and triadimefon the activity was doubled on 4th day. There after showed a gradual increase in the specific activity. On 24th day the specific activity was about 5 times as that of initial activity.

The specific activity was maximum in roots of mercury treated plants and a combination of mercury and triadimefon treated plants. They showed the same pattern of activity.

5. BIOACCUMULATION STUDIES

5.1 Bioaccumulation of Mercury

The accumulation of mercury was maximum in roots of mercury treated plants followed by stem and leaves (Table 20; Fig. 31A). The accumulation of mercury in root and stem of treated plants was increasing gradually but insignificantly during the growth up to 24 days. The bioaccumulation in the leaves showed a very gradual increase up to 12th day and reduced thereafter.

The accumulation of mercury in the roots of plants treated with a combination of mercury and triadimefon remained almost unchanged during the growth (Table 20; Fig. 31B). In the stem, accumulation of mercury

showed only insignificant changes throughout the growth. In the leaf tissue mercury content was gradually but insignificantly decreased.

When a comparison is made between the $HgCl_2$ treatment and a combination of $HgCl_2$ + triadimefon, mercury accumulation of root and shoot tissue showed lesser quantities in tissues of plants treated with mercuric chloride + triadimefon during all intervals. But mercury content of leaf tissue was significantly reduced in all the intervals of mercuric chloride + triadimefon treatment.

In the mercury treatment, where the additional doses (0.6mg of mercury at each intervals) were given a gradual increase in the accumulation was observed in the root, stem and leaf tissues during the period of growth. This increase was proportional to the quantity of additional doses. Maximum accumulation of mercury was observed in the root tissue followed by the stem and leaf tissues (Table 21; Fig. 32A).

When plants treated with a combination of mercury and triadimefon, the accumulation of mercury in the root, stem and leaf tissue was significantly reduced compared to those tissues in the mercury treatment (Table 21; Fig. 32B). In this treatment also the accumulation of mercury was maximum in the roots and was followed by stem and leaves.

For bioaccumulation studies of mercury, the plants were grown in Hoagland solution containing a known quantity of mercury. Hence a

comparison between accumulation (content/ tissue cultivated) and quantity of metal retained (residual) in the medium during the growth was made to ascertain the pattern of distribution of mercury (Table 22 & 23).

When single application of mercury was made the accumulation per plant remained unchanged during the growth (Table 22; Fig. 33A). The mercury content of residual solution was decreased gradually during growth. However, small quantities of mercury is found to be lost and the mercury content lost during growth was maximum in the samples of 20th and 24th days samples.

In plants treated with a combination of mercury and triadimefon, mercury accumulation was less than the plants treated with mercury alone (Table 22; Fig. 33B). The residual solution showed more quantity of mercury than that of mercury treatment alone though the quantity of which did not change during growth upto 24th day. Hence the loss of mercury was not significantly increased in combination treatment compared to the treatment of mercury alone.

In the mercury treatment where additional doses were given, accumulation of mercury remained almost unchanged at all intervals as in single dose (Table 23; Fig. 34A). Here the residue contains mercury at a range of 36-28%.

In plants treated with a combination of mercury and triadimefon the residue contained more mercury content than that of the treatment of mercury

alone (Fig. 34B).

5.2 Relation between Bio Concentration Factor (BCF) and Translocation Factor (TF)

A comparison of BCF and TF of mercury accumulation in *Centella* plants showed very low value of BCF, i. e, less than one (Table 24; Fig. 35). Nevertheless, TF values were more than one, which was found slightly increased up to 12th day and there after decreased. Treatment of a combination of mercury and triadimefon also exhibited more or less similar values of BCF and TF as that of the treatment with mercury alone. But there occurred slight reduction of both BCF and TF values invariably in whole samples of plants treated with mercury and triadimefon.

Plants treated with additional doses of mercury showed a trend of reduction in the values of both BCF and TF in mercury + triadimefon treated plants compared to the treatment with mercury alone (Table 25; Fig. 36). During growth the values of BCF were remained unchanged but the TF values found initially decreased and then increased.

Table 2: Effect of mercury and triadimefon on the root length (cms) in *Centella asiatica*

Treatment	Days of Sampling						
	0	**4**	**8**	**12**	**16**	**20**	**24**
C	11.10 ± 1.1	12.26 ± 1.2	12.44 ± 1.0	12.74 ± 1.2	13.19 ± 1.3	13.50 ± 1.0	13.60 ± 1.2
Hg	11.10 ± 1.1	11.30 ± 1.1	11.32 ± 1.1	11.41 ± 1.3	11.53 ± 1.3	11.63 ± 1.1	11.74 ± 1.4
Tri	11.10 ± 1.1	12.20 ± 1.2	12.50 ± 1.5	12.60 ± 1.3	12.88 ± 1.7	13.30 ± 1.5	13.46 ± 1.5
Hg + Tri	11.10 ± 1.1	11.80 ± 1.2	11.88 ± 1.1	11.93 ± 1.2	11.96 ± 1.0	12.04 ± 1.2	12.17 ± 1.1

Fig. 1: Effect of mercury and triadimefon on the root length (cms) in *Centella asiatica*

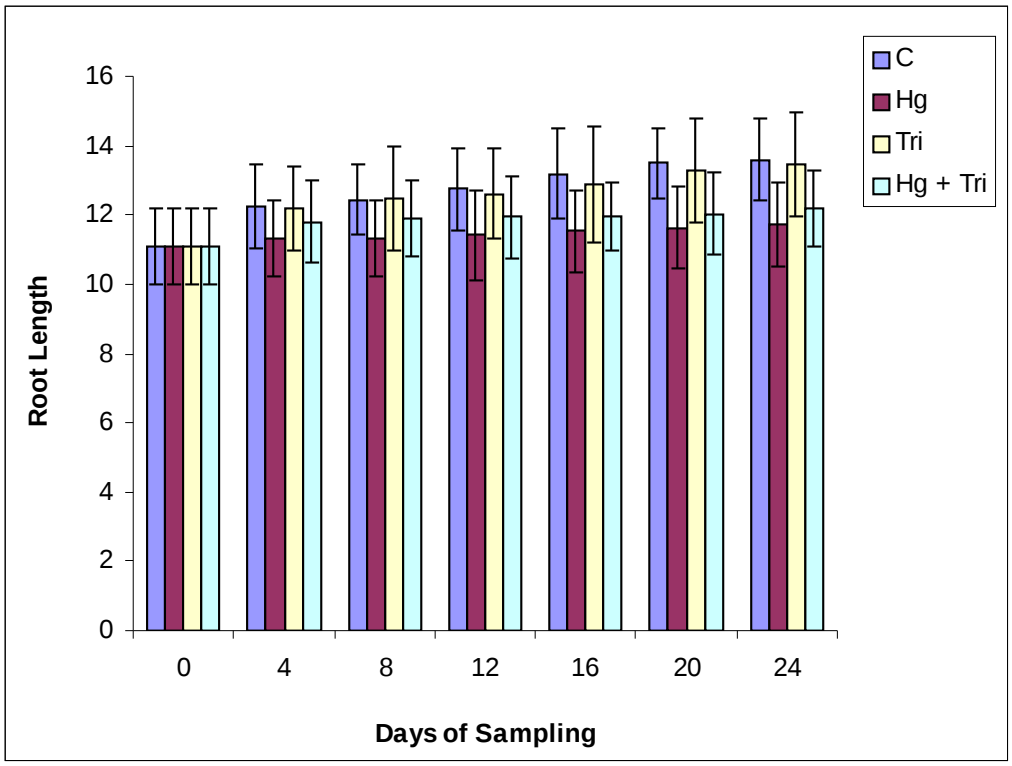


Table 3: Effect of mercury and triadimefon on the runner length (cms) in *Centella asiatica*

Treatment	Days of Sampling						
	0	**4**	**8**	**12**	**16**	**20**	**24**
C	20.28 ± 2.6	25.86 ± 2.8	33.32 ± 3.1	37.80 ± 3.0	42.32 ± 3.5	46.92 ± 4.3	53.70 ± 4.9
Hg	20.28 ± 2.6	22.78 ± 2.0	24.56 ± 2.2	27.66 ± 2.7	29.50 ± 2.5	31.80 ± 3.0	32.80 ± 2.9
Tri	20.28 ± 2.6	25.65 ± 2.1	30.75 ± 2.8	35.40 ± 3.0	39.88 ± 3.3	44.80 ± 3.9	48.95 ± 4.2
Hg + Tri	20.28 ± 2.6	23.38 ± 2.5	26.62 ± 2.3	29.84 ± 3.0	33.04 ± 3.1	37.16 ± 3.2	41.21 ± 3.6

Fig. 2: Effect of mercury and triadimefon on the runner length (cms) in *Centella asiatica*

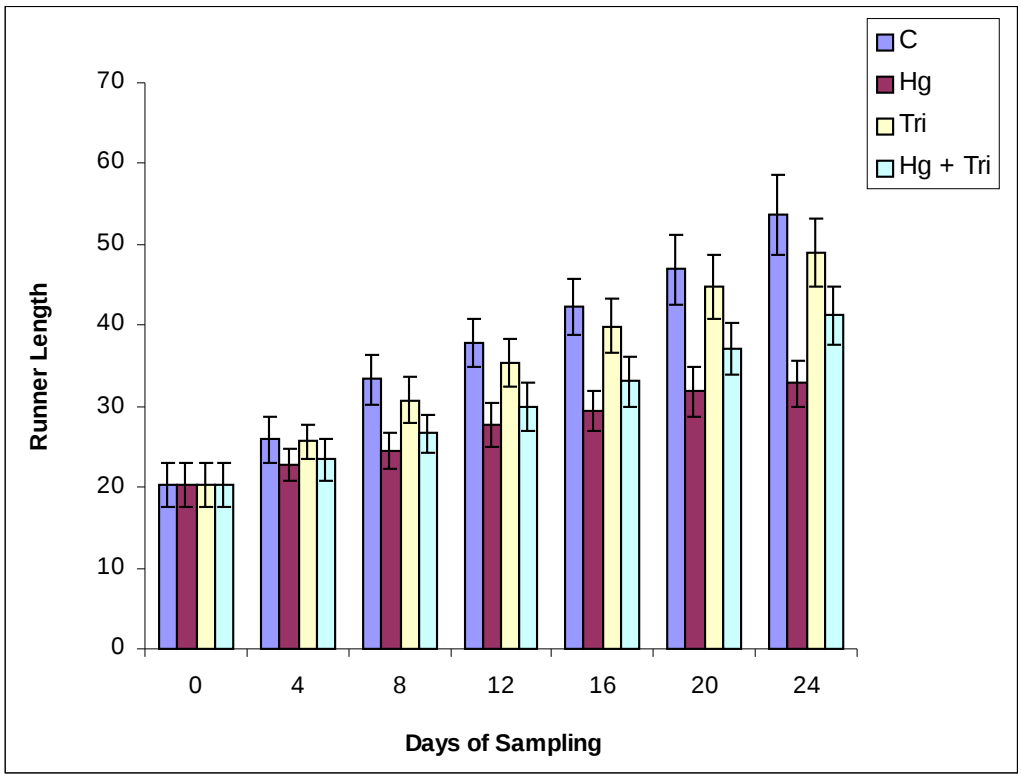


Table 4: Effect of mercury and triadimefon on the petiolar length (cms) in *Centella asiatica*

Treatment	Days of Sampling						
	0	**4**	**8**	**12**	**16**	**20**	**24**
C	5.32 ± 0.56	7.60 ± 0.58	7.95 ± 0.73	8.25 ± 0.74	8.83 ± 0.83	9.15 ± 0.96	9.88 ± 0.82
Hg	5.32 ± 0.56	5.56 ± 0.51	5.72 ± 0.51	5.78 ± 0.55	5.78 ± 0.57	5.78 ± 0.54	5.78 ± 0.52
Tri	5.32 ± 0.56	6.60 ± 0.67	7.15 ± 0.77	8.00 ± 0.81	8.80 ± 0.82	9.30 ± 0.88	9.65 ± 0.78
Hg + Tri	5.32 ± 0.56	6.10 ± 0.64	6.30 ± 0.64	6.36 ± 0.43	6.77 ± 0.52	7.16 ± 0.73	7.53 ± 0.73

Fig. 3: Effect of mercury and triadimefon on the petiolar length (cms) in *Centella asiatica*

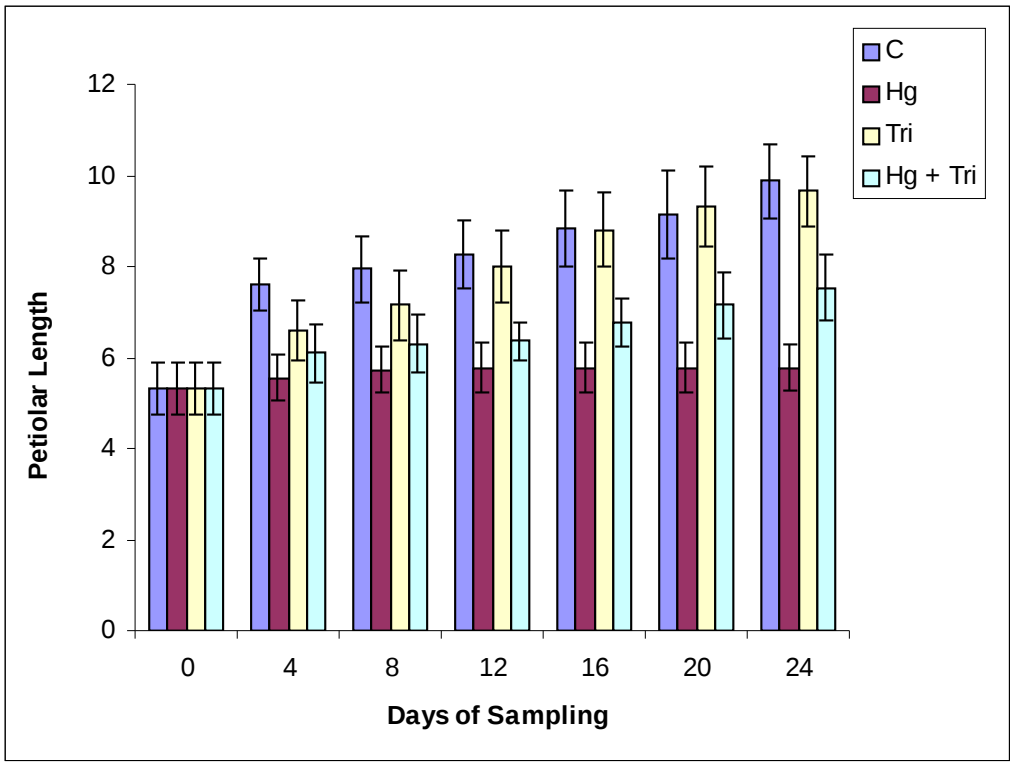


Table 5: Effect of mercury and triadimefon on the leaf area (mm^2) in *Centella asiatica*

Treatment	Days of Sampling						
	0	**4**	**8**	**12**	**16**	**20**	**24**
C	124.2 ± 11.7	290.6 ± 28.6	439.2 ± 39.3	555.6 ± 47.4	621.0 ± 60.1	666.8 ± 67.7	727.2 ± 64.8
Hg	124.2 ± 11.7	180.0 ± 14.7	217.5 ± 23.5	242.74 ± 23.6	260.50 ± 24.0	281.35 ± 25.8	306.50 ± 23.9
Tri	124.2 ± 11.7	260.00 ± 27.5	356.89 ± 27.9	434.54 ± 38.3	512.00 ± 49.1	584.74 ± 44.9	649.20 ± 57.8
Hg + Tri	124.2 ± 11.7	188.40 ± 23.9	242.74 ± 24.5	284.00 ± 27.1	339.10 ± 36.6	376.11 ± 35.9	403.70 ± 33.8

Fig. 4: Effect of mercury and triadimefon on the leaf area (mm^2) in *Centella asiatica*

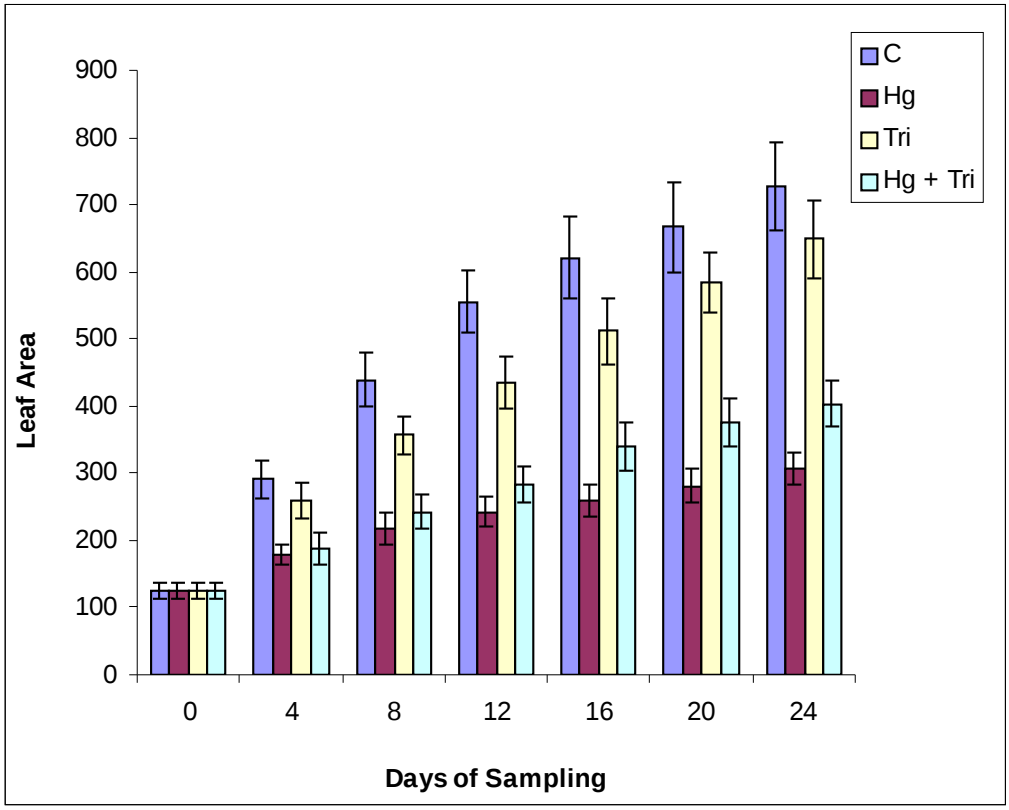


Table 6: Effect of mercury and triadimefon on the tolerance index percentage with respect to root length, runner length and leaf area in *Centella asiatica*

Plant Part	Treatment	Days of Sampling						
		0	**4**	**8**	**12**	**16**	**20**	**24**
Root Length	C	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
	Hg	100 ± 0.0	92.16 ± 0.97	91.00 ± 0..83	88.85 ± 0.76	85.82 ± 0.61	83.85 ± 0.69	83.24 ± 0.85
	Tri	100 ± 0.0	99.51 ± 0.30	99.37 ± 063	98.90 ± 0.77	97.65 ± 0.77	98.52 ± 0.69	98.97 ± 0.69
	Hg + Tri	100 ± 0.0	96.25 ± 0.76	95.50 ± 0.79	93.64 ± 0.52	90.68 ± 0.59	89.19 ± 0.75	89.49 ± 0.81
Runner Length	C	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
	Hg	100 ± 0.0	88.09 ± 0.88	73.71 ± 0.76	73.18 ± 0.65	69.71 ± 0.79	67.78 ± 0.53	61.08 ± 0.69
	Tri	100 ± 0.0	99.19 ± 0.78	92.29 ± 0.63	93.65 ± 0.51	94.23 ± 0.50	95.48 ± 0.60	91.16 ± 0.52
	Hg + Tri	100 ± 0.0	90.41 ± 0.73	79.89 ± 0.77	78.94 ± 0.89	78.07 ± 0.69	79.20 ± 0.50	76.74 ± 0.88
Leaf Area	C	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
	Hg	100 ± 0.0	61.94 ± 0.47	49.52 ± 0.37	43.69 ± 0.36	41.95 ± 0.40	42.19 ± 0.40	42.15 ± 0.34
	Tri	100 ± 0.0	89.47 ± 0.75	81.26 ± 0.89	78.21 ± 0.79	82.45 ± 0.81	87.69 ± 0.88	89.27 ± 0.61
	Hg + Tri	100 ± 0.0	64.83 ± 0.53	55.27 ± 0.44	51.12 ± 0.68	54.61 ± 0.55	56.41 ± 0.66	55.51 ± 0.42

Fig. 5A: Effect of mercury and triadimefon on the tolerance index percentage with respect to root length in *Centella asiatica*

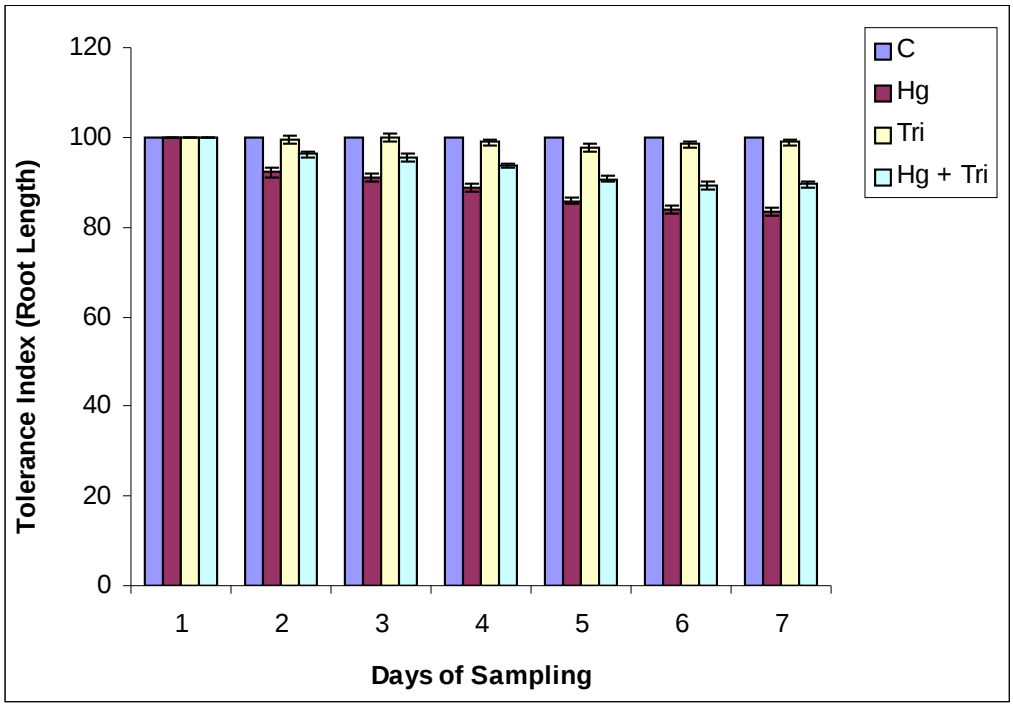


Fig. 5B: Effect of mercury and triadimefon on the tolerance index percentage with respect to runner length in *Centella asiatica*

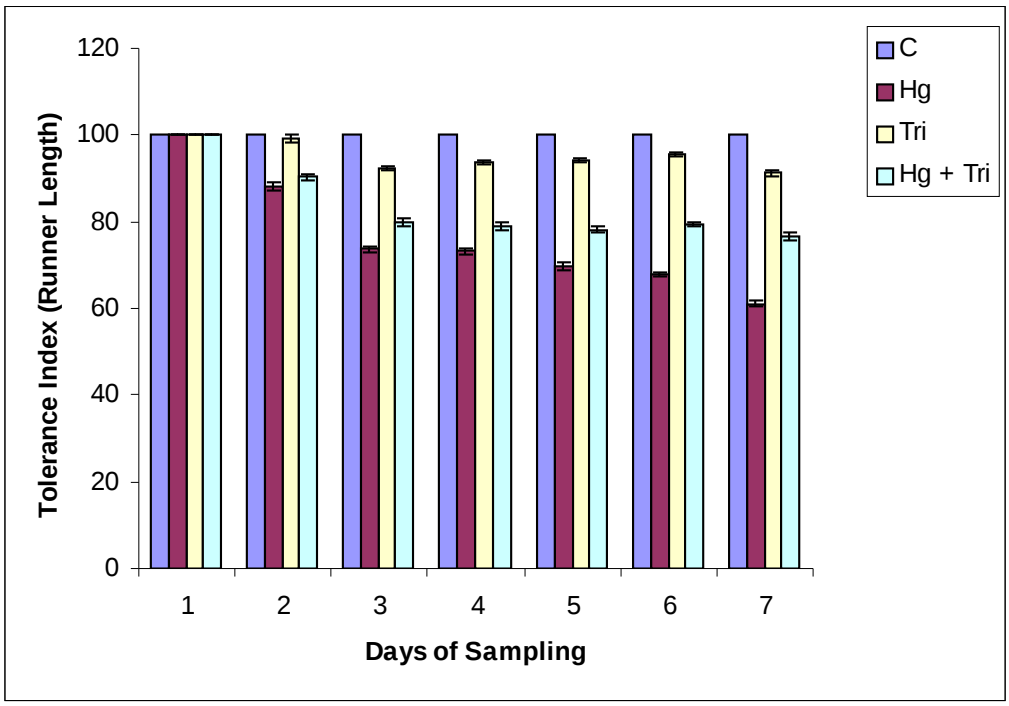


Fig. 5C: Effect of mercury and triadimefon on the tolerance index percentage with respect to leaf area in *Centella asiatica*

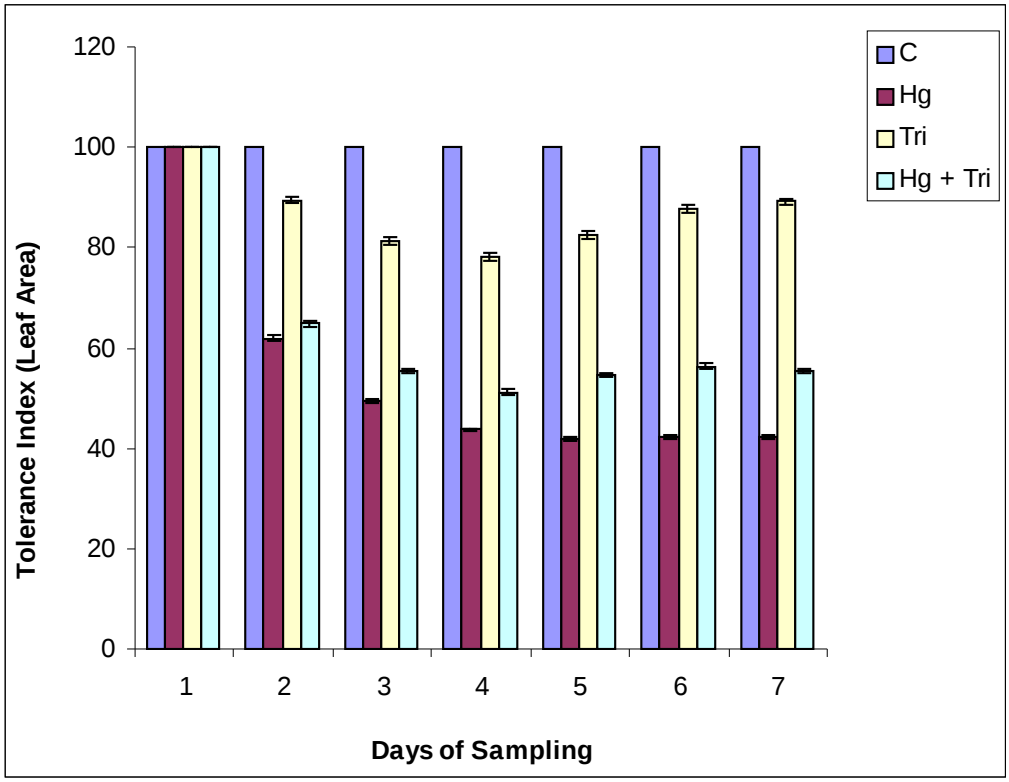


Table 7: Effect of mercury and triadimefon on the stomatal index in *Centella asiatica*

Treatment		Days of Sampling						
		0	**4**	**8**	**12**	**16**	**20**	**24**
C	Lower	10.25 ± 1.25	12.00 ± 2.50	13.25 ± 1.25	14.00 ± 1.75	16.25 ± 1.50	19 .00 ± 1.70	20.25 ± 1.25
	Upper	9.25 ± 1.30	10.50 ± 0.70	11.25 ± 1.40	12.75 ± 1.33	14.00 ± 0.80	14.75 ± 2.50	14.80 ± 1.20
Hg	Lower	10.25 ± 1.25	19.50 ± 1.25	22.25 ± 0.75	24.75 ± 2.00	25.50 ± 2.50	26.75 ± 1.50	27.60 ± 2.25
	Upper	9.25 ± 1.30	11.00 ± 0.75	13.75 ± 1.5	14.75 ± 1.75	16.75 ± 1.25	16.75 ± 1.00	17.15 ± 1.75
Tri	Lower	10.25 ± 1.25	11.00 ± 1.00	12.75 ± 1.25	13.75 ± 1.50	14.75 ± 2.0	17.00 ± 1.70	18.50 ± 1.20
	Upper	9.25 ± 1.30	9.25 ± 1.20	8.00 ± 1.90	11.75 ± 1.60	11.75 ± 1.38	13.33 ± 1.60	14.50 ± 1.25
Hg + Tri	Lower	10.25 ± 1.25	14.00 ± 1.75	16.25 ± 1.35	17.00 ± 1.65	19.25 ± 1.00	19.75 ± 1.50	23.00 ± 1.40
	Upper	9.25 ± 1.30	11 .00 ± 1.25	13.25 ± 1.50	13.75 ± 2.00	14.00 ± 2.00	14.66 ± 1.75	16.35 ± 1.50

Fig. 6A: Effect of mercury and triadimefon on the stomatal index (lower side) in *Centella asiatica*

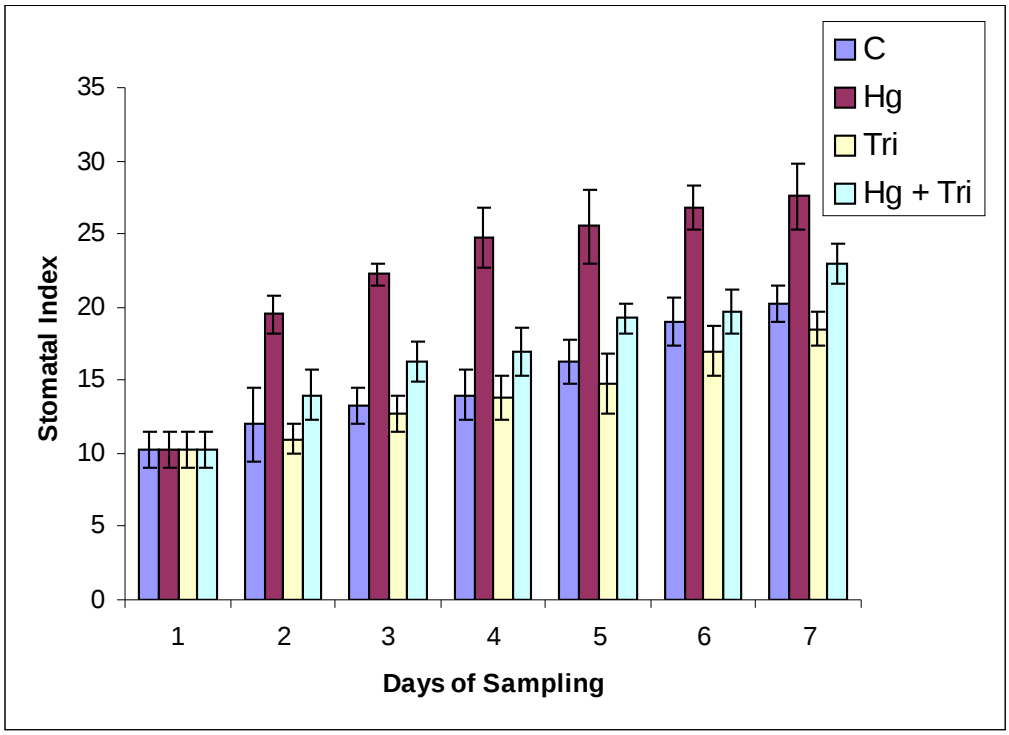


Fig. 6B: Effect of mercury and triadimefon on the stomatal index (upper side) in *Centella asiatica*

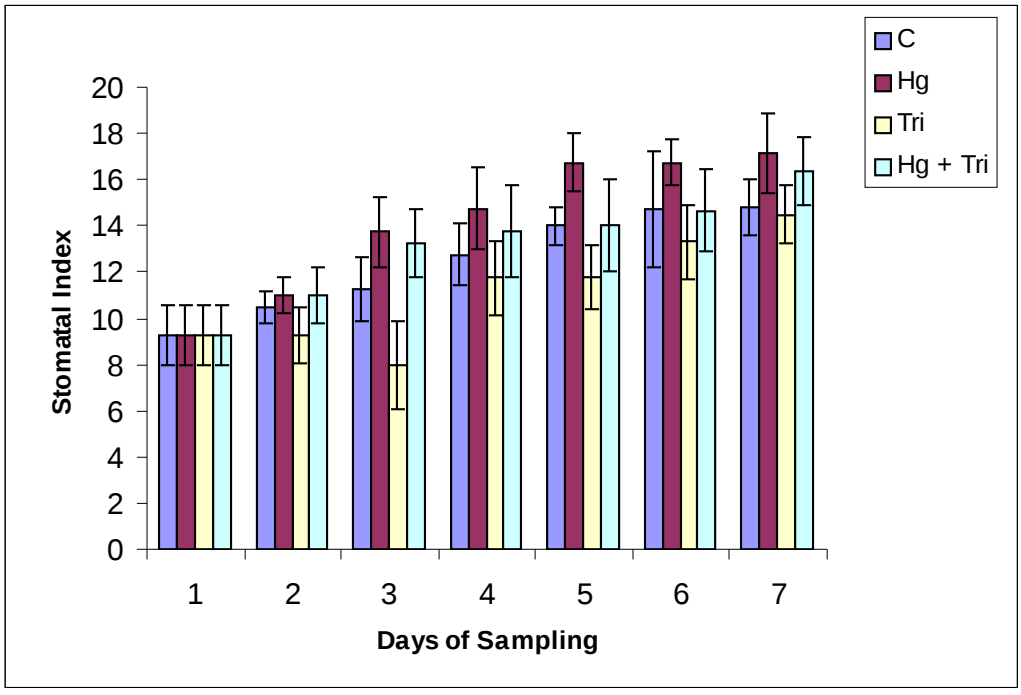


Table 8: Effect of mercury and triadimefon on the biomass of root, stem and leaf tissues in *Centella asiatica*

Plant Part	Treatment	Days of Sampling						
		0	**4**	**8**	**12**	**16**	**20**	**24**
Root	C	0.53 ± 0.04	0.63 ± 0.04	0.78 ± 0.05	0.85 ± 0.03	0.96 ± 0.03	1.20 ± 0.05	1.31 ± 0.04
	Hg	0.53 ± 0.04	0.58 ± 0.06	0.61 ± 0.07	0.63 ± 0.04	0.69 ± 0.03	0.73 ± 0.04	0.75 ± 0.03
	Tri	0.53 ± 0.04	0.59 ± 0.05	0.75 ± 0.04	0.98 ± 0.06	1.23 ± 0.07	1.49 ± 0.04	1.68 ± 0.07
	Hg + Tri	0.53 ± 0.04	0.57 ± 0.05	0.65 ± 0.07	0.69 ± 0.05	0.72 ± 0.04	0.78 ± 0.06	0.82 ± 0.03
Stem	C	0.87 ± 0.04	1.03 ± 0.05	1.27 ± 0.07	1.48 ± 0.08	1.67 ± 0.04	1.89 ± 0.04	2.13 ± 0.05
	Hg	0.87 ± 0.04	1.15 ± 0.04	1.18 ± 0.05	1.26 ± 0.04	1.34 ± 0.05	1.49 ± | 0.06	1.57 ± 0.03
	Tri	0.87 ± 0.04	1.10 ± 0.05	1.31 ± 0.05	1.45 ± 0.06	1.66 ± 0.05	1.84 ± 0.04	2.10 ± 0.03
	Hg + Tri	0.87 ± 0.04	1.11 ± 0.06	1.17 ± 0.07	1.26 ± 0.04	1.37 ± 0.06	1.47 ± 0.05	1.54 ± 0.04
Leaf	C	1.55 ± 0.06	1.67 ± 0.04	1.99 ± 0.06	2.41 ± 0.03	2.92 ± 0.04	3.45 ± 0.05	3.67 ± 0.03
	Hg	1.55 ± 0.06	1.62 ± 0.04	1.69 ± 0.05	1.77 ± 0.04	1.99 ± 0.06	2.17 ± 0.05	2.21 ± 0.04
	Tri	1.55 ± 0.06	1.60 ± 0.03	1.87 ± 0.06	2.35 ± 0.03	2.70 ± 0.04	3.33 ± 0.06	3.61 ± 0.05
	Hg + Tri	1.55 ± 0.06	1.64 ± 0.04	1.71 ± 0.04	1.85 ± 0.05	2.09 ± 0.05	2.20 ± 0.04	2.32 ± 0.05

Fig. 16A: Effect of mercury and triadimefon on the biomass of root tissues in *Centella asiatica*

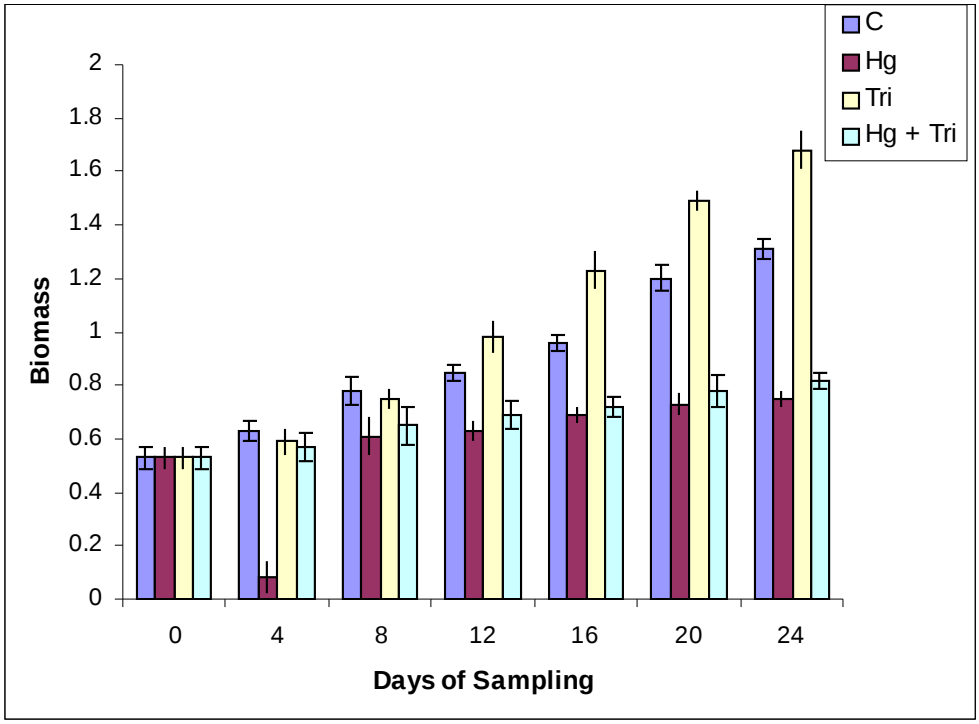


Fig. 16 B: Effect of mercury and triadimefon on the biomass of stem tissues in *Centella asiatica*

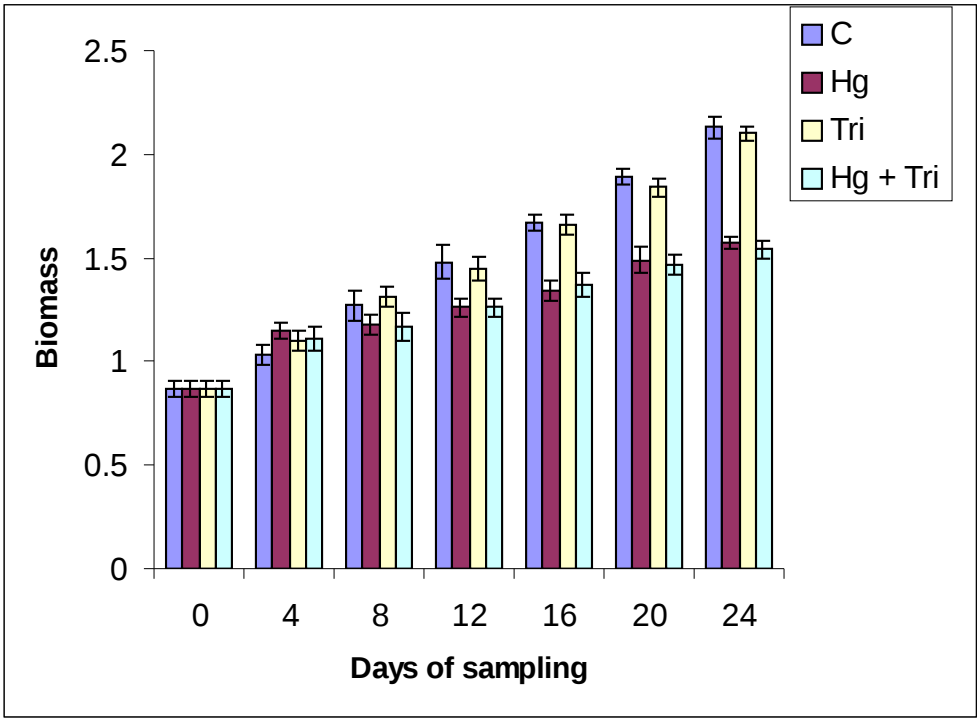


Fig. 16C: Effect of mercury and triadimefon on the biomass of leaf tissues in *Centella asiatica*

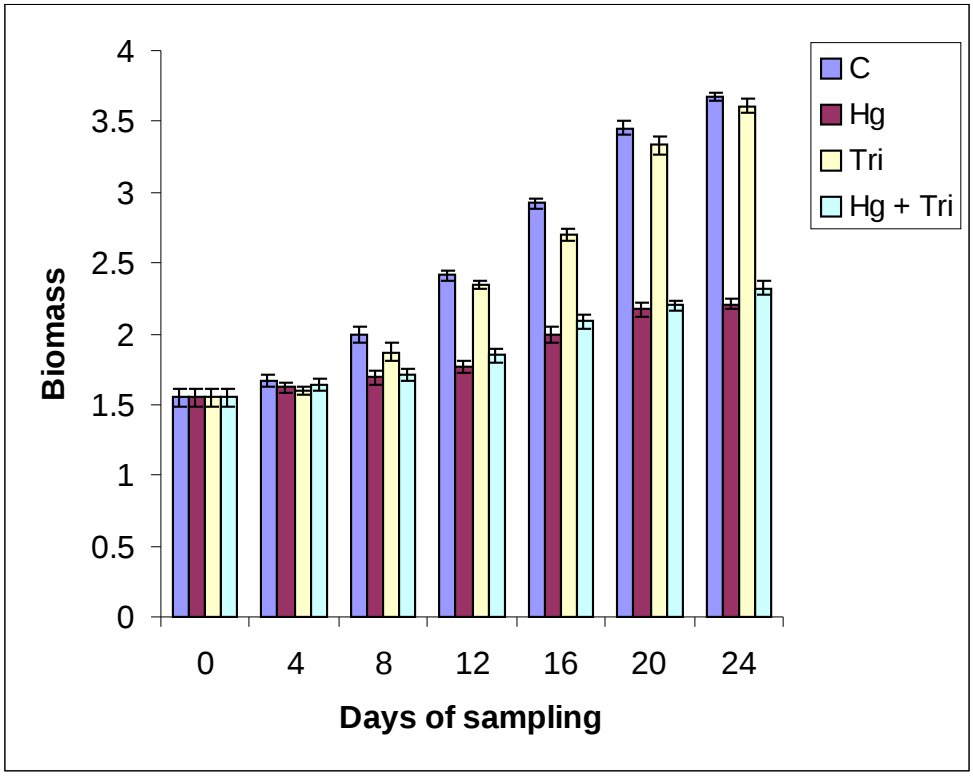


Table 9: Effect of mercury and triadimefon on the total biomass content in *Centella asiatica*

Treatment	Days of Sampling						
	0	**4**	**8**	**12**	**16**	**20**	**24**
C	2.95 ± 0.11	3.33 ± 0.09	4.04 ± 0.13	4.74 ± 0.07	5.55 ± 0.17	6.54 ± 0.13	7.11 ± 0.07
Hg	2.95 ± 0.11	3.35 ± 0.14	3.48 ± 0.07	3.66 ± 0.10	4.02 ± 0.14	4.39 ± 0.09	4.53 ± 0.09
Tri	2.95 ± 0.11	3.29 ± 0.11	3.93 ± 0.17	4.78 ± 0.15	5.59 ± 0.09	6.66 ± 0.13	7.39 ± 0.11
Hg + Tri	2.95 ± 0.11	3.32 ± 0.14	3.53 ± 0.13	3.80 ± 0.10	4.18 ± 0.11	4.45 ± 0.13	4.68 ± 0.12

Fig. 17: Effect of mercury and triadimefon on the total biomass content in *Centella asiatica*

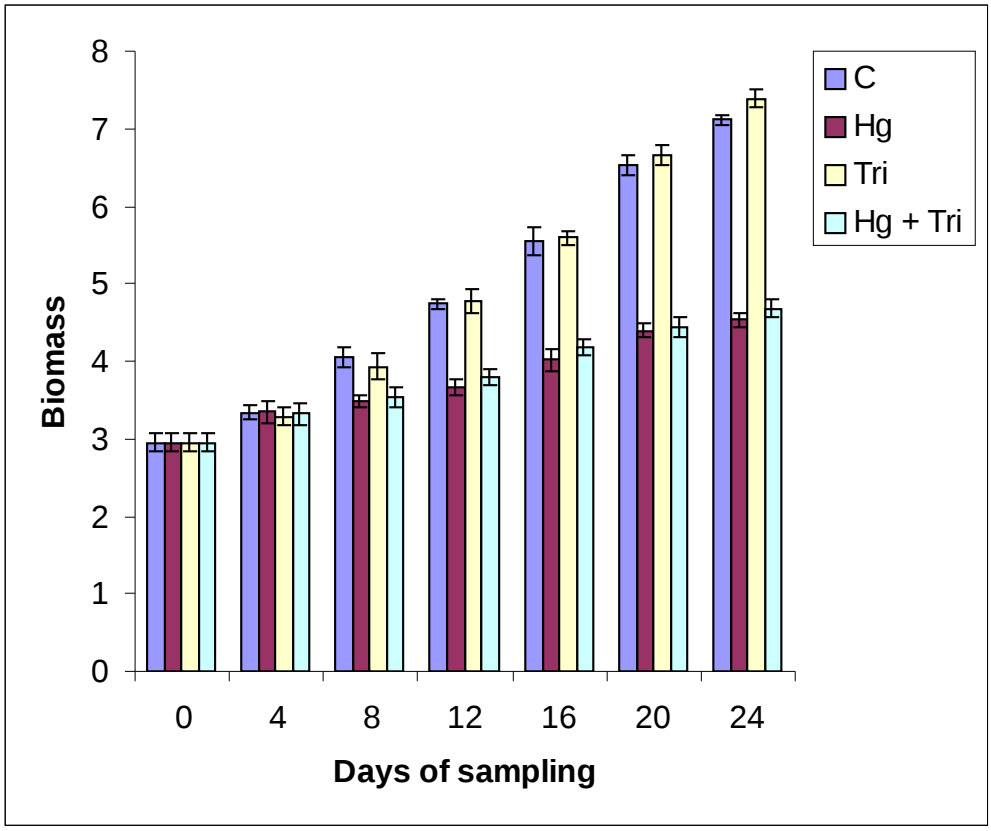


Table 10: Effect of mercury and triadimefon on the root / shoot ratio in *Centella asiatica*

Treatment	Days of Sampling						
	0	**4**	**8**	**12**	**16**	**20**	**24**
C	0.22	0.23	0.24	0.22	0.21	0.23	0.23
Hg	0.22	0.21	0.21	0.21	0.21	0.20	0.20
Tri	0.22	0.22	0.24	0.26	0.28	0.29	0.29
Hg + Tri	0.22	0.21	0.23	0.22	0.21	0.21	0.21

Fig.18: Effect of mercury and triadimefon on the root / shoot ratio in *Centella asiatica*

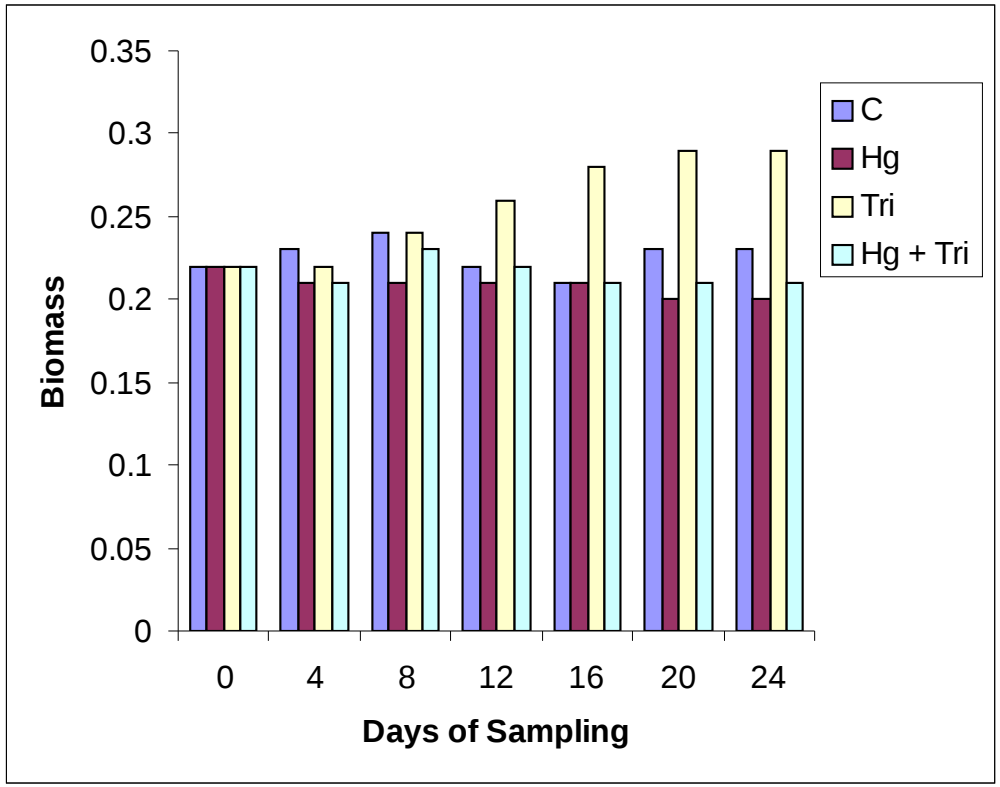


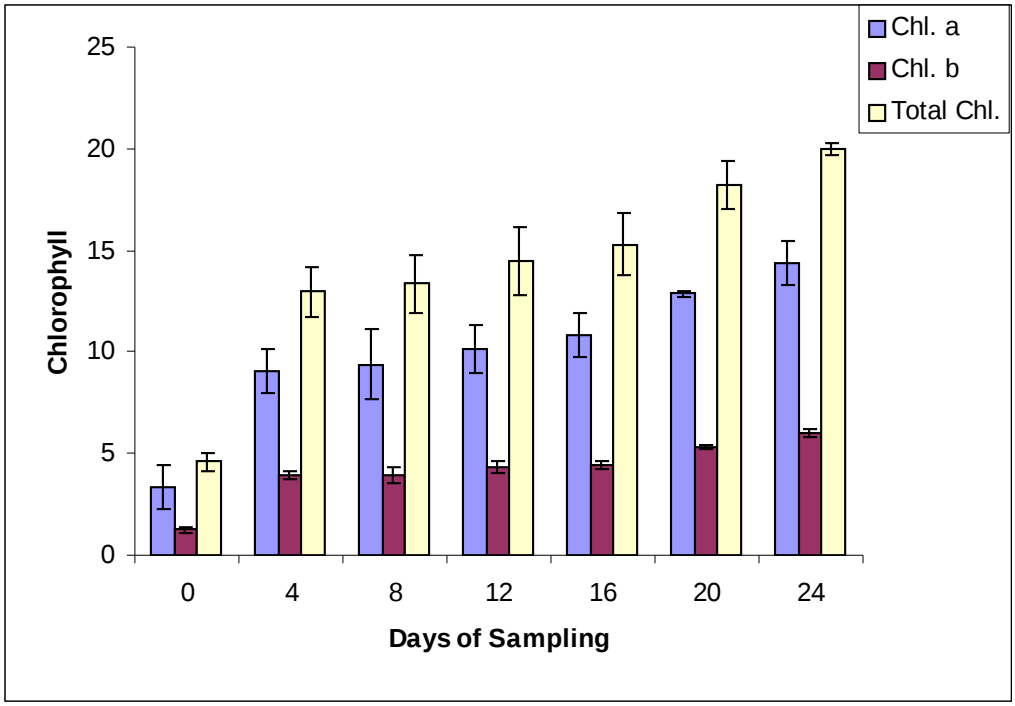
Table 11: Effect of mercury and triadimefon on the chlorophyll content in *Centella asiatica* leaves (mg g^{-1} dry weight)

Plant Part	Treatment	Days of Sampling

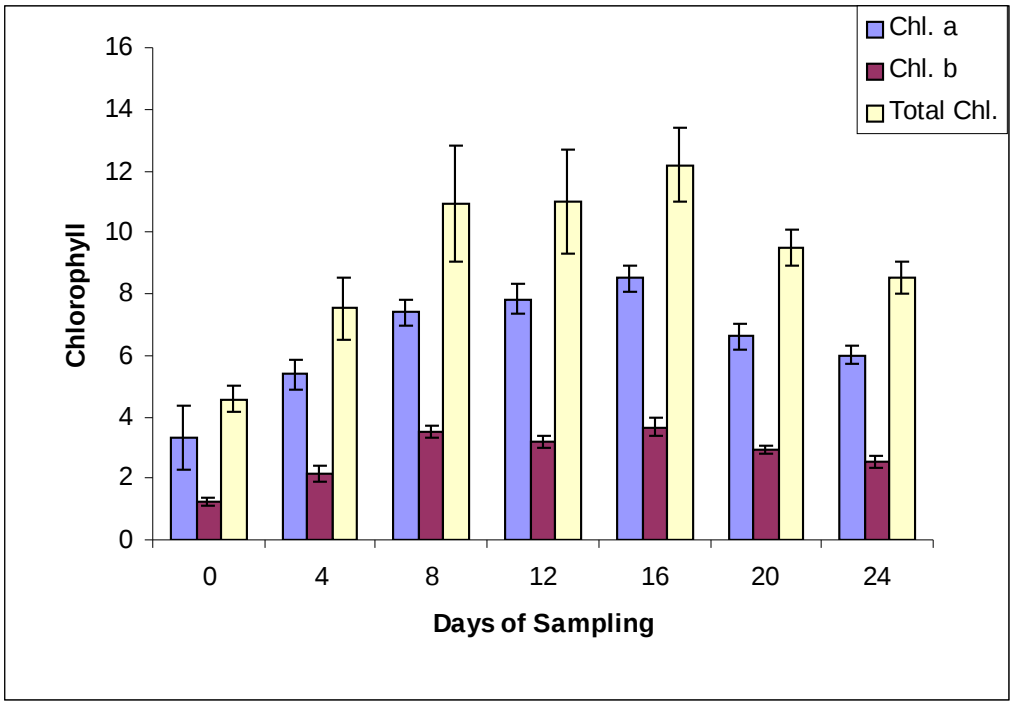
		0	4	8	12	16	20	24
C	Chl. a	3.33 ± 1.05	9.04 ± 1.10	9.39 ± 1.70	10.12 ± 1.21	10.85 ± 1.10	12.87 ± 0.17	14.40 ± 1.10
	Chl. b	1.25 ± 0.13	3.91 ± 0.20	3.95 ± 0.40	4.35 ± 0.29	4.43 ± 0.23	5.32 ± 0.13	5.97 ± 0.19
	Total Chl.	4.58 ± 0.40	12.95 ± 1.20	13.34 ± 1.40	14.47 ± 1.71	15.28 ± 1.51	18.19 ± 1.21	20.01 ± 0.31
Hg	Chl. a	3.33 ± 1.05	5.37 ± 0.49	7.40 ± 0.42	7.82 ± 0.50	8.50 ± 0.42	6.61 ± 0.41	6.00 ± 0.29
	Chl. b	1.25 ± 0.13	2.16 ± 0.25	3.51 ± 0.17	3.18 ± 0.18	3.67 ± 0.27	2.90 ± 0.13	2.52 ± 0.18
	Total Chl.	4.58 ± 0.40	7.53 ± 1.00	10.91 ± 1.90	11.00 ± 1.70	12.17 ± 1.21	9.51 ± 0.57	8.52 ± 0.51
Tri	Chl. a	3.33 ± 1.05	10.91 ± 1.70	11.42 ± 1.10	12.49 ± 1.20	12.96 ± 1.23	13.94 ± 1.52	16.79 ± 1.70
	Chl. b	1.25 ± 0.13	4.47 ± 0.43	4.93 ± 0.29	4.92 ± 0.39	5.13 ± 0.28	5.58 ± 0.18	6.35 ± 0.39
	Total Chl.	4.58 ± 0.40	15.38 ± 1.20	16.35 ± 0.96	17.41 ± 1.32	18.09 ± 1.51	19.52 ± 1.10	23.14 ± 0.19
Hg + Tri	Chl. a	3.33 ± 1.05	5.97 ± 0.18	7.57 ± 0.41	8.38 ± 0.81	10.29 ± 1.11	11.89 ± 1.60	12.18 ± 1.20
	Chl. b	1.25 ± 0.13	3.98 ± 0.51	3.37 ± 0.43	4.48 ± 0.42	4.40 ± 0.25	5.19 ± 0.43	5.72 ± 0.15
	Total Chl.	4.58 ± 0.40	9.95 ± 1.00	10.94 ± 1.31	12.86 ± 1.13	14.69 ± 1.51	17.08 ± 1.10	17.90 ± 1.31

Fig. 19: Effect of mercury and triadimefon on the total chlorophyll content in *Centella asiatica* leaves (mg g^{-1} dry weight)

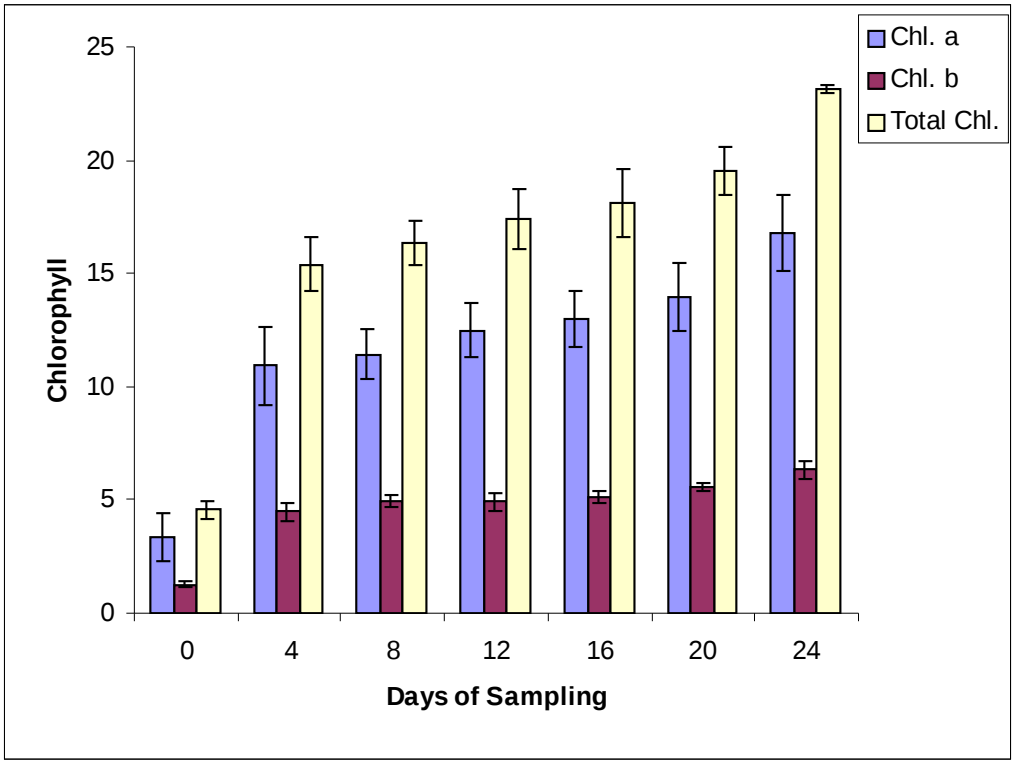
A) Control leaf



B) Mercury treated leaf



C) Mercury + Triadimefon treated leaf



D) Triadimefon treated leaf

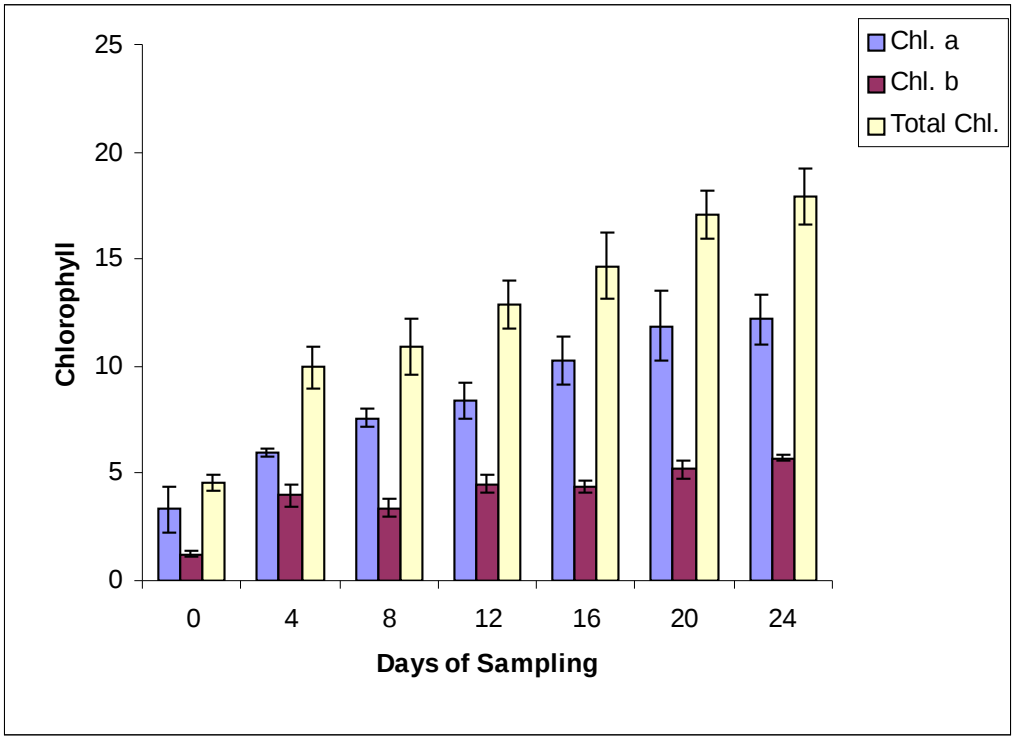


Fig. 19E: Effect of mercury and triadimefon on the total chlorophyll content in *Centella asiatica* leaves (mg g^{-1} dry weight)

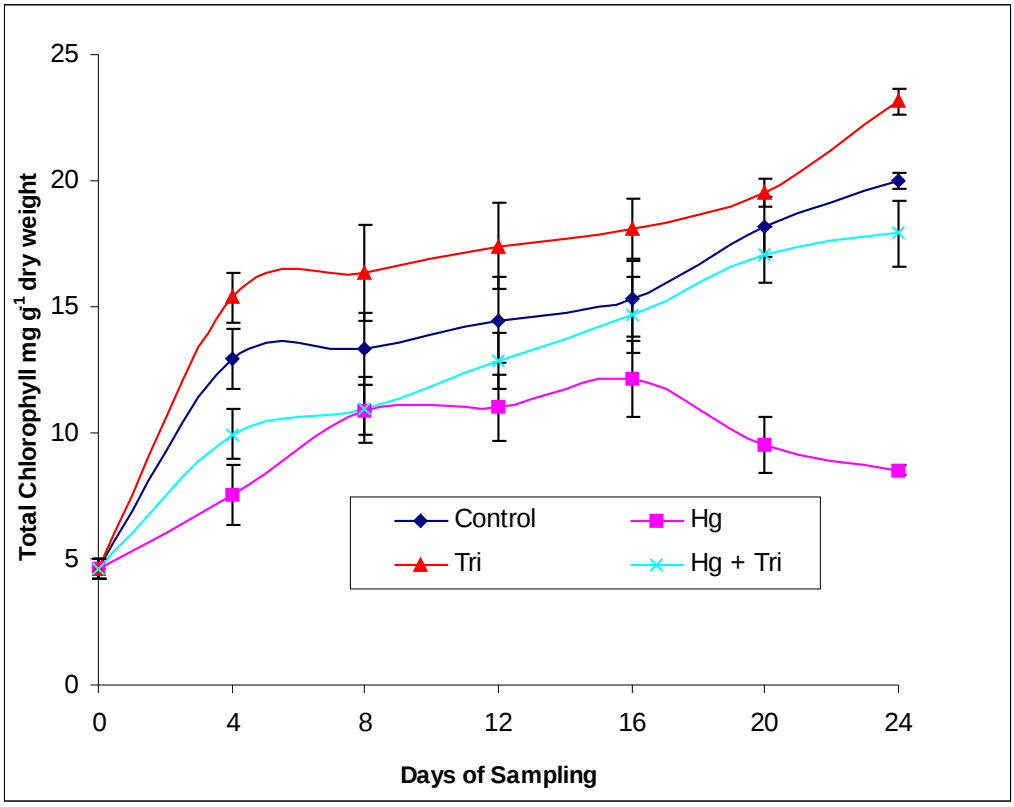


Table 12: Effect of mercury and triadimefon on the chlorophyll a/b ratio in *Centella asiatica* leaves

Plant Part	Treatment	Days of Sampling						
		0	**4**	**8**	**12**	**16**	**20**	**24**
Leaf	C	2.66 ± 0.14	2.31 ± 0.16	2.38 ± 0.31	2.33 ± 0.16	2.46 ± 0.30	2.42 ± 0.19	2.41 ± 0.21
	Hg	2.66 ± 0.14	2.49 ± 0.17	2.11 ± 0.15	2.46 ± 0.23	2.32 ± 0.19	2.28 ± 0.17	2.38 ± 0.21
	Tri	2.66 ± 0.14	2.44 ± 0.17	2.32 ± 0.16	2.54 ± 0.13	2.53 ± 0.27	2.50 ± 0.18	2.64 ± 0.23
	Hg + Tri	2.66 ± 0.14	1.52 ± 0.19	2.25 ± 0.17	1.87 ± 0.21	2.34 ± 0.13	2.29 ± 0.14	2.13 ± 0.17

Fig. 20: Effect of mercury and triadimefon on the chlorophyll a/b ratio in *Centella asiatica* leaves

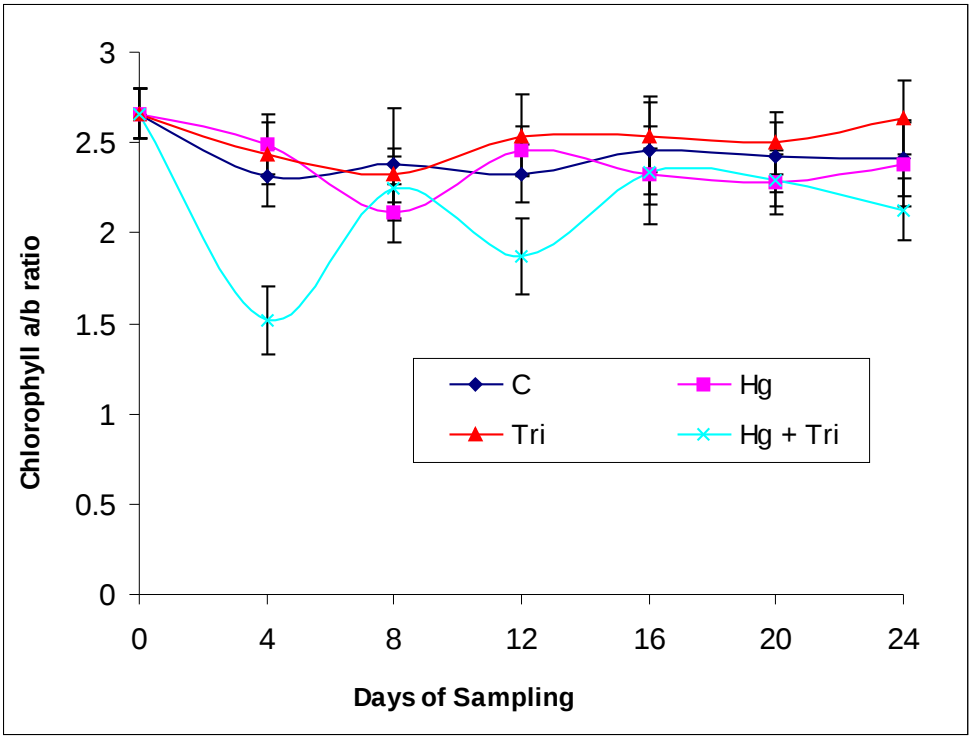


Table 13: Effect of mercury and triadimefon on total protein content in *Centella asiatica* (mg g^{-1} dry weight)

Plant Part	Treatment	Days of Sampling						
		0	4	8	12	16	20	24
Root	C	119.00 ± 3.70	147.84 ±14.3	176.60 ± 21.7	283.32 ± 31.1	304.97 ± 17.6	300.71 ± 25.6	281.92 ± 31.1
	Hg	119.00 ± 3.70	110.13 ± 12.6	121.27 ± 12.8	204.11 ±23.1	249.42 ± 23.9	252.81 ± 20.8	226.22 ± 19.0
	Tri	119.00 ± 3.70	159.72 ±13.7	234.30 ± 24.1	295.76 ± 36.3	323.83 ± 27.1	313.10 ± 19.0	298.19 ± 30.0
	Hg + Tri	119.00 ± 3.70	128.85 ± 11.7	138.91 ± 12.1	260.38 ± 22.5	289.49 ± 22.1	273.29 ± 25.9	271.46 ± 31.5
Stem	C	21.10 ± 1.12	31.51 ± 1.2	48.53 ± 4.9	68.66 ± 5.7	88.39 ± 8.1	85.10 ± 7.5	71.93 ± 7.6
	Hg	21.10 ± 1.12	28.76 ± 1.7	35.93 ± 2.5	63.83 ± 4.5	81.39 ± 5.9	82.89 ±7.5	65.57 ± 7.3
	Tri	21.10 ± 1.12	36.44 ± 3.2	56.90 ± 5.7	71.75 ± 5.5	94.30 ± 9.0	90.41 ± 8.3	82.47 ± 5.3
	Hg + Tri	21.10 ± 1.12	30.43 ± 2.5	46.23 ± 3.1	65.92 ± 5.5	83.76 ± 8.7	83.14 ± 7.7	67.46 ± 5.2
Leaf	C	121.15 ± 2.10	152.21 ± 11.9	196.74 ± 20.1	253.41 ± 20.7	291.85 ± 23.7	283.72 ± 31.1	224.42 ± 20.7
	Hg	121.15 ± 2.10	124.18 ± 012.7	172.88 ± 15.8	217.88 ± 21.5	280.07 ± 27.7	210.98 ± 25.5	183.72 ± 19.1
	Tri	121.15 ± 2.10	158.49 ± 11.9	207.21 ± 19.3	283.63 ± 22.1	335.19 ± 32.1	292.81 ± 32.1	248.82 ± 22.8
	Hg + Tri	121.15 ± 2.10	132.66 ± 16.7	182.22 ± 16.3	247.23 ± 21.1	286.72 ± 31.9	276.15 ± 29.9	216.26 ± 21.1

Fig. 21A: Effect of mercury and triadimefon on total protein content in the root of *Centella asiatica* (mg g^{-1} dry weight)

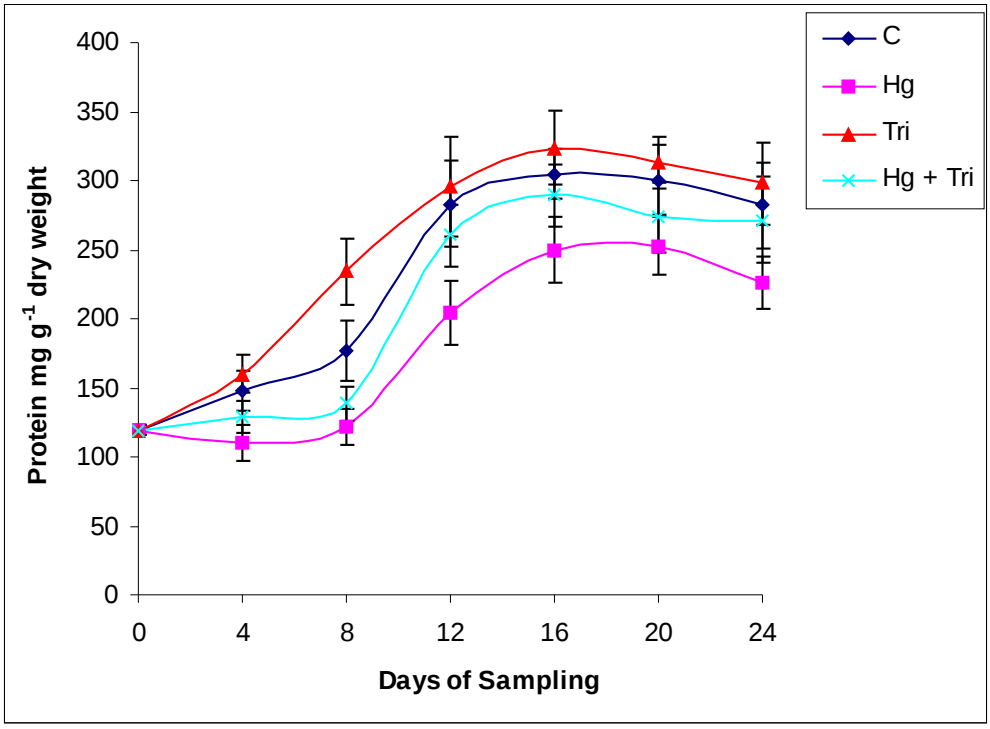


Fig. 21B: Effect of mercury and triadimefon on total protein content in the stem of *Centella asiatica* (mg g^{-1} dry weight)

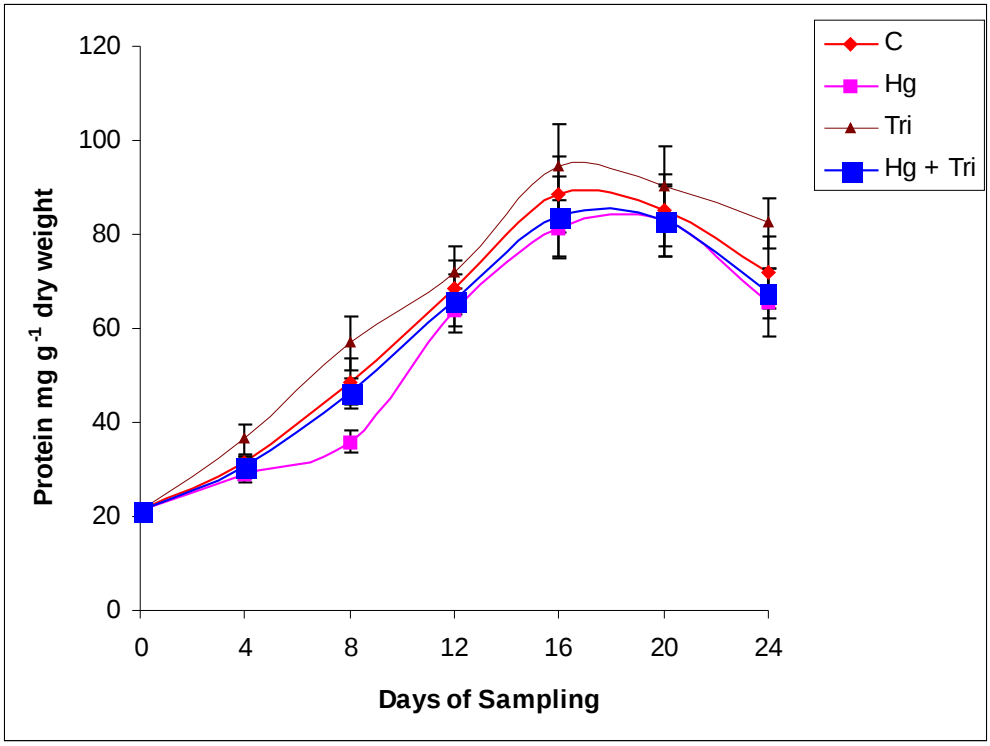


Fig. 21C: Effect of mercury and triadimefon on total protein content in the leaf of *Centella asiatica* (mg g^{-1} dry weight)

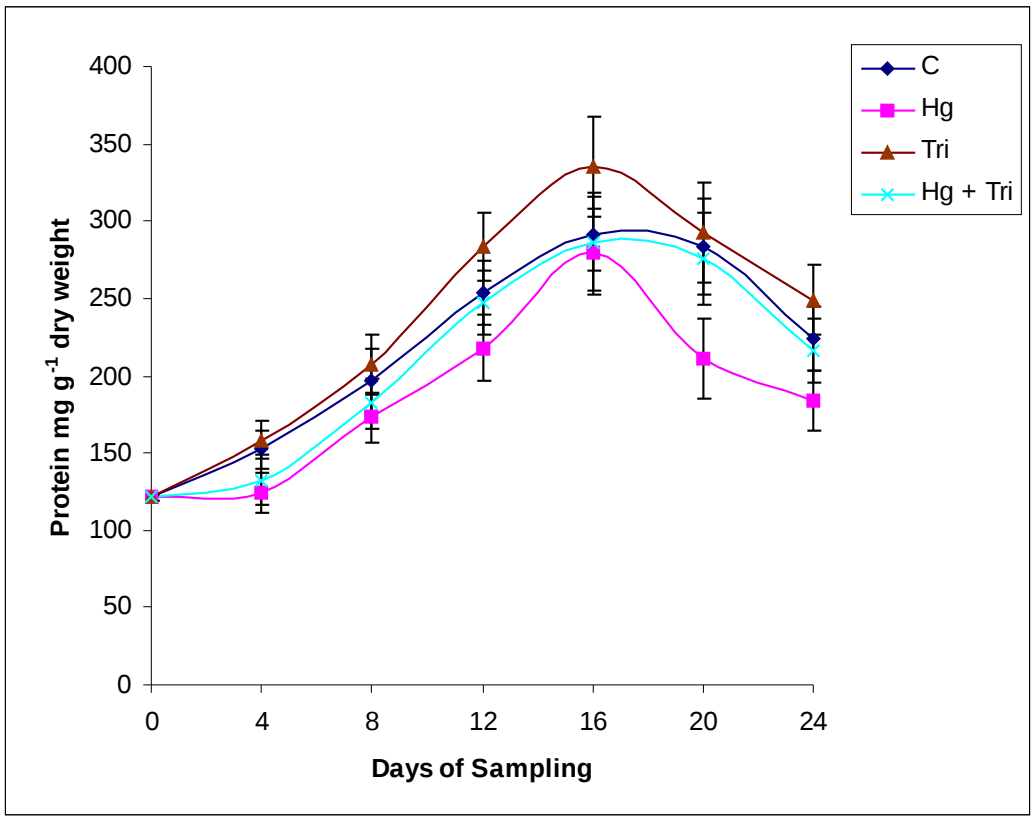


Table 14: Effect of mercury and triadimefon on soluble protein content in *Centella asiatica* (mg g^{-1} dry weight)

Plant Part	Treatment	Days of Sampling						
		0	4	8	12	16	20	24
Root	C	27.19 ± 2.5	33.26 ±2.7	50.36 ± 5.5	63.12 ± 6.7	71.89 ± 8.1	49.80 ± 5.2	44.44 ± 4.1
	Hg	27.19 ± 2.5	22.08 ± 2.3	38.11 ± 4.5	41.41 ± 3.9	51.36 ± 5.6	45.73 ± 5.3	37.04 ± 3.5
	Tri	27.19 ± 2.5	39.52 ± 4.1	56.50 ± 5.3	75.76 ± 8.3	87.12 ± 7.8	50.68 ± 4.9	45.18 ± 4.7
	Hg + Tri	27.19 ± 2.5	25.84 ± 3.0	44.30 ± 4.8	53.72 ± 6.2	64.01 ± 6.1	47.30 ± 5.2	38.06 ± 4.0
Stem	C	9.67 ± 0.37	12.05 ± 2.1	21.46 ± 2.1	28.18 ± 3.1	32.63 ± 3.2	28.56 ± 3.1	25.06 ± 3.2
	Hg	9.67 ± 0.37	9.99 ± 1.1	20.17 ± 1.8	24.56 ± 2.9	25.74 ± 2.6	25.71 ± 3.0	20.08 ± 2.3
	Tri	9.67 ± 0.37	14.00 ± 1.3	22.93 ± 1.9	29.88 ± 3.7	36.22 ± 3.7	31.80 ± 2.9	27.22 ± 2.7
	Hg + Tri	9.67 ± 0.37	11.11 ± 1.7	21.26 ± 2.4	27.72 ± 4.1	29.48 ± 3.1	29.46 ± 3.2	24.97 ± 2.1
Leaf	C	37.61 ± 0.13	49.41 ± 5.1	59.72 ± 6.3	64.27 ± 7.3	88.21 ± 7.9	70.34 ± 6.9	66.43 ± 7.1
	Hg	37.61 ± 0.13	35.01 ± 3.7	37.08 ± 3.5	60.14 ± 6.1	68.23 ± 7.3	61.27 ± 6.5	42.73 ± 4.5
	Tri	37.61 ± 0.13	51.33 ± 4.8	63.26 ± 7.1	69.81 ± 7.2	98.21 ± 8.7	78.65 ± 7.4	67.99 ± 6.7
	Hg + Tri	37.61 ± 0.13	42.53 ± 4.7	45.04 ± 5.1	62.10 ± 5.9	72.41 ± 6.8	66.86 ± 6.3	48.06 ± 4.7

Fig. 22A: Effect of mercury and triadimefon on soluble protein content in the root of *Centella asiatica* (mg g^{-1} dry weight)

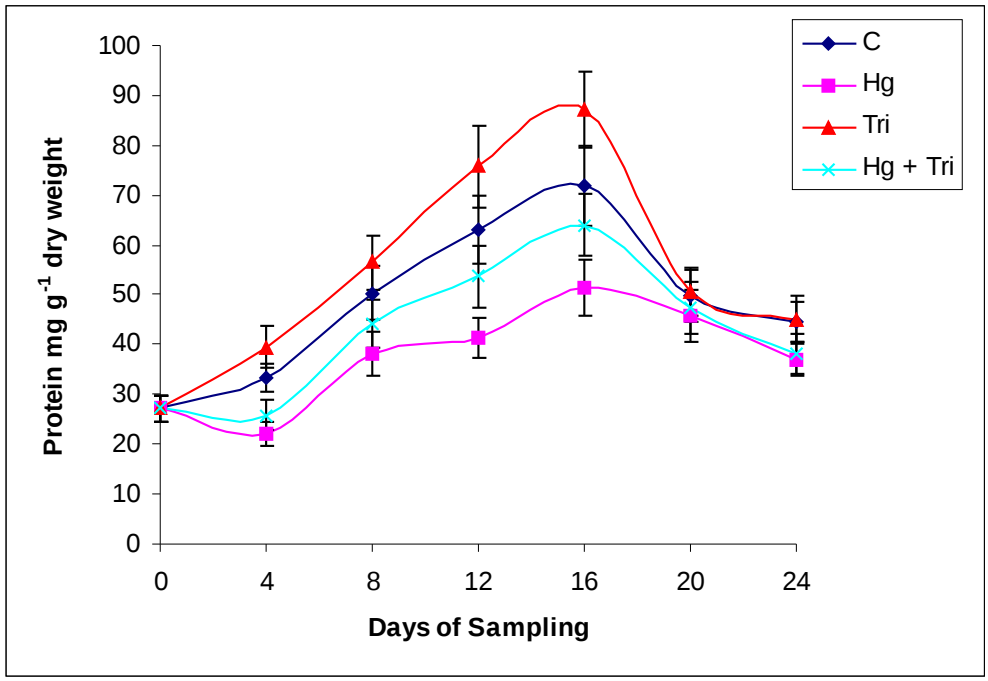


Fig. 22B: Effect of mercury and triadimefon on soluble protein content in the stem of *Centella asiatica* (mg g^{-1} dry weight)

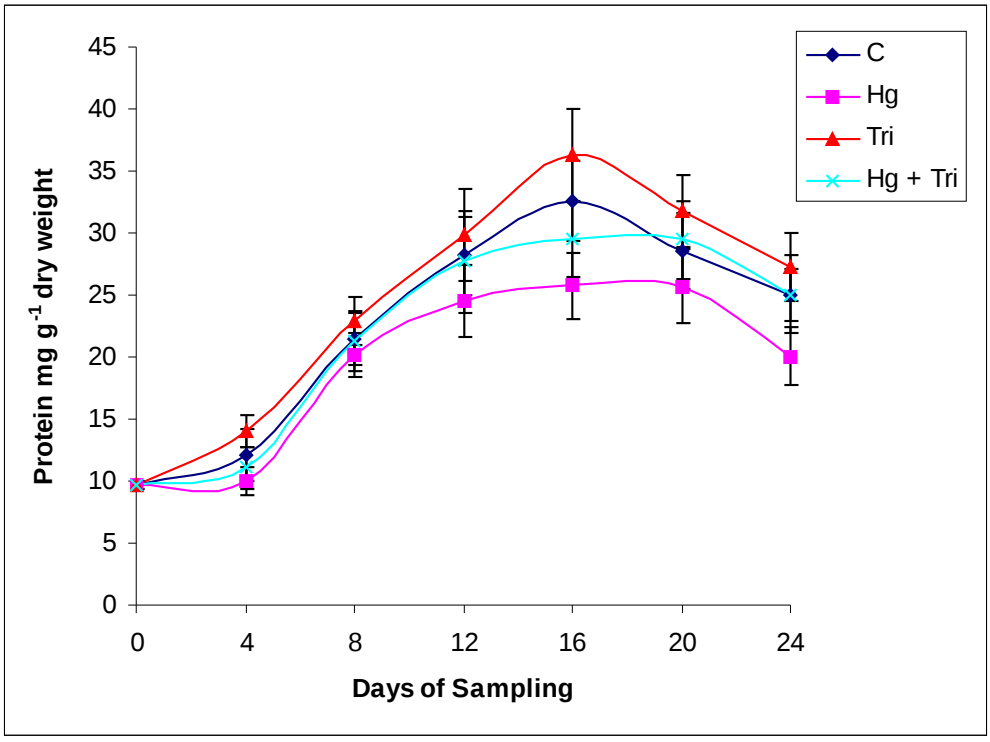


Fig. 22C: Effect of mercury and triadimefon on soluble protein content in the leaf of *Centella asiatica* (mg g^{-1} dry weight)

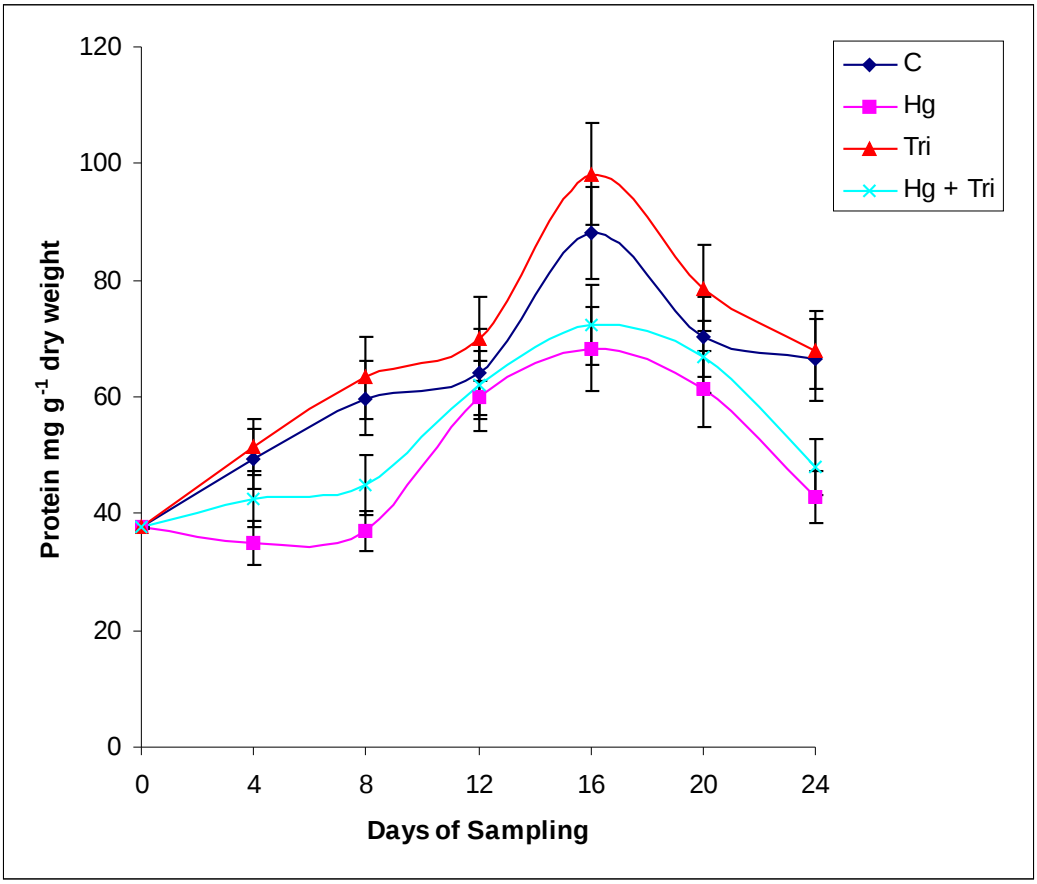


Table 15: Effect of mercury and triadimefon on total aminoacid content in *Centella asiatica* (mg g^{-1} dry weight)

Plant Part	Treatment	Days of Treatment						
		0	**4**	**8**	**12**	**16**	**20**	**24**
Root	C	1.30 ± 0.07	1.60 ± 0.11	1.70± 0.0.14	2.24 ±0.09	2.61 ± 0.0.07	3.31 ± 0.17	3.31 ± 0.24
	Hg	1.30 ± 0.07	2.80 ± 0.07	3.10 ±0.11	3.31 ±0.15	3.60 ± 0.15	4.23 ± 0.16	5.21 ± 0.34
	Tri	1.30 ± 0.07	1.71 ±0.09	2.41 ±0.13	2.70 ±0.12	2.91 ±0.14	3.61 ±0.15	3.62 ±0.27
	Hg + Tri	1.30 ± 0.07	2.31 ±0.14	2.90 ±0.07	3.20 ±0.16	3.26 ±0.11	3.72 ±0.18	3.81 ±0.29
Stem	C	2.36 ±0.11	3.02 ±0.07	3.13 ±0.21	5.30 ±0.16	5.32 ±0.09	5.92 ±0.15	6.23 ±0.45
	Hg	2.36 ±0.11	5.80 ±0.18	5.90 ±0.35	6.13 ±0.41	7.32 ±0.37	7.81 ±0.24	8.74 ±0.67
	Tri	2.36 ±0.11	2.90 ± 0.08	3.31 ±0.07	4.30 ±0.11	4.80 ±0.13	5.31 ±0.05	5.90 ±0.21
	Hg + Tri	2.36 ±0.11	4.80 ±0.09	5.62 ±0.06	5.80 ±0.04	5.81 ±0.39	6.12 ±0.41	8.03 ±0.37
Leaf	C	3.15 ± 0.12	3.84 ±0.16	3.93 ±0.21	4.10 ±0.39	4.10 ±0.31	4.22 ±0.41	4.41 ±0.43
	Hg	3.15 ± 0.12	4.71 ±0.19	4.94 ±0.12	5.33 ±0.11	6.12 ± 0.43	6.51 ± 0.40	7.80 ± 0.47
	Tri	3.15 ± 0.12	4.01 ± 0.26	4.20 ± 0.33	4.30 ± 0.28	4.44 ± 0.37	5.11 ± 0.42	5.40 ± 0.35
	Hg + Tri	3.15 ± 0.12	4.60 ± 0.33	4.95 ± 0.27	5.00 ± 0.27	5.51 ± 0.33	5.70 ± 0.41	5.72 ± 0.43

Fig. 26A : Effect of mercury and triadimefon on the amino acid content in the root tissue of *Centella asiatica* (mg g^{-1} dry weight)

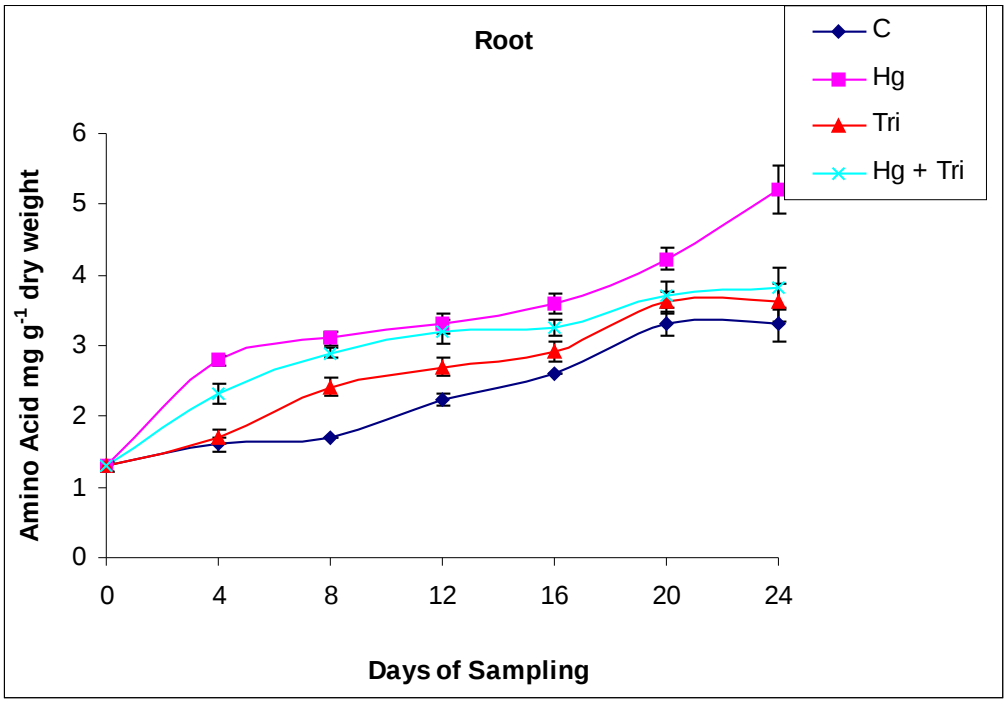


Fig. 26 B: Effect of mercury and triadimefon on the amino acid content in the stem tissue of *Centella asiatica* (mg g^{-1} dry weight)

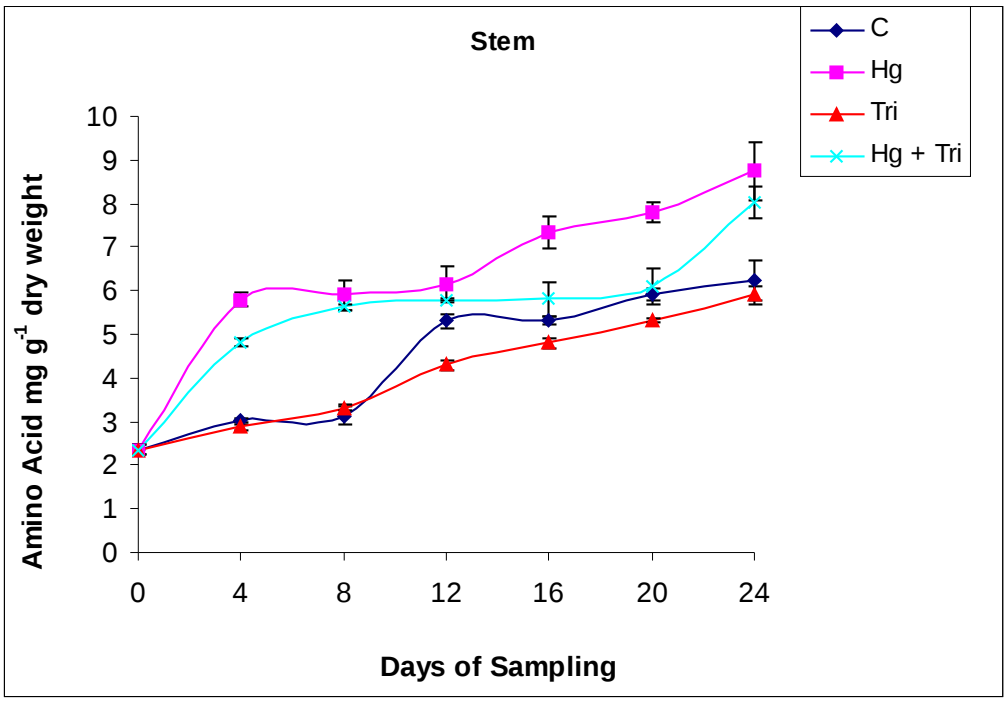


Fig. 26C: Effect of mercury and triadimefon on the amino acid content in the leaf tissue of *Centella asiatica* (mg g^{-1} dry weight)

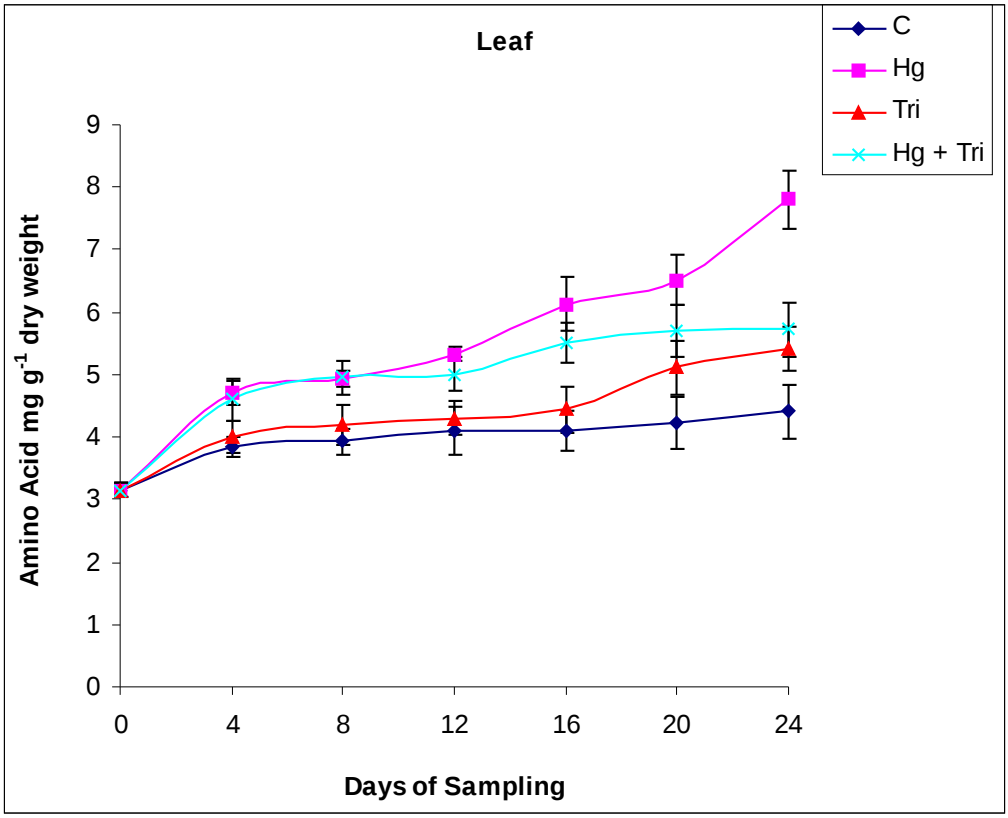


Table 16: Effect of mercury and triadimefon on proline content in *Centella asiatica* (mg g^{-1} dry weight)

Plant Part	Treatment	Days of Treatment						
		0	4	8	12	16	20	24
Root	C	0.165 ± 0.007	0.180 ± 0.006	0.191 ± 0.006	0.212 ± 0.021	0.261 ± 0.012	0.291 ± 0.011	0.350 ± 0.020
	Hg	0.165 ± 0.007	0.240 ± 0.013	0.261 ± 0.017	0.294 ± 0.009	0.319 ± 0.017	0.364 ± 0.017	0.379 ± 0.017
	Tri	0.165 ± 0.007	0.150 ± 0.007	0.161 ± 0.021	0.173 ± 0.020	0.185 ± 0.016	0.210 ± 0.016	0.261 ± 0.013
	Hg + Tri	0.165 ± 0.007	0.220 ± 0.017	0.242 ± 0.020	0.250 ± 0.011	0.30 ± 0.017	0.334 ± 0.012	0.356 ± 0.020
Stem	C	0.101 ± 0.003	0.120 ± 0.016	0.160 ± 0.016	0.203 ± 0.011	0.242 ± 0.013	0.261 ± 0.021	0.294 ± 0.014
	Hg	0.101 ± 0.003	0.153 ± 0.013	0.244 ± 0.031	0.283 ± 0.033	0.301 ± 0.030	0.440 ± 0.022	0.547 ± 0.021
	Tri	0.101 ± 0.003	0.094 ± 0.017	0.117 ± 0.017	0.119 ± 0.032	0.232 ± 0.013	0.241 ± 0.031	0.271 ± 0.013
	Hg + Tri	0.101 ± 0.003	0.141 ± 0.020	0.211 ± 0.030	0.271 ± 0.031	0.291 ± 0.020	0.397 ± 0.020	0.473 ± 0.021
Leaf	Control	0.093 ± 0.004	0.101 ± 0.016	0.111 ± 0.022	0.127 ± 0.012	0.167 ± 0.017	0.206 ± 0.012	0.224 ± 0.014
	C	0.093 ± 0.004	0.134 ± 0.014	0.139 ± 0.021	0.181 ± 0.011	0.274 ± 0.018	0.299 ± 0.021	0.396 ± 0.022
	Hg	0.093 ± 0.004	0.090 ± 0.017	0.109 ± 0.013	0.114 ± 0.013	0.160 ± 0.021	0.200 ± 0.011	0.201 ± 0.031
	Tri	0.093 ± 0.004	0.120 ± 0.021	0.130 ± 0.014	0.160 ± 0.017	0.237 ± 0.020	0.260 ± 0.013	0.351 ± 0.027

Fig. 27A : Effect of mercury and triadimefon on proline content in the root tissue of *Centella asiatica* (mg g^{-1} dry weight)

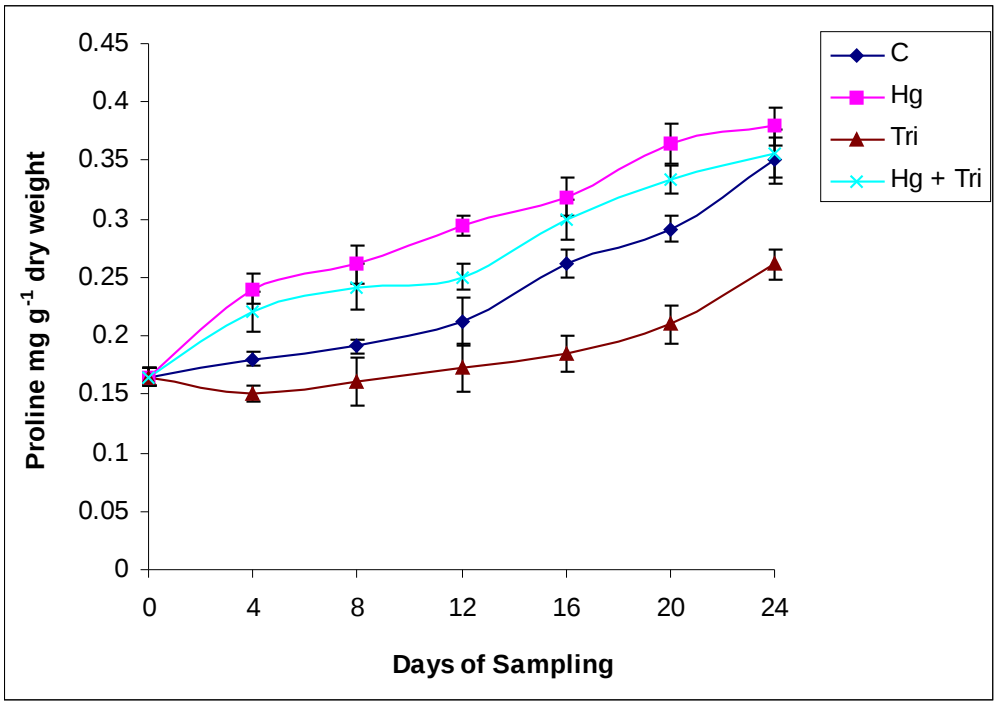


Fig. 27B: Effect of mercury and triadimefon on proline content in the stem tissue of *Centella asiatica* (mg g^{-1} dry weight)

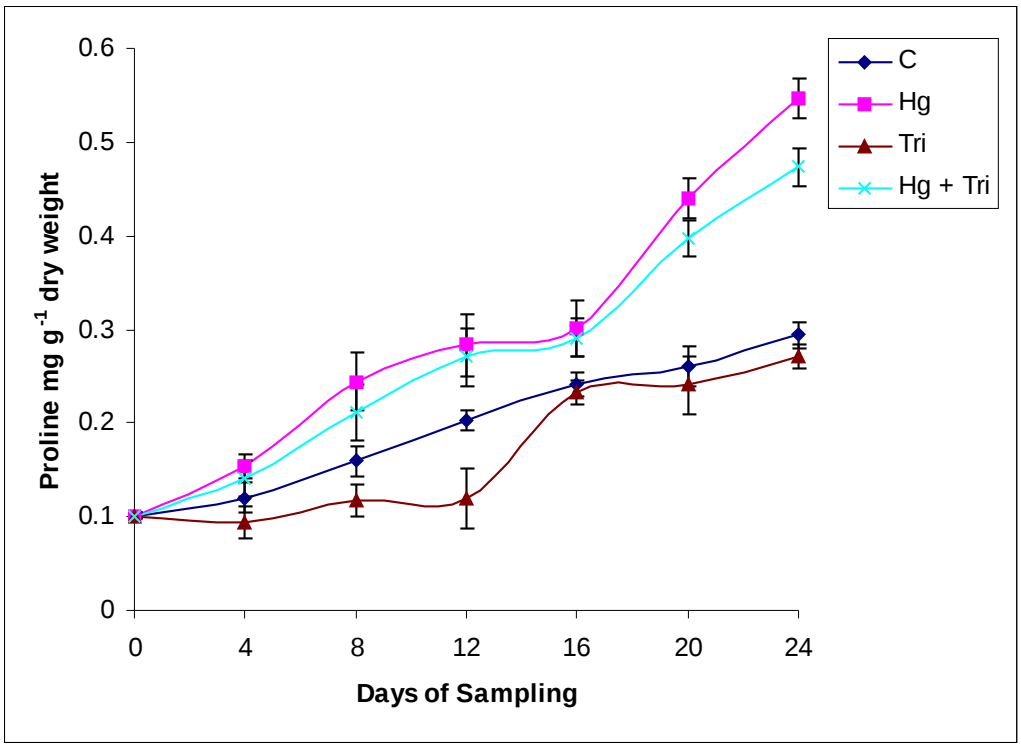


Fig. 27C: Effect of mercury and triadimefon on proline content in the leaf tissue of *Centella asiatica* (mg g^{-1} dry weight)

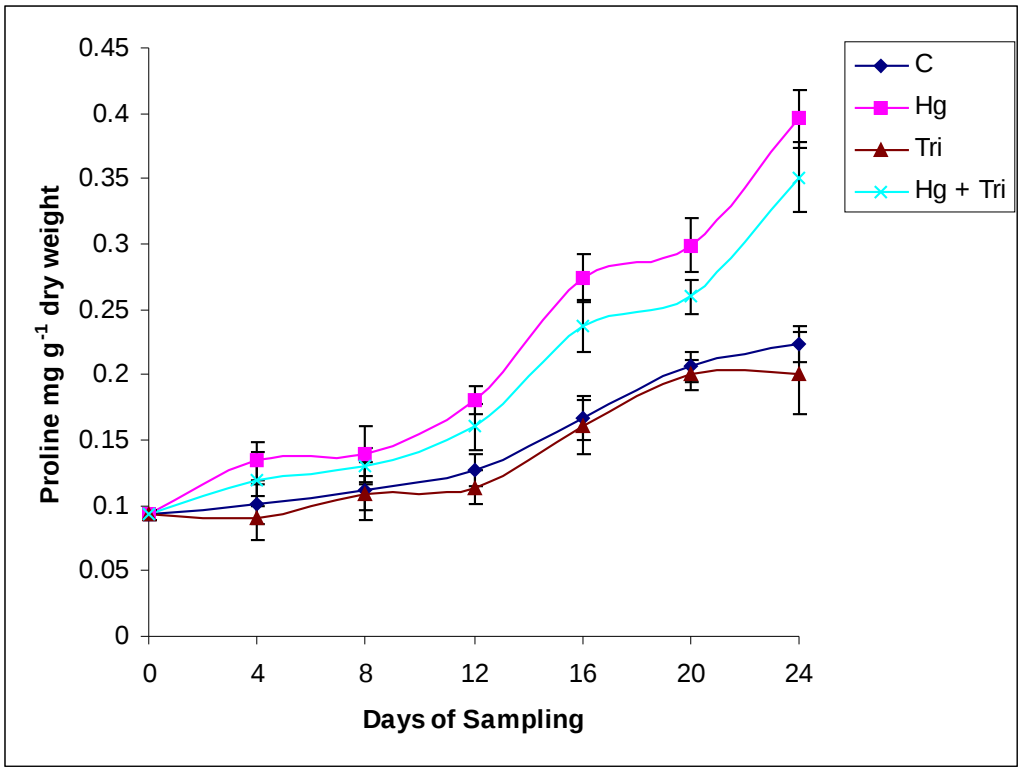


Table 17: Effect of mercury and triadimefon on phenolic content in *Centella asiatica* (mg g^{-1} dry weight)

Plant Part	Treatment	Days of Treatment						
		0	4	8	12	16	20	24
Root	C	0.68 ± 0.015	0.83 ± 0.017	1.39 ± 0.160	1.46 ± 0.017	1.56 ± 0.054	1.73 ± 0.014	1.85 ± 0.050
	Hg	0.68 ± 0.015	0.93 ± 0.031	1.66 ± 0.160	1.73 ± 0.011	1.84 ± 0.014	1.89 ± 0.030	1.99 ± 0.018
	Tri	0.68 ± 0.015	0.74 ± 0.013	1.16 ± 0.120	1.35 ± 0.031	1.40 ± 0.024	1.49 ± 0.027	1.61 ± 0.012
	Hg + Tri	0.68 ± 0.015	0.90 ± 0.011	1.47 ± 0.220	1.69 ± 0.031	1.80 ± 0.017	1.87 ± 0.041	1.90 ± 0.013
Stem	C	0.91 ± 0.06	1.01 ± 0.017	1.18 ± 0.130	1.25 ± 0.034	1.47 ± 0.050	1.58 ± 0.019	1.65 ± 0.017
	Hg	0.91 ± 0.06	1.28 ± 0.013	1.36 ± 0.130	1.43 ± 0.041	1.56 ± 0.090	1.67 ± 0.021	1.77 ± 0.024
	Tri	0.91 ± 0.06	0.99 ± 0.018	1.10 ± 0.100	1.19 ± 0.170	1.30 ± 0.150	1.45 ± 0.024	1.59 ± 0.019
	Hg + Tri	0.91 ± 0.06	1.08 ± 0.019	1.21 ± 0.170	1.40 ± 0.041	1.51 ± 0.100	1.64 ± 0.016	1.69 ± 0.011
Leaf	C	0.63 ± 0.04	0.78 ± 0.016	1.02 ± 0.040	1.18 ± 0.017	1.27 ± 0.050	1.93 ± 0.030	2.85 ± 0.024
	Hg	0.63 ± 0.04	1.04 ± 0.024	1.66 ± 0.170	1.85 ± 0.030	1.87 ± 0.023	2.39 ± 0.017	3.79 ± 0.018
	Tri	0.63 ± 0.04	0.69 ± 0.021	0.89 ± 0.130	0.99 ± 0.024	1.25 ± 0.032	1.30 ± 0.022	1.80 ± 0.016
	Hg + Tri	0.63 ± 0.04	1.02 ± 0.017	1.54 ± 0.170	1.62 ± 0.013	1.74 ± 0.031	2.25 ± 0.015	2.85 ± 0.023

Fig. 28A: Effect of mercury and triadimefon on phenolic content of root of *Centella asiatica* (mg g^{-1} dry weight)

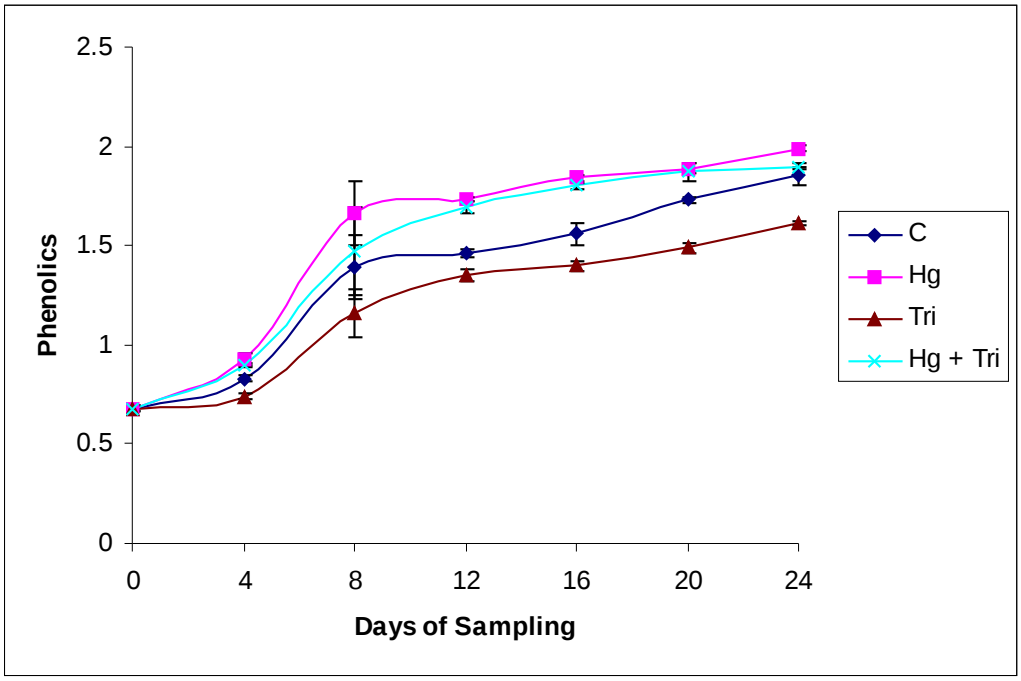


Fig. 28B: Effect of mercury and triadimefon on phenolic content of stem of *Centella asiatica* (mg g^{-1} dry weight)

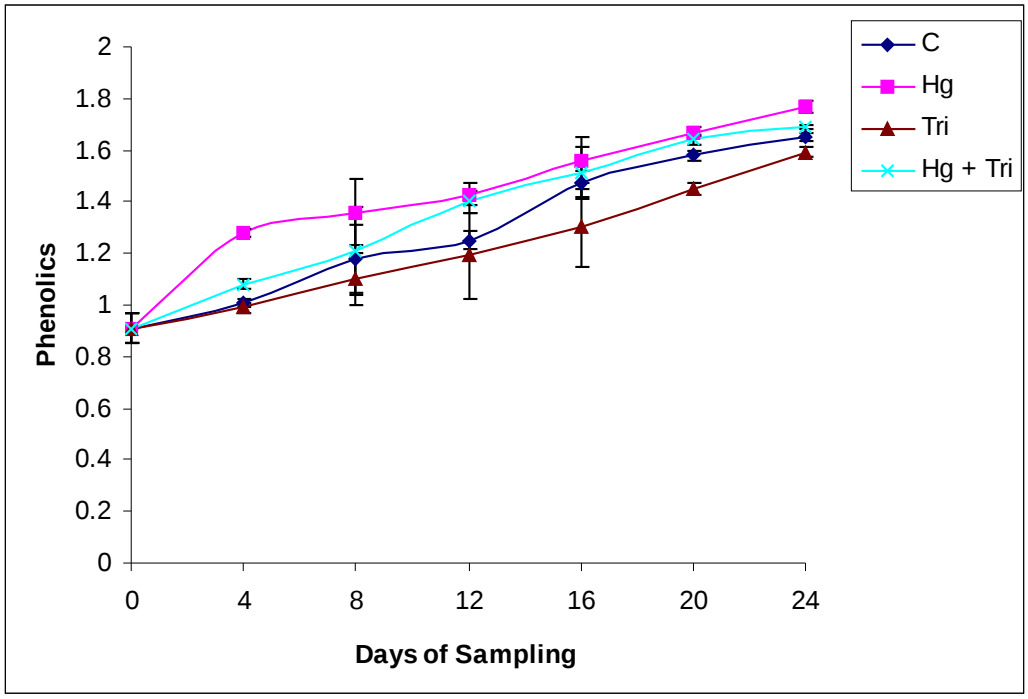


Fig. 28C: Effect of mercury and triadimefon on phenolic content of leaf of *Centella asiatica* (mg g^{-1} dry weight)

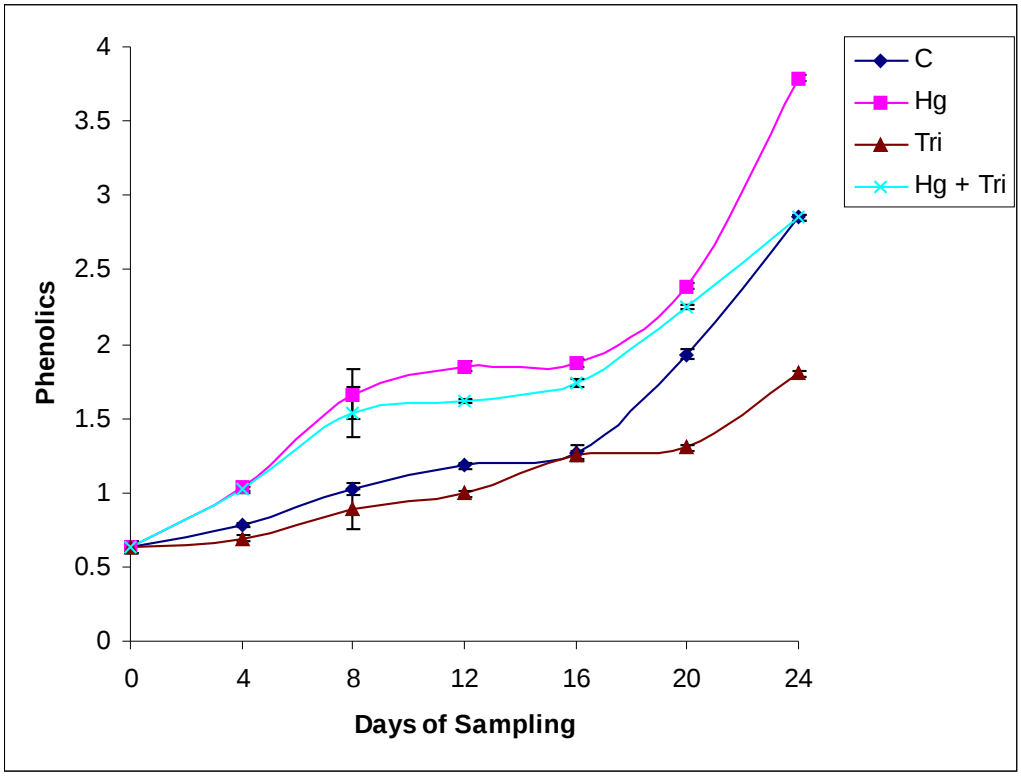

Phenolics
4
3.5
3
2.5
2
1.5
1
0.5
0
0
4
8
12
16
20
24
Days of Sampling
C
Hg
Tri
Hg + Tri

Table 18: Effect of mercury and triadimefon on the unit activity of peroxidase in *Centella asiatica* (Units g^{-1} Fresh weight)

Plant Part	Treatment	Days of Treatment						
		0	4	8	12	16	20	24
Root	C	7.38 ± 0.36	7.45 ± 0.48	7.93 ± 0.35	8.17 ± 0.38	8.27 ± 0.3 9	8.56 ± 0.31	8.71 ± 0.13
	Hg	7.38 ± 0.36	13.26 ± 0.54	13.90 ± 0.4 6	14.11 ± 0.2 8	14.60 ± 0.0 9	14.97 ± 0.11	15.60 ± 0.13
	Tri	7.38 ± 0.36	6.19 ± 0.32	7.18 ± 0.33	7.75 ± 0.3 4	8.10 ±0.2 9	8.19 ± 0.17	8.47 ± 0.24
	Hg + Tri	7.38 ± 0.36	13.16 ± 0.41	13.75 ± 0.5 9	13.98 ± 0.4 8	14.21 ± 0.2 7	14.69 ± 0.15	14.89 ± 0.23
Stem	C	2.00 ± 0.05	2.08 ± 0.47	3.16 ± 0.24	3.95 ± 0.2 4	4.11 ± 0.1 3	4.37 ± 0.14	4.63 ± 0.16
	Hg	2.00 ± 0.05	4.79 ± 0.35	4.97 ± 0.3 7	5.18 ± 0.3 4	5.31 ± 0.2 6	5.60 ± 0.14	5.91 ± 0.15
	Tri	2.00 ± 0.05	2.54 ± 0.13	3.69 ± 0.2 5	3.73 ± 0.2 4	4.00 ± 0.2 3	4.14 ± 0.24	4.51 ± 0.13
	Hg + Tri	2.00 ± 0.05	3.13 ± 0.28	4.18 ± 0.2 6	5.07 ± 0.2 7	5.27 ±0.1 4	5.35 ± 0.15	5.67 ± 0.14
Leaf	C	1.40 ± 0.03	1.80 ± 0.12	2.39 ±0.1 8	2.20 ± 0.0 1 3	2.56 ± 0.23	2.69 ± 0.13	2.91 ± 0.16
	Hg	1.40 ± 0.03	2.34 ± 0.09	2.87 ± 0.1 7	4.57 ± 0.27	4.90 ± 0.17	4.98 ± 0.11	5.23 ± 0.13
	Tri	1.40 ± 0.03	1.13 ± 0.13	2.22 ± 0.14	2.34 ± 0.34	2.50 ± 0.14	2.60 ± 0.13	2.87 ± 0.15
	Hg + Tri	1.40 ± 0.03	2.78 ± 0.11	2.81 ± 0.15	3.56 ± 0.3 1	4.13 ± 0.16	4.17 ± 0.15	5.12 ± 0.17

Fig. 29A : Effect of mercury and triadimefon on the unit activity of peroxidase in the root of *Centella asiatica* (Units g^{-1} Fresh weight)

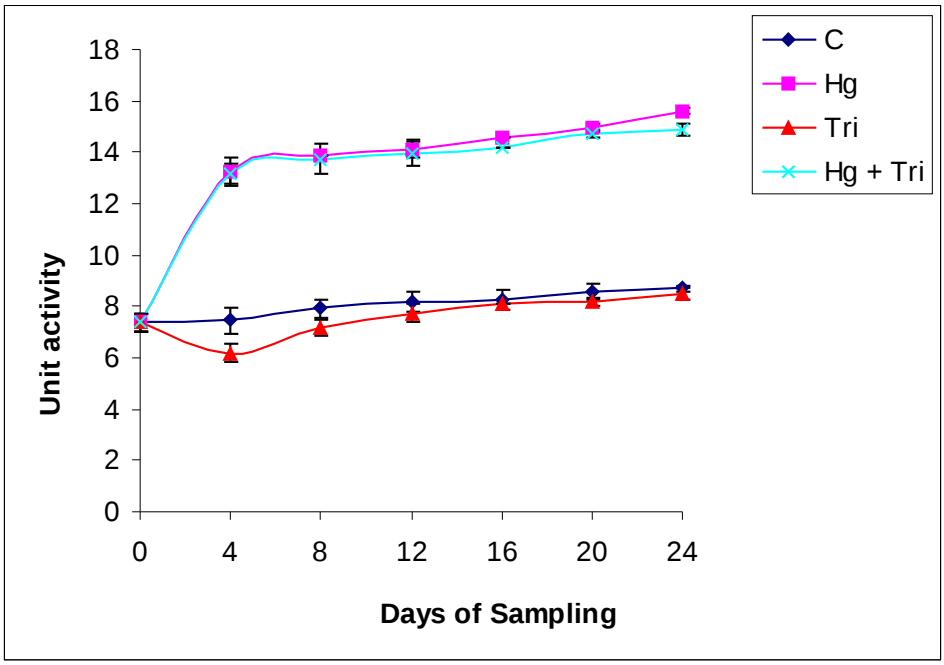


Fig. 29B : Effect of mercury and triadimefon on the unit activity of peroxidase in the stem of *Centella asiatica* (Units g^{-1} Fresh weight)

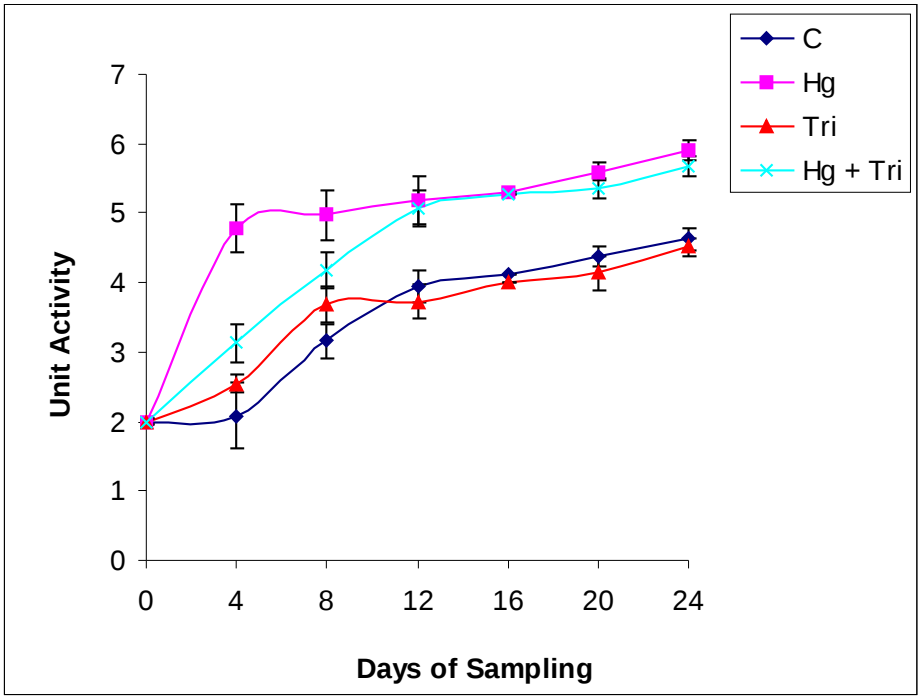


Fig. 29C: Effect of mercury and triadimefon on the unit activity of peroxidase in the leaf of *Centella asiatica* (Units g^{-1} Fresh weight)

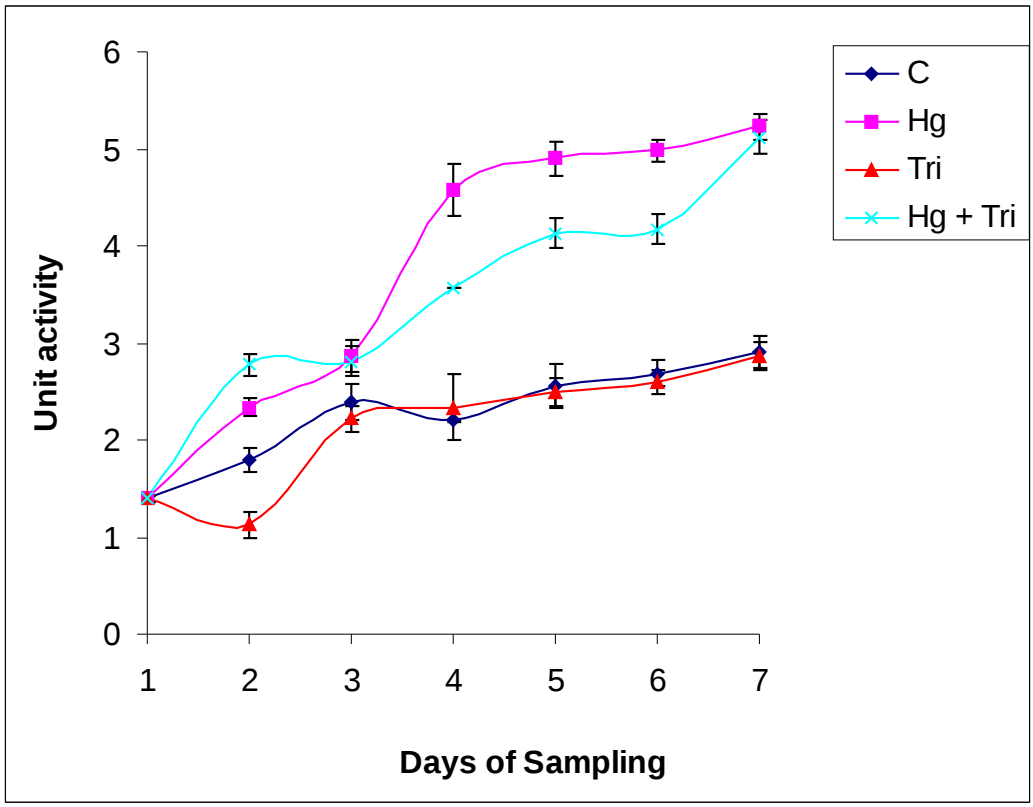


Table 19: Effect of mercury and triadimefon on the specific activity of peroxidase in *Centella asiatica* (Units mg^{-1} Protein)

Plant Part	Treatment	Days of Treatment						
		0	**4**	**8**	**12**	**16**	**20**	**24**
Root	C	2. 88 ± 0.07	3. 25 ± 0.26	2. 47 ± 0.23	2.46 ± 0.19	2.68 ± 0.17	4.12 ± 0.25	4.73 ± 0.25
	Hg	2. 88 ± 0.07	6.56 ± 0.12	4.26 ± 0.31	4.20 ± 0.15	4.92 ± 0.26	5.87 ± 0.29	8.13 ± 0.21
	Tri	2. 88 ± 0.07	2.31 ± 0.13	2.07 ± 0.19	1.99 ± 0.11	2.18 ± 0.17	4.04 ± 0.27	5.72 ± 0.16
	Hg + Tri	2. 88 ± 0.07	6.07 ± 0.37	3.76 ± 0.29	3.64 ± 0.26	4.31 ± 0.24	5.97 ± 0.31	8.27 ± 0.23
Stem	C	0.90 ± 0.09	1.03 ± 0.10	0.96 ± 0.03	0.97 ± 0.02	0.95 ± 0.04	1.18 ± 0.16	1.57 ± 0.14
	Hg	0.90 ± 0.09	2.55 ± 0.17	1.40 ± 0.19	1.32 ± 0.16	1.36 ± 0.07	1.50 ± 0.11	2.21 ± 0.16
	Tri	0.90 ± 0.09	1.14 ± 0.18	1.10 ± 0.09	0.88 ± 0.09	0.91 ± 0.04	1.08 ± 0.08	1.51 ± 0.09
	Hg + Tri	0.90 ± 0.09	1.53 ± 0.12	1.12 ± 0.11	1.20 ± 0.10	1.18 ± 0.13	1.25 ± 0.09	1.77 ± 0.14
Leaf	C	0.23 ± 0.14	0.27 ± 0.04	0.41 ± 0.07	0.36 ± 0.01	0.34 ± 0.03	0.48 ± 0.01	0.60 ± 0.02
	Hg	0.23 ± 0.14	0.42 ± 0.03	0.67 ± 0.04	0.70 ± 0.07	0.73 ± 0.04	0.91 ± 0.03	1.39 ± 0.13
	Tri	0.23 ± 0.14	0.21 ± 0.04	0.37 ± 0.03	0.36 ± 0.06	0.28 ± 0.04	0.47 ± 0.03	0.60 ± 0.05
	Hg + Tri	0.23 ± 0.14	0.46 ± 0.04	0.55 ± 0.05	0.58 ± 0.05	0.61 ± 0.03	0.73 ± 0.01	1.27 ± 0.11

Fig. 30A: Effect of mercury and triadimefon on the specific activity of peroxidase in the root of *Centella asiatica*

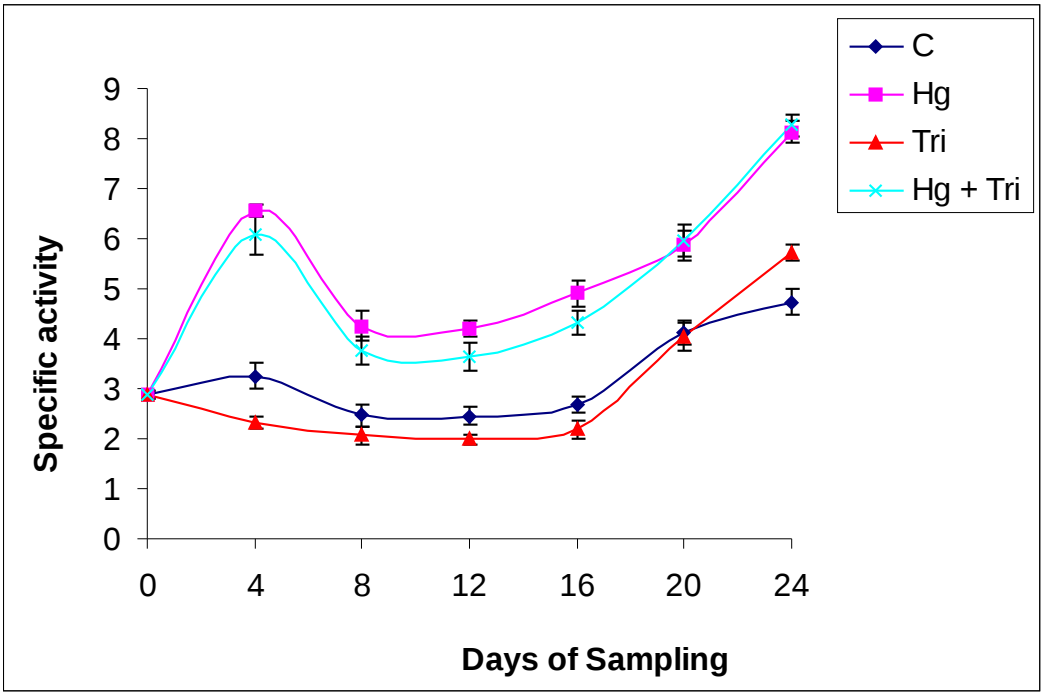


Fig. 30B: Effect of mercury and triadimefon on the specific activity of peroxidase in the stem of *Centella asiatica*

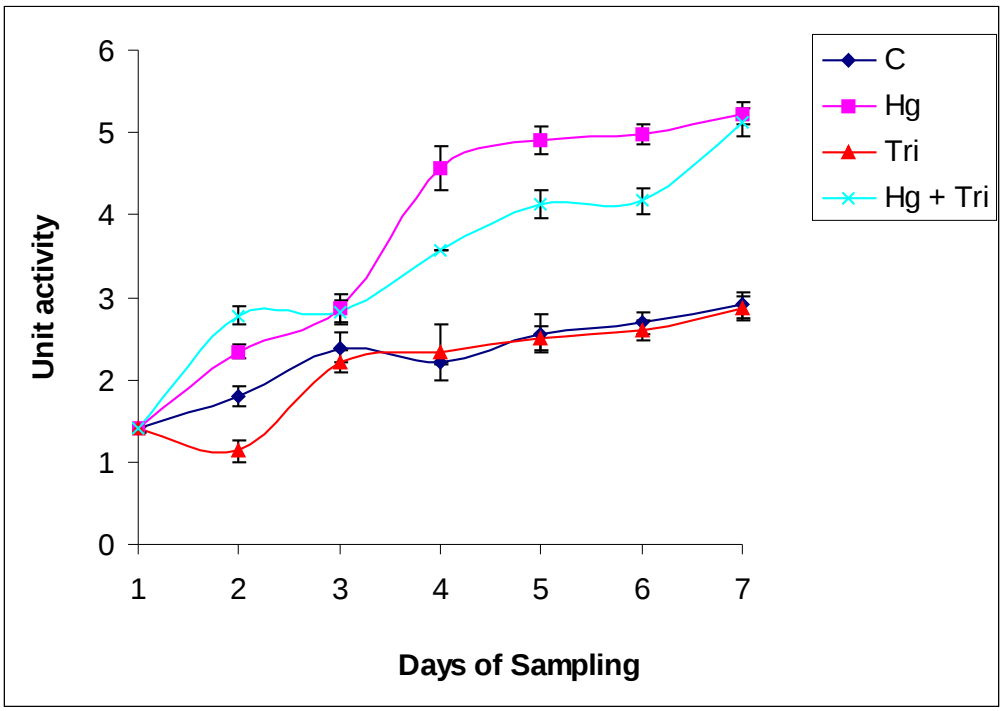


Fig. 30C: Effect of mercury and triadimefon on the specific activity of peroxidase in the leaf of *Centella asiatica*

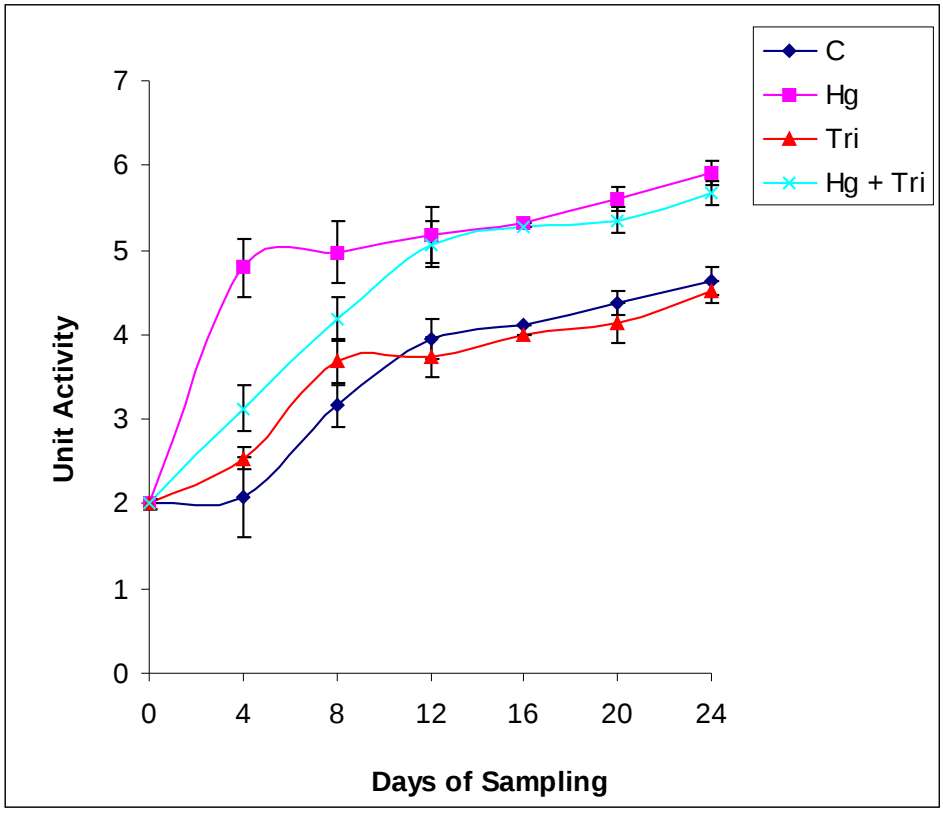


Table 20: Accumulation of mercury in different plant parts of *Centella asiatica* treated with mercury and a combination of mercury and triadimefon (mg g^{-1} dry weight)

Treatment	Plant parts	Intervals in days						
		0	**4**	**8**	**12**	**16**	**20**	**24**
Hg (9mg of Hg)	Root	NA	2.01 ± 0.04	2.01 ± 0.11	2.00 ± 0.03	2.11 ± 0.13	2.16 ± 0.16	2.39 ± 0.14
	Stem	NA	1.90 ± 0.09	1.80 ± 0.12	1.95 ± 0.14	1.89 ± 0.17	2.15 ± 0.20	2.26 ± 0.19
	Leaf	NA	1.50 ± 0.08	1.69 ± 0.11	1.75 ± 0.17	1.74 ± 0.15	1.35 ± 0.13	1.03 ± 0.21
H+ Tri (9mg of Hg)	Root	NA	1.80 ± 0.12	1.70 ± 0.31	1.81 ± 0. 21	1.78 ± 0.17	1.89 ± 0.15	2.04 ± 0.17
	Stem	NA	1.50 ± 0.14	1.69 ± 0.13	1.60 ± 0.18	1.59 ± 0.13	1.70 ± 0.15	1.30 ± 0.14
	Leaf	NA	1.19 ± 0.17	1.07 ± 0.17	1.13 ± 0.13	1.09 ± 0.14	1.00 ± 0.10	1.00 ± 0.19

Fig. 31A: Accumulation of mercury in different plant parts of *Centella asiatica* treated with mercury

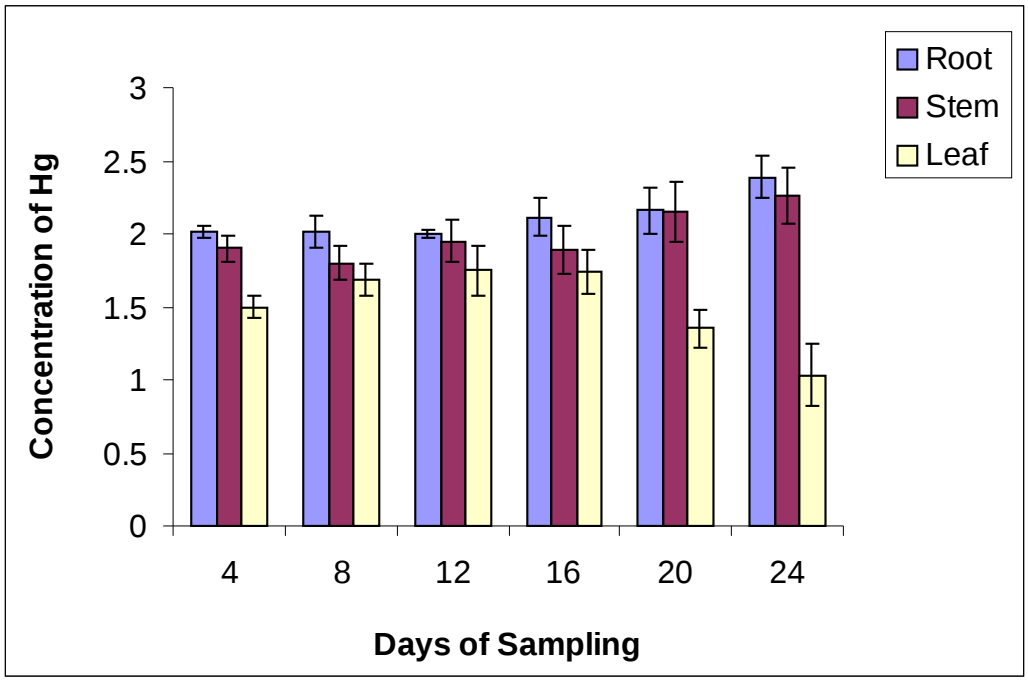


Fig. 31B: Accumulation of mercury in different plant parts of *Centella asiatica* treated with a combination of mercury and triadimefon

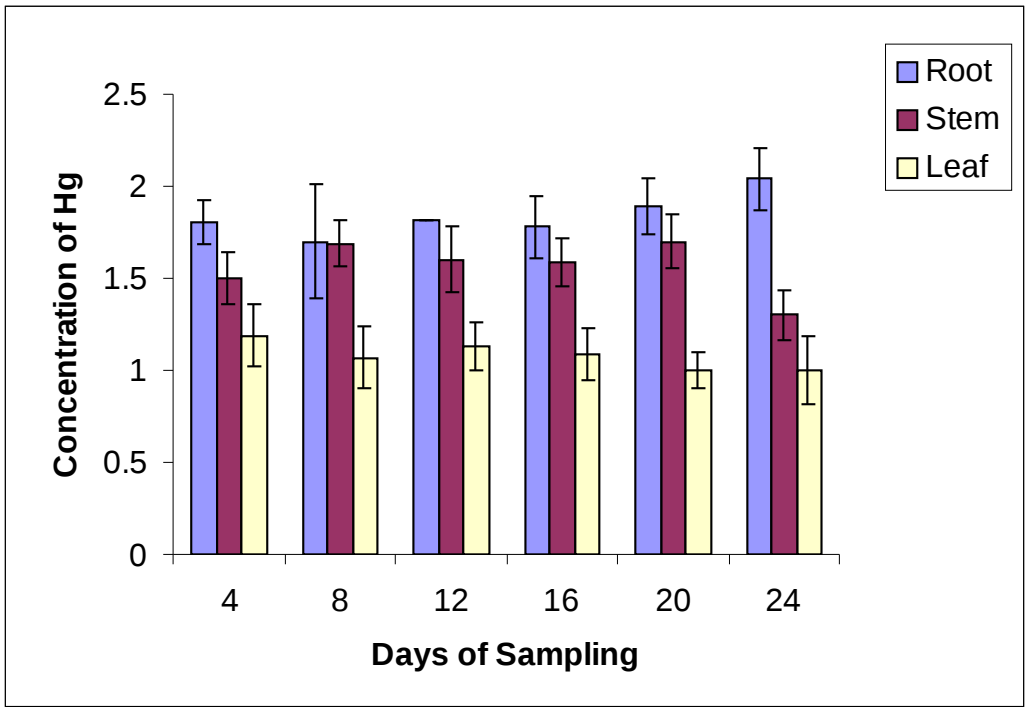


Table 21: Accumulation of mercury in different plant parts of *Centella asiatica* by additional application of mercury and a combination of mercury and triadimefon

Treatment	Plant parts	Intervals in days						
		0	**4**	**8**	**12**	**16**	**20**	**24**
		Concentration of Hg						
		0mg	**9.0mg**	**9.6 mg**	**10.2mg**	**10.8mg**	**11.4mg**	**12.0mg**
Hg	Root	NA	2.01 ± 0.04	2.31 ± 0.12	2.69 ± 0.22	2.81 ± 0.17	3.01 ± 0.21	3.13 ± 0.09
	Stem	NA	1.90 ± 0.09	1.91 ± 0.19	2.16 ± 0.19	2.49 ± 0.13	2.36 ± 0.19	2.49 ± 0.11
	Leaf	NA	1.50 ± 0.08	1.46 ± 0.17	1.58 ± 0.14	1.65 ± 0.14	1.78 ± 0.13	1.90 ± 0.21
Hg + Tri	Root	NA	1.80 ± 0.12	2.00 ± 0.15	2.09 ± 0.24	2.10 ± 0.20	2.47 ± 0.18	2.49 ± 0.20
	Stem	NA	1.50 ± 0.14	1.19 ± 0.09	1.26 ± 0.21	1.61 ± 0.17	1.81 ± 0.09	1.95 ± 0.17
	Leaf	NA	1.19 ± 0.17	1.13 ± 0.11	1.17 ± 0.18	1.30 ± 0.19	1.41 ± 0.09	1.50 ± 0.14

Fig. 32A: Accumulation of mercury in different plant parts of *Centella asiatica* by additional application of mercury

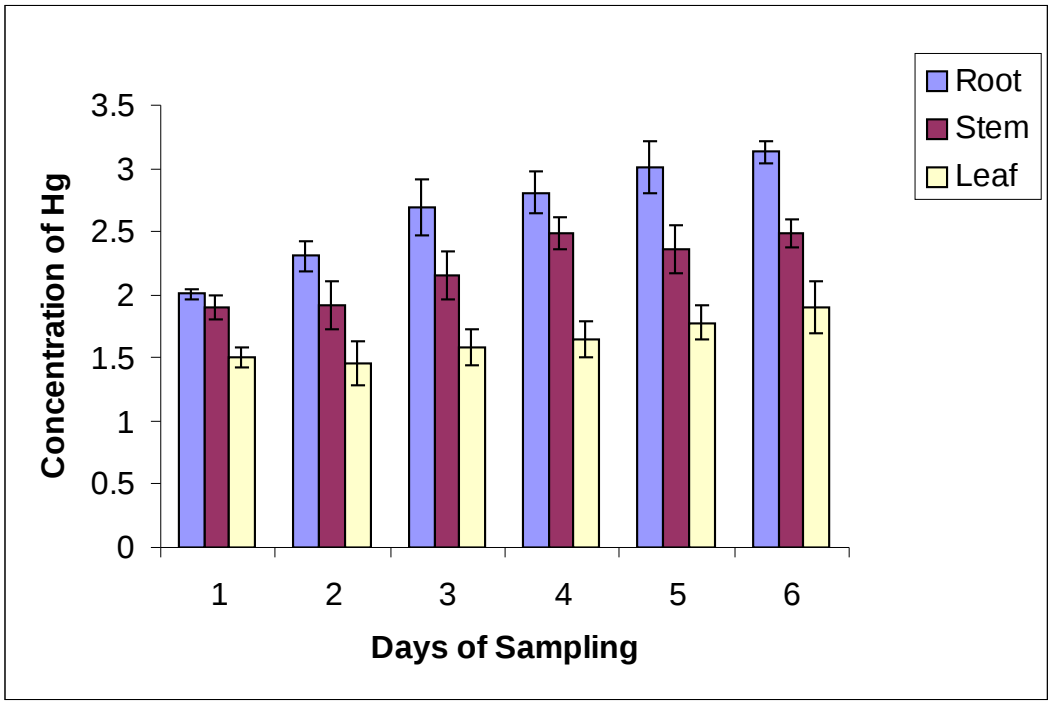


Fig. 32B: Accumulation of mercury in different plant parts of *Centella asiatica* by additional application of mercury and triadimefon

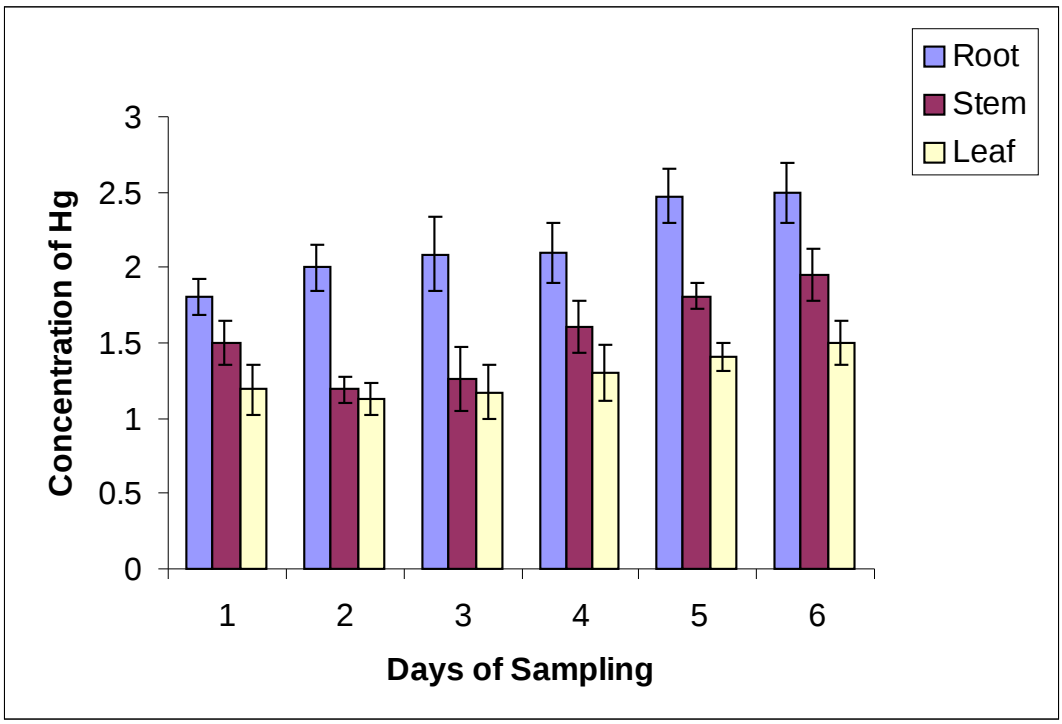


Table 22: Accumulation of mercury in *Centella asiatica* by the application of mercury and a combination of mercury and triadimefon in relation to the availability and loss during growth (mg/whole plant)

Treatment	Mercury	Intervals in days					
		4	**8**	**12**	**16**	**20**	**24**
Hg (9mg of Hg)	A	5.41 (60.1)	5.50 (61.1)	5.75 (63.9)	5.74 (63.8)	5.66 (62.9)	5.68 (63.1)
	R	3.27 (36.3)	3.05 (33.9)	2.90 (32.2)	2.60 (28.9)	2.43 (27.0)	2.39 (26.6)
	L	0.32 (3.6)	0.45 (5.0)	0.35 (3.9)	0.66 (7.3)	0.91 (10.1)	0.93 (10.3)
Hg + Tri (9mg of Hg)	A	4.49 (49.9)	4.46 (49.6)	4.54 (50.4)	4.46 (49.6)	4.59 (51.0)	4.64 (51.6)
	R	4.09 (45.5)	3.90 (43.3)	4.01 (44.6)	3.88 (43.1)	3.69 (41.0)	3.82 (42.4)
	L	0.42 (4.6)	0.64 (7.1)	0.45 (5.0)	0.66 (7.3)	0.72 (8.0)	0.54 (6.0)

Values in the parenthesis represents percentage distribution

A - Total accumulation in plants (mg/ whole tissue)

R - Residual content (mg) present in the medium during growth

L - Quantity (mg) lost during growth

Fig. 33A: Accumulation of mercury in *Centella asiatica* plants treated with mercury in relation to the availability and loss

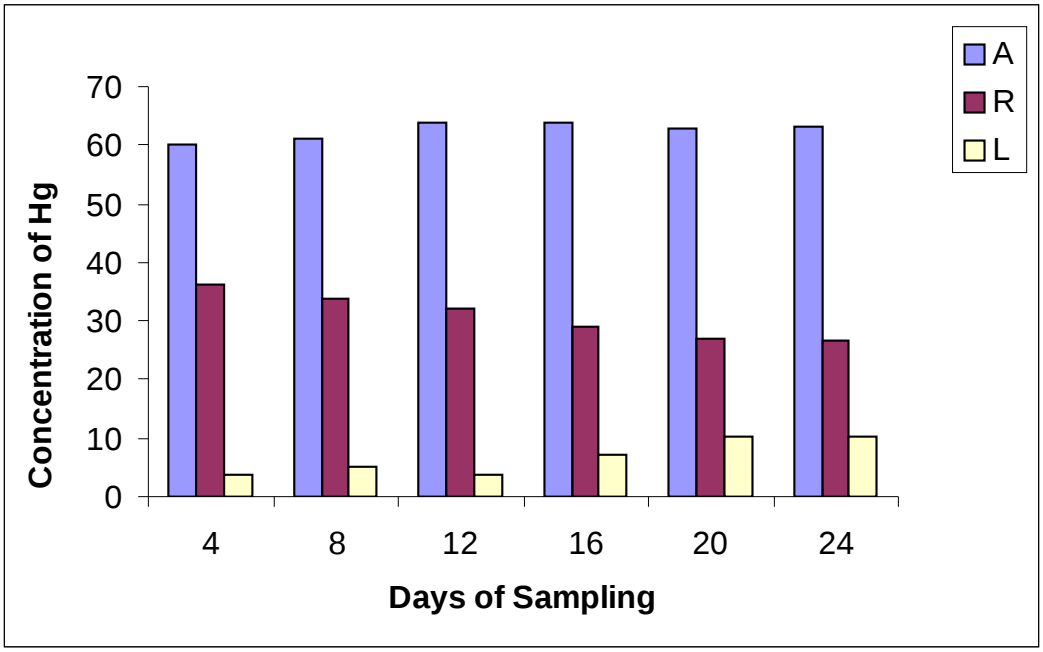
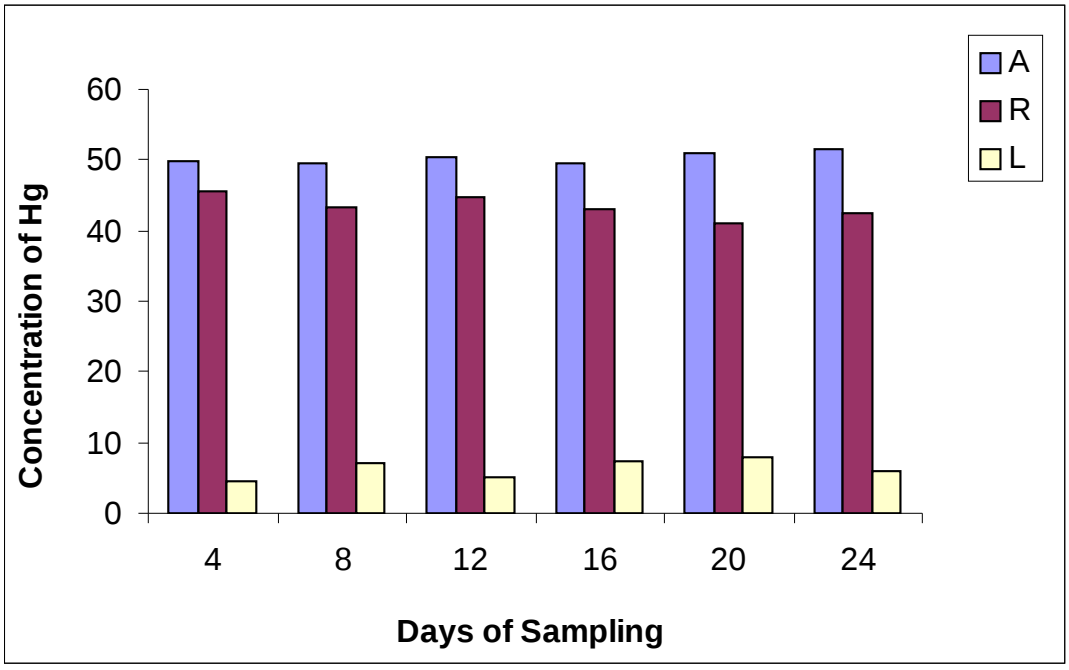


Fig. 33B: Accumulation of mercury in *Centella asiatica* plants treated with a combination of mercury and triadimefon in relation to the availability and loss.



A – Total accumulation in plants (mg/whole tissue)
R – Residual content (mg) present in medium during growth
L – Quantity (mg) lost during growth

Table 23: Accumulation of mercury in *Centella asiatica* by additional application of mercury and a combination of mercury and triadimefon in relation to the availability and loss

Treatment	Mercury	Intervals in days					
		4	**8**	**12**	**16**	**20**	**24**
		Concentration of Hg					
		9.0mg	**9.6 mg**	**10.2mg**	**10.8mg**	**11.4mg**	**12.0mg**
Hg	A	5.41 (60.1)	5.68 (59.17)	5.73 (56.18)	6.95 (64.4)	7.15 (62.7)	7.52 (62.7)
	R	3.27 (36.3)	3.71 (38.7)	3.99 (39.12)	3.67 (34.0)	3.50 (30.7)	3.38 (28.2)
	L	0.32 (3.6)	0.21 (2.20)	0.48 (4.7)	0.18 (1.7)	0.75 (6.6)	1.1 (9.2)
Hg + Tri	A	4.49 (49.9)	4.32 (45.0)	4.52 (44.3)	5.01 (46.4)	5.69 (49.9)	5.94 (49.5)
	R	4.09 (45.5)	4.93 (51.4)	5.01 (49.0)	5.37 (49.7)	5.50 (48.3)	5.67 (47.3)
	L	0.42 (4.6)	0.35 (3.7)	0.67 (6.6)	0.42 (3.9)	0.21 (1.8)	0.39 (3.3)

Values in the parenthesis represents percentage distribution

A - Total accumulation in plants (mg/ whole tissue)

R - Residual Content (mg) present in the medium during growth

L - Quantity (mg) lost during growth

Fig. 34A: Accumulation of mercury in *Centella asiatica* plants by additional application of mercury

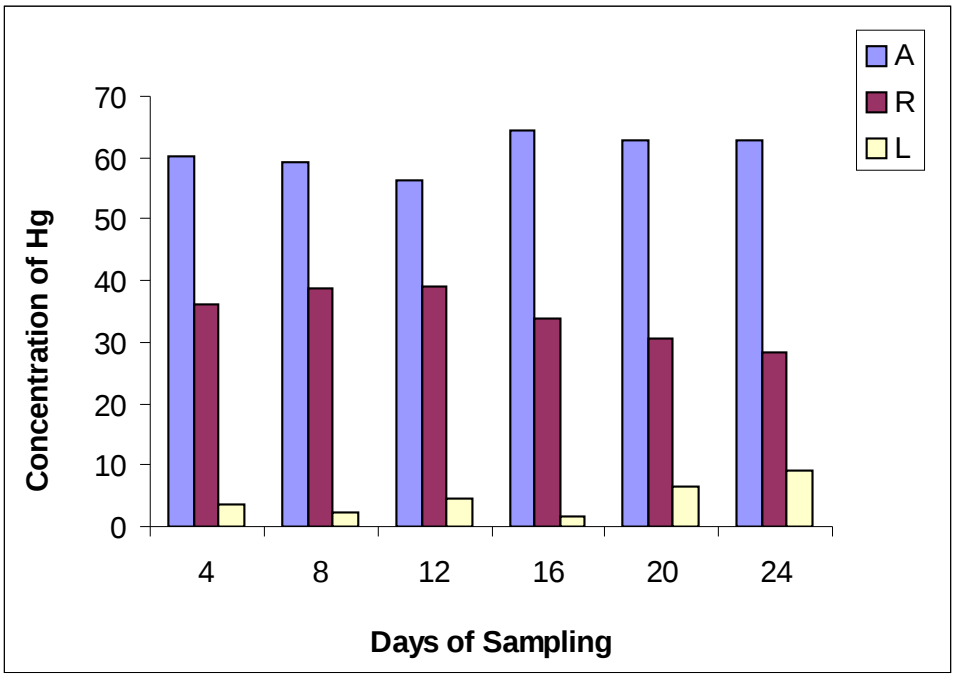


Fig. 34B: Accumulation of mercury in *Centella asiatica* plants treated with additional application of a combination of mercury and triadimefon

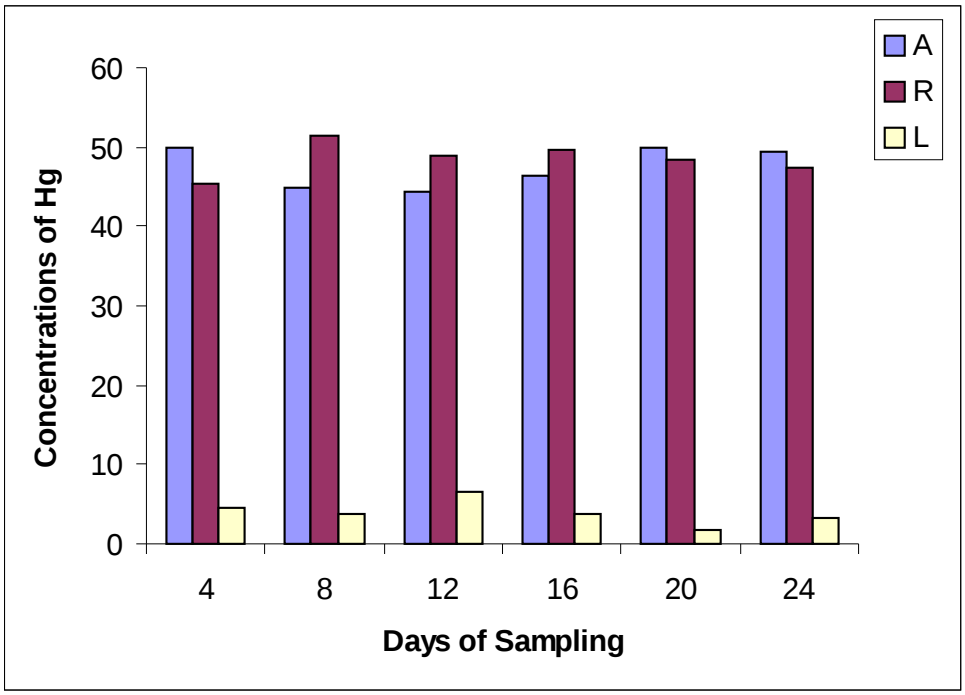


Table 24: Effect of mercury and a combination of mercury and triadimefon on BCF and TF in *Centella asiatica*

	Treatments (9mg of Hg)	**Intervals in days**					
		4	**8**	**12**	**16**	**20**	**24**
Hg	BCF	0.22	0.22	0.22	0.23	0.24	0.27
	TF	1.69	1.74	1.85	1.72	1.62	1.38
Hg + Tri	BCF	0.20	0.19	0.20	0.20	0.21	0.23
	TF	1.49	1.62	1.51	1.51	1.43	1.13

Ref: Yoon *et al.* (2006)

BCF Bio Concentration Factor (Accumulation ratio of growth medium to root)
TF Trans Location Factor (Accumulation ratio of root to shoot)

Fig. 35: Effect of mercury and a combination of mercury and triadimefon on BCF and TF in *Centella asiatica*

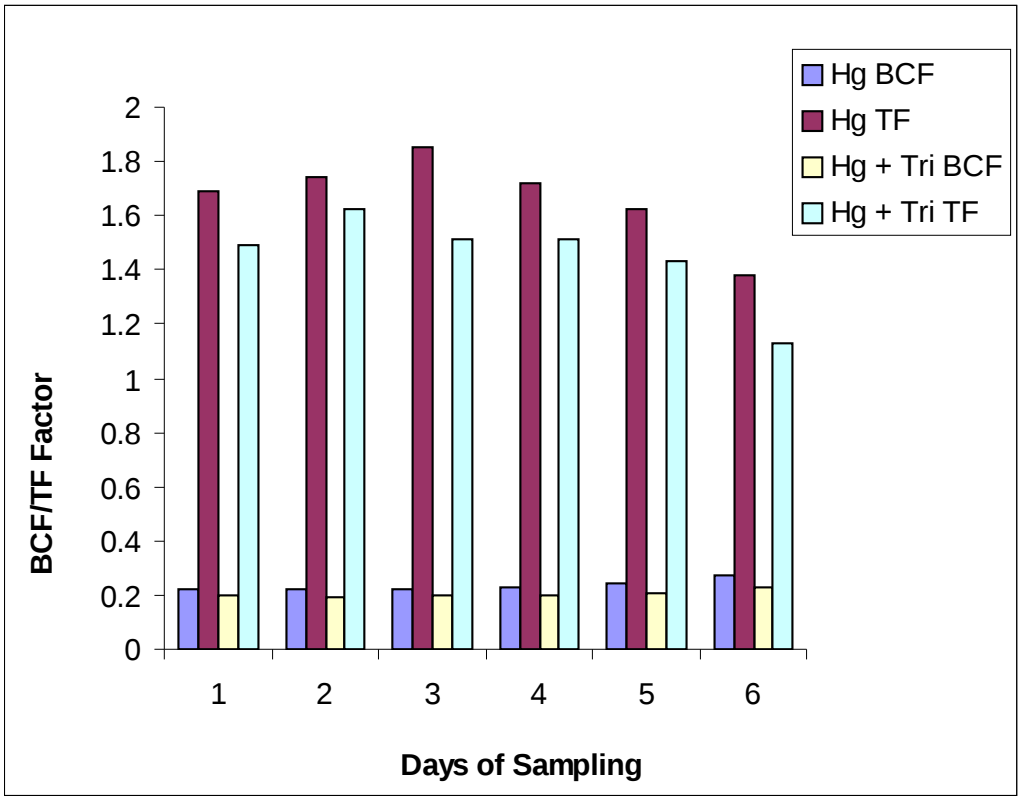


Table 25: Effect of additional application of mercury and a combination of mercury and triadimefon on BCF and TF in *Centella asiatica*

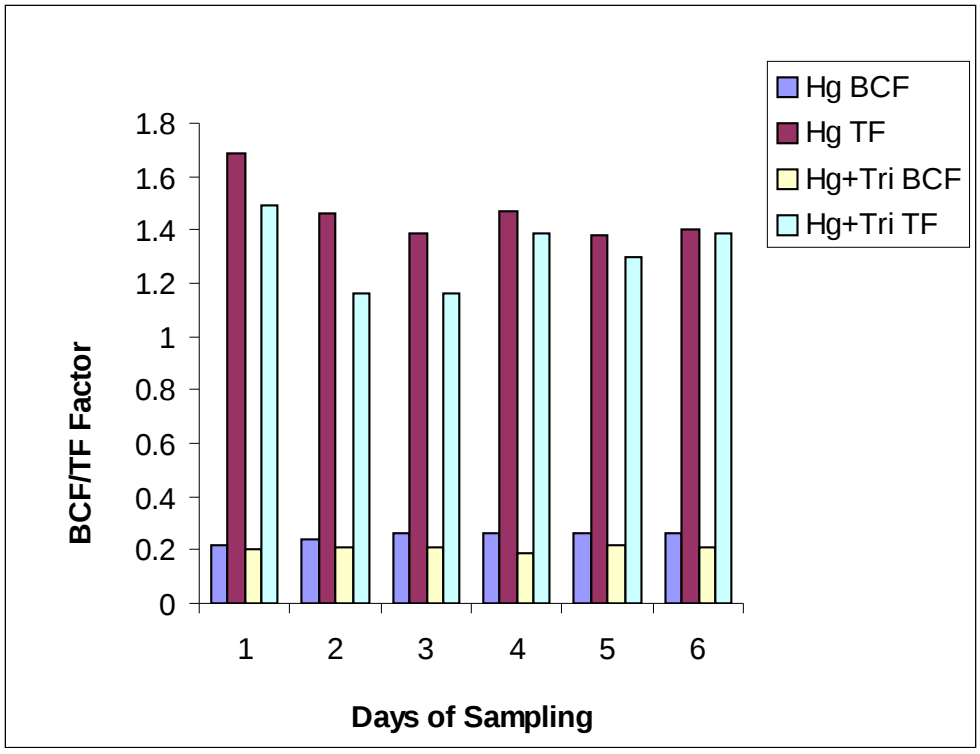
	Treatments	**Intervals in days**					
		4	**8**	**12**	**16**	**20**	**24**
		Concentration of Hg					
		9.0mg	**9.6 mg**	**10.2mg**	**10.8mg**	**11.4mg**	**12.0mg**
Hg	BCF	0.22	0.24	0.26	0.26	0.26	0.26
	TF	1.69	1.46	1.39	1.47	1.38	1.40
Hg + Tri	BCF	0.20	0.21	0.21	0.19	0.22	0.21
	TF	1.49	1.16	1.16	1.39	1.30	1.39

Ref: Yoon *et al.* (2006)

BCF Bio Concentration Factor (Accumulation ratio of growth medium to root)

TF Trans Location Factor (Accumulation ratio of root to shoot)

Fig. 36: Effect of additional application of mercury and a combination of mercury and triadimefon on BCF and TF in *Centella asiatica*



DISCUSSION

Screening experiments with different concentrations of mercury to impart visible toxic symptoms on *Centella asiatica* grown in Hoagland nutrient solution artificially contaminated with $HgCl_2$ revealed that treatment with 15 µM $HgCl_2$ resulted in exhibiting about fifty percent growth retardation. According to Foy *et al.* (1978) sensitivity/ tolerance of plants towards heavy metals vary from species to species and metal to metal and for the present investigation on the effect of heavy metal toxicity, optimal concentrations are being selected based on trial experiment with various concentrations and/or grade, which exhibit about 50% growth retardation. In *C. asiatica* inhibition of plant growth due to mercury toxicity in general was shown by the parameters including growth in root length (Table 2), runner length (Table 3), petiolar length (Table 4) and leaf area (Table 5). These observations are in consistent with the findings of Shaw (1995), who reported inhibition of root growth in *Phaseolus aureus* treated with 0.5 – 20 µM mercury.

Inhibitory role of mercury on plant growth and development has already been reported in *Cyperus rotundus* (Lenka *et al*., 1993), *Sesamum* sps. (Singh *et al*., 1994), *Raphanus sativus* (Khanna and Rai, 1995), *Rubia tinctorum* (Maitani *et al*., 1996) and *Chromolaena odorata* (Velasco-

Alinsug *et al*., 2005). Suszcynsky and Shann (1995) observed growth inhibition in tobacco plant root and shoot as mercury was applied either as volatile or ionic forms.

According to Orcutt and Nilsen (2000) a critically toxic level of mercury in most plants to affect growth is considered to be 1 – 8 ppm. Mercury has been reported to inhibit growth in many sensitive plants like *Raphanus sativus* (Khanna and Rai, 1995) at 0.5 – 1 ppm and *Phaseolus aureus* at 0.5-20 µM (Shaw, 1995). But mercury tolerant plants like *Chloris barbata* exhibited inhibition of root growth only at 500 μgl^{-1} and *Cyperus rotundus* at 100 μgl^{-} (Lenka *et al*., 1993).

Studies on the effect of mercury on plants revealed that treatment of 5-10µM $HgCl_2$ on *Pisum sativum* (Beauford *et al*., 1977) and 1-2µM $Hg(NO_3)_2$ on *Chromolaena odorata* (Velasco-Alinsug *et al*., 2005) are effective not only in inducing growth retardation but also considerable bioaccumulation of mercury was observed in those plants.

In *C. asiatica* the root system appear as “fibrous”, since a tuft of small roots originate from the node. In the present study the rooted cuttings were subjected to the treatments and length of roots and runner of plants treated with mercury showed very slow growth upto 24^{th} day of treatment compared to the control plants (Table 2 & 3; Fig 1 & 2). According to Wong and Bradshaw (1982) the growth retardation in the root is considered to be the

most rapid response to toxic concentration of heavy metals, and frequently used in many tolerance tests of plants. Wilkins (1978) opined that primary toxic effect of heavy metals on plant growth is inhibition of root growth. According to the author tolerance index is an ideal strategy to measure the degree of tolerance of plants subjected to metal toxicity.

Due to the inhibitory effect of mercury, petiolar length of the *C. asiatica* plants showed slightly reduced values compared to the control. Significant reduction of leaf area is observed during the first interval (4 days) and further growth also is very low compared to that of the control plants (Table 5). Toxic effect of mercury in the growth of leaves has already been reported in *Abelmoschus esculentus* (Ganesan and Manoharan, 1983).

Tolerance index values calculated on the basis of root length ratio of experimental and control do not show much variation in *C. asiatica* because the root system appears as a tuft of “fibrous like” and the elongation growth is very limited and so root length cannot be taken as criterion for tolerance index calculation (Table 6; Fig. 5A). Runner length also exhibited only slight variation in the net tolerance index (Fig. 5B). However, leaf area is found to be adversely affected by the treatment with $HgCl_2$ and hence the tolerance index values exhibit significant variation between experimental and control plants (Fig. 5C). The tolerance index values expressed on the leaf area basis is significantly reduced in the plants under mercury toxicity.

Stomatal index values of upper and lower epidermises of *C. asiatica* do not show significant variation in the control plants. However, increased values are shown by lower epidermis. In the plants treated with mercuric chloride a significant increase is observed in the stomatal indices of lower epidermis, whereas values of upper epidermis remain almost unchanged (Table 7; Fig. 6 A & 6 B). Effect of heavy metals on plants like *Arabidopsis thaliana* has been shown to render stomatal conductance by osmoregulation of guard cell-water relations (Perfus-Barbeoch *et al.*, 2002). In *C. asiatica* increased stomatal index values due to mercury treatment may induce enhanced transpiration rate and/ or interfere with bioaccumulation potential and / or phytovolatilization process of mercury in the leaves. This aspect will be discussed in detail in the bioaccumulation part. Reduced stomatal index in the leaves of plants treated with cadmium has been reported in plants, which are not accumulators of this metal (Prasad, 1995). Contradictory to this view, *C. asiatica* shows increased stomatal index and hence the stomatal index and heavy metal accumulation are found to be related each other.

Histochemical studies showed that in the roots of plants treated with mercury the outer layer of cortex and xylem vessels are found filled with many orange red spots, which are absent in control (Fig. 7). These deposits are found to be complexes of mercury as reported in plants like *Pisum sativum* (Beauford *et al*., 1977). Velasco-Alinsug *et al*. (2005) reported that in *Chromolaena odorata* treated with mercuric nitrate granular dark deposits

were present in the piliferous layer of roots. In *C. asiatica* the disappearance of these stained spots in the stem tissue are very clear on 24th day (Fig. 12), which may probably be due to the loss of mercury from the shoot system through stomata by phytovolatilization process as mentioned earlier.

In the stem of plants treated with mercury there are orange spots in the xylem vessels and cortical cells upto 12^{th} day of treatments and development of sclerenchymatous bundle cap consisting of thick and compact cells (Fig. 10, 11 & 12). Even though cell wall thickness was not evident in the cells of hypodermis and cortex, sclerenchymatous bundle sheath cells exhibited more thickness in the plants treated with mercury compared to the control. More or less similar observation was made in *Triticum aestivum* treated with 0.5-2mM $HgCl_2$ resulted in the reduction of stem diameter and vascular bundles (Setia *et al*., 1994).

In plants treated with mercury the increase in biomass was very slow and gradual compared to the control (Table 8, Fig. 16A). The stem and leaf tissues also showed the same trend (Fig. 16B & 16C). Significant reduction of biomass has been reported as an important effect of heavy metal stress (Kumar *et al*., 1995). *Triticum aestivum* plants treated with mercury exhibited retardation of root and shoot growth, which resulting in decreased dry weight of entire seedling (Iqbal and Majeed, 1991). According to Linger *et al*. (2005)

Cannabis sativa plants cultivated in soil contaminated with cadmium exhibited a significant reduction of biomass.

Chlorophyll content is often measured as a parameter in order to assess the impact of environmental stresses on plants, since the changes of pigments are linked with visual symptoms of growth disorders and photosynthetic productivity (Parekh *et al*., 1990). In *C. asiatica* under mercuric chloride stress chlorophyll a, chlorophyll b and total chlorophyll showed significant reduction compared to the control (Table 11; Fig. 19A to 19E). More or less similar observation has been reported in *Bacopa monnieri* treated with different concentrations of mercury for different periods (Sinha *et al*., 1996; Hussain, 2007) according to whom reduction of chlorophyll is an important effect of mercury toxicity. Chlorophyll a/b ratio of control and plants treated with mercury remained almost same throughout the developmental period under $HgCl_2$ stress (Table 12; Fig. 20). Inhibition of chlorophyll synthesis has been reported in water plants by various authors (Kupper *et al*., 1998; Mysliwa-Kurdziel and Strzalka, 2002). Mercury is known to interact with light harvest proteins of chlorophyll in spinach leaves (Ahmed and Tajmir-Riahi, 1993). According to Oncel *et al*. (2000) in wheat treated with heavy metals, total chlorophyll content was decreased to 50-70% and this reduction might be the result of inhibition of enzymes responsible for chlorophyll biosynthesis. Hyper-accumulation of heavy metals also interfere with chlorophyll synthesis in plants (Stiborova *et al*., 1986).

Toxic effect of mercury was shown by the significant variation in the distribution of metabolites during growth. Treatment with $HgCl_2$ resulted in a significant reduction of the protein content in the root tissue (Table 13). Slight reduction in the protein was observed in the stem tissues compared to that of control (Table 13; Fig. 21B). Inhibitory effect of mercury ions in metabolic reactions of protein synthesis is well established in living systems (Reddy and Prasad, 1992a; Orcutt and Nilsen, 2000; Cseh, 2002). Ions of mercury (Hg^{2+}) have high affinity for sulfhydryl (-SH) groups and consequently disturb almost every functions where critical proteins are involved (Clarkson, 1972). Mercury ion can bind to two sites of the protein molecule without deforming the chain or it may bind two neighbouring chain together. High concentration of mercury may lead to protein precipitation.

Leaf protein content was also reduced in *C. asiatica* plants treated with mercury compared to the control (Fig. 21C). Reduced protein content due to impaired synthesis in the presence of mercury and other heavy metals have been reported in many plants (Reddy and Prasad, 1992a; Prasad, 1997). Soluble protein fraction also showed almost similar trend in the control and mercury treated plants (Table 14). As a result of mercury treatment in *Triticum aestivum* reduction of soluble protein content was observed (Zhang *et al.*, 1989).

According to Ma (1998) mercury was found to inhibit water channels in the membranes of higher plant cells and due to membrane peroxidation, disruption of membranes, structural integrity and increased membrane permeability occur in ripe seedlings. Mercuric chloride is found to reduce the hydraulic conductivity of wheat root cells and rapidly depolarise the membrane potential of the root cells (Zhang and Tyerman, 1999).

Results of the PAGE studies revealed that the protein profile of *C. asiatica* plants treated with mercury showed only minor changes compared to the control plants (Fig. 23, 24 & 25). Appearance of new bands was evident only in the stem tissues. The new bands may be representing phytochelatins, which are related to metal toxicity. Synthesis of new proteins such as phytochelatins is known to sequester the metal toxicity in plants (Rauser, 1987; Reddy and Prasad, 1990; Leopold *et al*., 1999; Kubota *et al*., 2000). However in *C. asiatica* specific protein are not much observed in plants treated with mercury.

An increase in the distribution of total free amino acid exhibited in the root tissues of plants treated with mercuric chloride (Table 15) is found to presumably be due to impaired protein synthesis in the presence of Hg^{2+} ions. Bishnoy *et al.* (1993) reported that mercury is an inhibitor of amino acid mobilization resulting in a transient accumulation in plants. In the stem tissue of *C. asiatica* also significant increase of total free amino acid was occurred

in the plants treated with mercury (Fig. 26B). This observation is corroborated with the reports in *Salvinia natans* (Sen and Mondel, 1987), where enhanced occurrence of free amino acid was observed. In the leaf tissue of *C. asiatica*, $HgCl_2$ treatment shows only insignificant change in free amino acid content up to 12^{th} day, there after significant increase is observed compared to that of control (Fig. 26C). The abundant occurrence of free amino acid may either be due to impaired protein synthesis in the presence of Hg^{2+} ions or synthesis of stress induced non-protein amino acid such as γ-aminobutyric acid (GABA), which is known to accumulate in stressed tissues (Heineke *et al.*, 1992; Breitkreuz and Shelp, 1995). Another possibility of increased amino acid content in *C. asiatica* may plausibly be due to enhanced proteolysis and accumulation of free amino acids as reported in rice seedlings treated with cadmium (Hsu and Kao, 2003).

Proline content of root tissue of plants treated with $HgCl_2$ was significantly higher than the control plants (Table 16). In the present study the proline content of stem and leaf tissues also showed increase in the plants treated with mercury (Fig. 27B & 27C). Proline accumulation is a well established characteristic feature of heavy metal stress in plants (Alia and Saradhi, 1991; Bassi and Sharma, 1993). According to Khanna and Rai (1995) the stress induced accumulations of aminoacids in general and proline in particular in *Raphanus sativus* has significant effect on uptake or ionic

balance, which in turn would help the plants in mitigating the stress. Alia *et al.* (2001) suggested that proline accumulation might be involved in the protection of plants against heavy metal stress. Increase in total free amino acid associated with increased proline content is found to be resultant to the proteolysis and impaired translocation of the products under heavy metal stress.

Mercury treatment resulted in a slightly increased phenolic content in the root tissue of *C. asiatica* (Table 17; Fig. 28A). In stem tissues negligible changes occurred in the total phenolic content compared to the control (Fig. 28B). But in the leaf tissue mercury treatment resulted in a significant increase of phenolic content compared to the control (Table 17; Fig. 28C). The increased phenolic content may be involved in the antioxidant activity to reduce mercury stress and / or induce growth retardation. According to Harbone (1980) phenolics are having a potentially inhibiting effect on growth as they are capable of auxin destruction by augmenting indole acetic acid oxidase. Phenolics may also interact with other hormones by synergism or inhibition. Role of phenolics with antioxidative status under dehydration stress have been reported in *Ramonda serbica* (Sgherri *et al*., 2004). Recent research reports highlight the potential role of phenolics as effective antioxidants against heavy metal toxicity (Michalak, 2006). According to this author phenolic synthesis is induced on exposure to stress and the antioxidant action resides mainly in their chemical structure.

Toxicity of mercury manifested in *C. asiatica* as reduced protein synthesis and increased free amino acids inclusive of proline and phenolics are found to be involved in a concerted process because the metabolic pathways of these biomolecules are inter related in such a way that the biosynthesis of phenolics begins with the aromatic amino acids phenyl alanine, tyrosine or tryptophan by a sequence of reactions and phenyl alanine ammonia lyase (PAL) is the key enzyme. This enzyme controls the diversion of carbon from the metabolism of protein into the production of phenolics. According to Santiago *et al.* (2002) PAL is generally stimulated in plant tissues exposed to heavy metal stress. Nevertheless, the specific role of this enzyme in the scenario of the metabolism of free amino acid, proline and phenolics in *C. asiatica* is yet to be investigated.

It is well known that excessive heavy metal induce the generation of reactive oxygen species (ROS) in plants and for the break down of the molecules scavenging enzymes including peroxidase, SOD and catalase are synthesised. Increase in the activity of guaiacol peroxidase in *C. asiatica* is found to be a general characteristic of mercury toxicity (Table 18 & 19). Anti-oxidant enzymes become active under stressfull condition as consequences of heavy metal toxicity. Increased peroxidase and polyphenol oxidase activities have been reported in *Azolla* exposed to mercury toxicity (Jana and Choudhury, 1982) and in *Oryza sativa* (Mishra and Choudhury, 1996). Zhang *et al.* (1989) reported that in wheat seedlings two peroxidase enzymes

were observed due to treatment with $HgCl_2$. According to Ma (1998) plants stressed due to the presence of mercury show increased levels of antioxidant enzymes like peroxidase and superoxide dismutase but at higher levels of mercury content the protection effect is disappeared. Induction of peroxidase activity under heavy metal stress has also been reported in various plants such as rye grass (Piquery *et al.,* 2000), spruce (Radotic *et al.,* 2000), *Bacopa monnieri* (Singh *et al.,* 2006), *Brassica juncea* and *Nicotiana tabaccum* (Wang *et al.,* 2008).

Guaiacol peroxidase activity was almost doubled in the root tissue of plants treated with mercury (Table 18). This observation is similar to that reported in *Brassica napus* subjected to metal toxicity exhibiting a significant increase in the peroxidase activity in root where maximum metal accumulation was occurred (Hosseini *et al.,* 2007). Ma (1998) suggested that mercury-stressed (1-10μg l^{-1}) plant cells showed increased activities of antioxidants like superoxide dismutase and catalase in varying degrees and rendering a positive endogenous protective effect. However, the protective effects disappear at higher levels of mercury (50 mg l^{-1}). Increased activities of peroxidases have been considered as a defensive mechanism against mercury toxicity and were reported in *Phaseolus vulgaris* (Parmar *et al*., 2002; Parmar and Chanda, 2005).

A linear increase of peroxidase activity occurred in the present study (Table 18 & 19) as a result of $HgCl_2$ treatment in *C. asiatica* up to 24 days growth, which coincides with stress induced signs of senescence reveals that enzymatic anti oxidation capacity is very feeble in *C. asiatica.* According to Wang *et al.* (2008) metal accumulator plants are equipped with antioxidative defence against heavy metal toxicity, where as in intolerant plants this enzyme cannot fully scavenge the reactive oxygen species generated by heavy metals. Hence, the protective role of peroxidase enzyme against mercury – stress in *C. asiatica* is very limited.

In addition to ROS scavenging potential of peroxidase in *C. asiatica* plants the role of this enzyme in the lignification of cell walls under stressed condition as suggested by Ferrara *et al.* (1989) cannot be ruled out because sclerenchymatous bundle sheath development is observed in the tissues of plants treated with mercury.

Quantitative analysis of mercury content by Atomic Absorption Spectrophotometry showed that accumulation of mercury was maximum in root tissues followed by stem and leaves in plants treated with $HgCl_2$ (Table 20 & 21). The accumulation of mercury in root and stem was increasing gradually but insignificantly during the growth up to 24 days (Fig. 31A). But in the leaves, the quantity of mercury remains unchanged during growth up to 24^{th} day. Studies on bioaccumulation of mercury in plants growing in

naturally polluted soil or cultivated in nutrient solution artificially contaminated with mercury compound reveal that no plants are found to be hyper accumulator of mercury (Henry, 2000; Orcutt and Nilsen, 2000).

Shaw and Panigrahi (1986) and Lenka *et al.* (1992) reported that in plant species growing around chlor-alkali factory of India, accumulated maximum quantity of mercury in leaves followed by stem and root. A significant correlation was noted between the mercury concentration of the soil and accumulation in the plant tissues as well as variation between different tissues. In an elaborate study on *Elodea densa* Maury-Brachet *et al*. (1990) showed that when the plants were cultivated in a natural sediment enriched with 4mg kg^{-1} CH_3HgCl, high accumulation of mercury was observed in the leaves, stem and roots. In long - term experiments, roots' absorption was the dominating factor of mercury accumulation, the leaves being the principal organ of storage. In fresh water aquatic plants, higher concentration of mercury accumulation in plants indicated proportional increase in the mercury level in the water (Mortimer, 1985; Tabbada *et al*., 1989).

Even though plants in general are not hyper accumulator of mercury (Henry, 2000; Raskin and Ensley, 2000), considerable quantity of this metal is reported to be accumulated in *Lycopersicum esculentum* (Zhou and Zhao, 1992), *Lolium* sps. (Singh and Jeng,1993), *Rosemarium officinalis*

(Barghigiani and Ristori, 1995) and *Chromolaena odorata* (Velasco-Alinsug *et al*., 2005). In most of these plants the amount of mercury entered the tissues are directly related to its concentration in the soil.

Generally there is a tendency for mercury to accumulate more in roots indicating that the roots serve as a barrier to mercury uptake (Gracey and Stewart, 1974) and the amount of mercury retained in the root is about 20 times that observed in the shoots (Lindberg *et al.*, 1979).

In the present investigation maximum accumulation of mercury was observed in the root tissue followed by stem and leaf tissues of *C. asiatica* (Table 20 & 21). Roots of aquatic plants like *Eriocaulon septangulare* cultivated in mercury contaminated medium showed maximum accumulation in the roots (Coquery and Welbourn, 1994). Similarly, *Eichhornia, Typha, Sparganium* and *Menyanthes* are known to absorb and accumulate mercury in their roots (Robichaud *et al.*, 1995). Maximum accumulation of mercury has been reported in roots of *Bacopa monnieri* cultivated in nutrient medium artificially contaminated with $HgCl_2$ (Hussain, 2007) and also in *Chromolaena odorata* subjected to treatment with mercuric nitrate (Velasco-Alinsug *et al*., 2005).

In the mercury treated plants, where additional doses (0.6mg of mercury at each interval) were applied, a gradual and significant ($p < 0.01$) increase was observed in the root, stem and leaf tissues during growth

(Table 21; Fig. 32 A & 32 B). This increase was promotional to the quantity of additional doses.

After harvest of the plants for bioaccumulation studies, the mercury content present is the residual nutrient solution showed that considerable quantity of this metal is lost compared to the total accumulation and content present in the residual solution and the loss is about 10% of the total added to the growth medium (Table 22). In the experiments where additional quantity of $HgCl_2$ was added the accumulation pattern followed more or less the same pattern as in the single application (Table 23). As mentioned earlier phytovolatalization of mercury occurs to considerable extend in some plants, growing in polluted area (Orcutt and Nilsen, 2000; Pilon-Smits, 2005) and in transgenic plants, which are genetically modified by introducing a bacterial gene coding mercury reductase (Rugh *et al*., 1996) to make the plant efficient to reduce ionic and methylated forms of mercury to the elemental mercury in order to volatalize to the atmosphere. Hammer *et al*. (1988) reported that mercury is released from sediments and aquatic plants like *Ceratophyllum demersum* and *Anodonta gardis*. Similarly, an aquatic plant *Posidonia oceanica* accumulates mercury from sediments and this plant is considered as an indicator of pollution (Ferrara *et al*., 1989). Baskin (2003) reported that mercury salts in the soil get reduced by biological and chemical reaction to metallic mercury or methylated form, which is volatalized and taken up by plants growing in the neighbourhood through leaves and this mode of entry is

found to be more efficient than absorption of Hg^{2+} ions through roots (Patra and Sharma, 2000).

Suszcynsky and Shann (1995) investigated bioaccumulation of mercury in tobacco by exposing the plants to mercury vapour through root and to ionic mercury (Hg^{2+}) and reported that plants exposed to mercury vapour accumulated the metal in the shoot with no movement to the roots, whereas root exposed plants showed the accumulation of mercury in the shoot. In *C. asiatica* translocation of mercury from roots to stem / shoot tissue is evident from the bioaccumulation pattern (Table 20 & 21). Histochemical localization of mercury as stained spots in the stem and leaves also reveals the translocation of mercury from roots and these results are in conformity with the observations in *Chromolaena odorata* in which localization of mercury complexes are present (Velasco - Alinsug *et al*., 2005).

The loss of mercury that presumed to occur after the growth for 24 days is comparatively low in the normal experiment and also when additional quantity of $HgCl_2$ was applied (Table 22 & 23). However, this loss in meagre amount can be attributed to the phytovolatalization in accordance with the view of Pilon –Smits (2005) according to whom phytovolatalization is the release of pollutants from the plant as gas or vapour and this process is considered as a cost effective method for phytoremediation.

A comprehensive study on the phytofiltration in plants such as *Brassica juncea*, *Phaseolous vulgaris* and *Vicia villosa* emphatically revealed that when expsed to environmental pollution with mercury, plants absorb and accumulate and/or volatalize mercury and comparative studies indicate that the accumulation process is specific to plant species. (Moreno *et al*., 2004, 2005a, b, c, 2008). According to Moreno *et al*., (2005 a) mercury accumulation pattern between root and shoot also vary in different species. The mercury accumulation patten of *Centella asiatica* is comparable with plants like *Phaseolou vulgaris, Brassica juncea* etc. since quantities accumulate in the root and shoot vary and volatalization or loss is meagre.

According to Patra and Sharma (2000) absorption of organic and inorganic mercury forms from soil by plant is low and large increase in the soil-mercury level results only moderate increase in bioaccumulation in different parts of the plant. Nevertheless, in *C. asiatica* only very small quantity of mercury is found to lost by phytovolatalization because about 60% is accumulated in the plant body (Table 22 & 23). The loss of mercury by phytovolatalization can be directly correlated to the increased stomatal index of *C. asiatica* plants treated with $HgCl_2$ (Table 7). According to Patra and Sharma (2000) Hg^{2+} ions get reduced inside the plant to metallic mercury and get volatalized to the atmosphere and the vapour enter other plants through leaves. However, the formation of metallic mercury by reducing Hg^{2+} ions involves the reduction process and in higher plants such enzymes have not yet

been elucidated. However, studies are being conduced in bacteria for the elucidation of specific cytoplasmic enzyme mercuric reductase responsible for reduction to elemental mercury (Patra and Sharma, 2000).

For the analysis of bioaccumulation potential in comparison with the availability of mercury in the growth medium, the plants were grown in Hoagland solution containing a specific quantity of mercury and the estimation of mercury content accumulated in the plant (content/ plant part) and quantity of metal retained (residual) in the medium during the growth were recorded (Table 22 & 23).

As mentioned earlier about 60% of the total quantity of mercury added to the growth medium is found to be accumulated in the plant body and get distributed in the order: root > stem > leaves. This accumulation pattern remains unchanged even when additional $HgCl_2$ was added. In addition to the mild phytovolatalization process, *C. asiatica* plants are characterised as bioaccumulator of mercury (Table 22). But plants are not tolerant to high concentration of mercury, since they did not survive in $HgCl_2$ above 15μM concentration and hence are neither tolerant nor hyper accumulator of mercury.

Triadimefon (1-(4-Chlorphenoxy) -3, 3- dimethyl -1-(1-1-1, 2,4 Trizole -1-yl)-2-butanol) is a triazole derivative and very potent systemic fungicides (Ragsdale, 1977; Siegel, 1981) and is reported to exhibit plant

growth regulating property (Buchenauer and Rohner, 1981). Triadimefon protects plants from drought desiccation, chilling and ozone stresses (Fletcher and Hofstra, 1985). Even though triadimefon is known to be a growth promoting substance (Buchenauer and Rohner, 1981), it is also widely used as an agent, which antagonize the inhibitory effect of stressful substances such as drought (Asare-Boamah *et al*., 1986), sodium chloride (Panneerselvam *et al*., 1997; Gopi *et al*., 1998; 1999; Sujatha *et al*., 1999a; 1999b; Karikalan *et al*., 1999) and UVB radiation (Chouhan *et al*., 2007). In the present study an attempt was made to see the antagonistic effect of triadimefon on the plants by exposing the plants to a combination of $HgCl_2$ and triadimefon.

Morphological parameters such as runner length, petiolar length and leaf area remained unchanged due to triadimefon treatment in spite of the reported role of triadimefon as hormones for growth promotion processes as suggested by several authors (Buchenauer and Rohner, 1981). However, the inhibitory effect on runner length, leaf area, etc., imposed by mercury was significantly reversed by the treatment of a combination of $HgCl_2$ + triadimefon (Table 3 & 5). It has been established that triazole compounds have a characteristic role in antagonising the stressful effects in general and due to heavy metals in particular (Panneerselvam *et al*., 1997; Gopi *et al*., 1998; 1999; Sujatha *et al*., 1999a; 1999b; Karikalan *et al*., 1999). This antagnoistic effect of triadimefon to reverse the growth inhibition due to $HgCl_2$ or deranged metabolism in *C. ascatica* was highly significant in the case of leaf growth (Table 5).

The tolerance indices calculated on the basis of parameters like runner length, root length, leaf area etc., also showed involvement of triadimefon in reducing the stressful effect of mercury in *C. asiatica* (Table 6). This observation is in consonance with the reports on the role of triadimefon in wheat plants under heavy metal stress (Babu and Singh, 1992).

An important observation in the distribution of stomata in general and that of the lower epidermis in particular, was significant increase due to mercury treatment (Table 7). But the triadimefon alone did not show any affect on the stomatal index, whereas the enhancement of stomatal index in plants treated with $HgCl_2$ was slightly reduced in the combination treatment (Fig. 6A & 6B). This observation is an indirect evidence to show the role of stomata in the escape or volatalization of mercury from leaf as reported in *Bacopa monnieri* plants treated with $HgCl_2$ (Hussain, 2007) since accumulation of mercury in the combination treatment (Hg+ Triadimefon) is comparatively lower than that of $HgCl_2$ treatment (Table 20). According to Sairam *et al*. (1989) and Fletcher *et al*. (2000) triadimefon treatment increased stomatal diffusive resistance and decreased transpiration in stressed plants. On the contrary in *C. asiatica* the distribution of stomatal index itself is controlled by triadimefon instead of inducing stomatal resistance.

In the root tissues of plants treated with mercury as well as a combination of mercury and triadimefon some stained spots are observed during histochemical studies (Fig. 7, 8 & 9). These spots (deposits) are found

to be complexes of mercury as reported in plants like *Pisum sativum* (Beauford *et al*., 1977). Similarly, in *Chromolaena odorata* treated with $HgCl_2$ granular dark deposits were observed in the piliferous layer of roots (Velasco- Alinsug *et al*., 2005).

Some spots are observed in the stem of plants treated with a combination of mercury and triadimefon during earlier intervals. But in the 24^{th} day of treatment such spots are not observed in the stem tissues of plants treated with mercury and mercury+ triadimefon (Fig. 10, 11 & 12) revealing the lack of absorption and/ or localization of mercury in the stem tissues presumably due to the inhibitory effect of triadimefon on mercury absorption.

The sections of leaves treated with mercury and mercury+ triadimefon showed thick xylem vessels compared to the plants treated with triadimefon and control plants (Fig. 13, 14 & 15). But stained spots, representing the presence of mercury were absent in the leaf tissues revealing the loss of mercury by phytovolatalization as mentioned earlier.

Biomass expressed on per plant basis showed a significant reduction in roots of plants treated with mercury, whereas treatment with triadimefon + mercury resulted in an enhancement of dry matter compared to the control (Table 8). Triadimefon treatment alone also showed significant increase. In this context the hormone-like activity to increase plant biomass(Fletcher *et al*., 2000) as well as the antagonistic effect to reduce the inhibitory effect of

mercury (Fletcher and Hofstra, 1985, Asare-Boamah *et al*., 1986) are found to be expressed by triadimefon in *C. asiatica*.

The inhibitory role of Hg^{2+}on protein synthesis was shown by reduced protein content in all the tissues (Table 13 & 14) and with the treatment of a combination of mercury + triadimefon the reduced protein synthesis was found reversed probably due to the role of triadimefon to check or inhibit the uptake of mercury because Hg^{2+} ions are well known inhibitor of protein synthesis. Triazoles, inclusive of triadimefon are very stable compound and hence catabolism is very slow in plants when applied to the plants (Davis and Curry, 1991). So the efficacy of triadimefon as growth regulator is continued and imposes protective influence on enzyme activity leading to normal metabolism. More or less similar results had been occurred in wheat plants treated with cadmium and exposed to triadimefon to reduce the toxic effect by this heavy metal on nitrogen metabolism (Babu and Singh, 1992).

In all parts of *C. asiatica* plant, enhanced accumulation of free amino acid was observed when treated with $HgCl_2$, but triadimefon did not impose any effect either singly or along with $HgCl_2$ (Table 15). Significantly increased content of proline in plants treated with $HgCl_2$ reveals the stressful condition of the plant, whereas triadimefon along with mercury did not affect proline content presumably due to the antagonistic effect of triadimefon on

the inhibitory role of Hg^{2+} ions, which induces proline synthesis as a protectant against stress (Table 16; 27A, 27B & 27C). However, a highly significant reduction of proline in the plants treated with triadimefon alone is found to be a characteristic of this triazole compound, the occurrence of which is essential as a protectant under a stressful condition (Fletcher and Hofstra, 1985), which coincides with proline accumulation in plant tissues. Sankhla *et al.* (1996) reported that stress induced changes such as proline level and lipid peroxiation are ameliorated by triazoles in the leaves of *Ziziphus* seedlings.

The effect of triadimefon on chlorophyll (a, b and total) distribution showed a slight stimulative effect than the control in the biosynthesis as reported in many plants (Fletcher *et al*., 2000), while mercury induced significant reduction of all forms of chlorophyll. In this context also the stimulatory as well as antagonistic effect of triadimefon are clearly exhibited.

Phenolics are more or less uniform in the root and stem tissues of all the treatments (Table 17). But the leaf shows significant changes with a maximum content in plants treated with mercury and minimum with triadimefon treatment, while intermediate quantity is present in control as well as a combination of mercury + triadimefon treatment revealing the protective role of triadimefon against mercury stress, which is otherwise ameliorated with abundant occurrence of phenolics.

Peroxidase activity is not at all affected by triadimefon treatment revealing the absence of any sort of stress in the tissues, whereas mercury treatment resulted in very high hike in the peroxidase activity (Table 18 &19). This abundant activity of peroxidase is directly related to the stress of mercury and in conformity with the well known role of this enzyme in antagonizing stressed conditions by scavenging highly reactive oxygen species as observed in *Phaseolus vulgaris* treated with heavy metal (Van Assche *et al*., 1988), *Oryza sativa* exposed to cadmium stress (Reddy and Prasad, 1992b). According to YiXian *et al.* (2000) peroxide induction is a general response of higher plants to the exposure of toxic level of Cd, Cu, Pb and Hg.

The treatment of mercury + triadimefon showed that peroxidase activity remained almost unchanged from 4th day onwards in all the tissues of plants compared to the mercury treatment (Fig. 29A). Detoxification of active oxygen species induced by stresses inclusive of heavy metal stress has been reported as an effect of triadimefon in plants (Fletcher *et al*., 2000). Similarly triadimefon affect activity of antioxidant enzymes in the detoxification process of reactive oxygen species (ROS) (Sankhla *et al*., 1992a; 1992b; 1997). The activity of guaiacol peroxidise in the plants treated with mercury and triadimefon (Table 18) is found to be insufficient to nullify the stress induced by mercury expressed as the enhanced activity of mercury toxicity.

According to Sankhla *et al.* (1996; 1997) in the absence of stress, thidiazuron, a triazole enhances the activity of ascorbate oxidase, peroxidase, catalase etc. The activities of these enzymes are conserved after stress imposed by heavy metals or any other stressful materials.

The specific activity of peroxidase was more both in plants treated with mercury as well as mercury + triadimefon (Fig. 30A) due to the significant reduction of protein content especially soluble proteins as described earlier.

When a comparison is made between the mercury accumulated in the whole plant (root + stem + leaf) of both the treatments (mercury and mercury + triadimefon), the plants treated with a combination of mercury and triadimefon showed a significantly reduced quantity of mercury accumulation in the root, stem and leaf tissues compared to those tissues of plants treated with mercury alone (Table 20 & 21). Hence, it is evident that mitigation of mercury toxicity in the presence of triadimefon induced the partial avoidance of mercury uptake. This observation is comparable to the restricted accumulation of toxic heavy metals in plants tolerant to those metals as opined by Fitz and Wenzel (2002). Puerner and Siegel (1972) proposed that the toxicity of mercuric chloride is mitigated or reduced when it is applied along with fluorescein in cucumber seedling.

The residual solution of Hg + triadimefon treatment showed more quantity of mercury, which remain unchanged during growth up to 24th day (Table 22). Hence, the loss of mercury was not significantly increased compared to the treatment of mercury alone, where the loss is gradually increased.

In the mercury treatment where additional doses are given, accumulation of mercury remains almost unchanged at all intervals as in single dose (Table 23). Here the residue contains mercury at a range of 36-28% from 4th to 24th days. In plants treated with a combination of mercury and triadimefon the residue contains more mercury content compared to the accumulation in the plants upto 16th day and there after remained uniform.

Yoon *et al*. (2006) proposed a pattern or scheme to asses the potential of plants to accumulate metals from soil or growth medium. According to those authors bioaccumulation factor (BCF) is defined as the ratio of metal concentration in the roots to that of soil and the ratio of metal concentration in the shoot to that of the root is called translocation factor (TF). The translocation factor represents the ability of plants to translocate metals from root to the shoot. Those authors further suggested that by comparing BCF and TF, the potential of different plants for metal absorption and translocation to the shoot can be assessed.

In *C. asiatica*, single treatment with mercury showed very low values of BCF during all the intervals revealing limited rate of absorption by the roots retaining much more quantity in the soil/ growth medium, whereas TF values are very high indicating low accumulation potential of root system compared to the shoot (stem + leaf). In other words, translocation of mercury from the root to the shoot is significant and this amount is distributed in the stem as well as leaves (Table 24).

A comparison of BCF and TF of mercury accumulation in *Centella* plants shows very low value of BCF, i.e., the ratio is less than one, revealing the lack of abundant accumulation in the plant body (Table 24 & 25). Nevertheless, TF values are more than one, exhibiting considerable translocation to the shoots retaining low amount of mercury in the root system compared to the shoot (stem +leaf). Almost same trend is observed in the samples of all the intervals.

Treatment of a combination of mercury and triadimefon also exhibited more or less similar values of BCF and TF (Table 24). But there occurred slight reduction of both BCF and TF values invariably in the whole samples of plants treated with mercury and triadimefon, revealing comparatively lesser accumulation in the roots than that of the plants treated with mercury alone.

Plants treated with mercury + triadimefon, BCF values are slightly reduced presumably due to retarded uptake of mercury to the root system. TF values also are comparatively lower than that of the plants treated with mercury confirming once again the antagonistic role of triadimefon in the absorption of mercury ions.

Tolerant plants restrict heavy metal absorption from soil to root and translocation from root to shoot resulting in reduced bioaccumulation, while hyper accumulators actively take up and translocate heavy metals to the above-ground biomass. According to Fitz and Wenzel (2002) plants exhibiting BCF and TF values less than one, are not suitable for phytoremediation. *Centella asiatica* plants treated with mercury and a combination of mercury + triadimefon show very low BCF values and hence accumulation of mercury in the root system is very low. So rhizofiltration process due to adherence of mercury ions to the roots as suggested by Pilon-Smits (2005) is obviously not occurring in the present study. However, TF values are above one, and hence the entire plant in general is found to be a moderate accumulator of mercury if at all the plant is not tolerant to a particular concentration of $HgCl_2$. Nevertheless, the bioaccumulation potential of this plant is not at all affected by the application of additional doze of $HgCl_2$ (Table 25). So it can be concluded that accumulation potential of *C. asiatica* is having a threshold level and above that no significant increase occurs further in the accumulation potential. Addition of triadimefon resulted in a reduction in BCF values compared to Hg treatment (Table 24 & 25). It

may be due to the inhibition induced by triadimefon on mercury absorption by roots, where more mercury content may be adsorbed/ absorbed.

Plants treated with additional doses of mercury showed the trend of reduced values of both BCF and TF in mercury + triadimefon treated plants compared to the treatment with mercury alone (Table 25). During growth, the BCF values become uniform during the entire period, in spite of the addition of more mercury.

The investigation enabled to show the sensitivity of *Centella asiatica* plants towards mercury toxicity. Metabolic adjustments to attain slight tolerance up to 15 μM concentration $HgCl_2$ in Hoaglands nutrient medium are exhibited by reduced biomass, photosynthesis, inhibition of protein synthesis, enhancement in free amino acid, proline and phenolic contents and also significant increase in peroxidise activity. Bio accumulation pattern of mercury in different parts of the plant revealed that about 60% of the available mercury content in the growth medium is accumulated in the plant body irrespective of the additional amount of $HgCl_2$ in the growth medium (Table 22 & 23). But this pattern or level of accumulation is sustainable only up to 15 μM concentration, because the plants are intolerant to higher concentration. Given the sensitivity of *C. asiatica* plants towards low concentration of mercury, significant bioaccumulation potential is a serious problem, since this plant is an important ingredient of many Ayurvedic medicines (Kakkar, 1988). More

over *C. asiatica* is often exposed to contaminated water/soil because it grows and/or is cultivated in wet lands or marshy areas, which are commonly used for waste water disposal and become an anthropogenic sources of environmental pollution. Hence, indiscriminate collection of *Centella asiatica* plants from wetlands, which often get polluted due to waste disposal and cultivation in those areas may lead to health hazards, since the plant is widely consumed by human beings through various Ayurvedic medicines.

SUMMARY AND CONCLUSIONS

To elucidate the toxic effect of mercury on *Centella asiatica*, an important medicinal plant, studies were conducted by cultivating the rooted cuttings of the plant in Hoagland's nutrient medium artificially contaminated with 15 µM $HgCl_2$. Samples of root, runner and leaves were collected at intervals of four days up to 24 days and parameters like morphological measurements, stomatal index, anatomical/ histochemical studies of root, stem and leaves, estimation of biomass, chlorophyll, protein, total free amino acids, proline and phenolics were followed. Apart from these parameters, PAGE studies of protein, guaiacol peroxidase assay and quantitative estimation of mercury bioaccumulation in various plant parts such as roots, runner and leaves were also undertaken.

Another aspect of the present study was treatment with 15 mgl^{-1} triadimefon in order to elucidate not only the growth stimulation effect of this triazole compound but to understand the antagonistic effect on mercury toxicity also. For a comparative study samples are collected from the treatments such as 15µM $HgCl_2$, 15 mgl^{-1} triadimefon and a combination of both 15µM $HgCl_2$ and 15mgl^{-1} triadimefon at comparable intervals of 4 days starting from the day of treatment, i.e., zero day to 24th day. All the parameters were followed to analyse in the roots, runner and leaves of

C.asiatica plants treated with triadimefon and a combination of $HgCl_2$ and triadimefon also.

Data obtained in this study revealed that rooted propagules of *Centella asiatica* cultivated in nutrient medium is very sensitive to mercuric chloride and at 15µM concentration about 50% visual growth retardation occurs. The plants are not tolerant to $HgCl_2$ above 15 µM since the plant does not recover from the toxic effect during prolonged period of growth. However, the inhibitory effects of mercury are shown by the plants in the manifestation of morphological, anatomical/ histochemical and biochemical variations in comparison with the behaviour of control plants cultivated in Hoagland's nutrient medium.

The salient features of mercury toxicity includes stunted growth, increased stomatal index, increased tolerance index, Histochemical localization of mercury content, reduction of biomass, chlorophyll content and protein; increased free amino acid, proline and phenolic content in all parts of the plant. Linear increase in peroxidase activity was another observation of plants treated with $HgCl_2$. Absorption and accumulation of mercury showed variations in the quantities accumulated in the tissues.

Triadimefon treatment exhibited only slight stimulatory effect, though not significant on almost all parameters followed in this investigation. Nevertheless, in combination with $HgCl_2$, triadimefon showed radical

changes, mostly reversible on growth retardation, anatomical changes, mercury localization and tolerance index. The inhibitory effect of mercury as shown by reduced biomass, protein, chlorophyll and increased proline content are also reversed by this triazole compound. But triadimefon does not impose much change in the distribution of free amino acids. Similarly, only slight increase was observed in the phenolic content which is significantly increased in the treatment with $HgCl_2$ alone. The activity of peroxidase also was not affected by triadimefon.

Bioaccumulation study in the plants treated with mercury show absorption and translocation of mercury to the root, stem and leaf. How ever, the mercury content in the residual solution of mercury + triadimefon treated plants showed more quantity revealing the retardation effect of triadimefon in the rate of absorption/ translocation of mercury. When additional amount of $HgCl_2$ is given to the growth medium, the accumulation pattern of mercury remains unchanged. By analysing the data obtained and correlating with literature, the following conclusions are made.

1. *Centella asiatica* is very sensitive to $HgCl_2$, since beyond 15 µM concentration the plants do not survive. Tolerance towards mercury toxicity is not acquired by this plant during prolonged growth.

2. Growth retardation is shown by all parameters like length of root and runner and leaf area.

3. Inhibitory effect of mercury is shown by reduced biomass, proteins and chlorophyll contents.

4. Enhanced synthesis of proline, free amino acids and phenolics are characteristic mechanisms to avoid or ameliorate mercury toxicity in *Centella asiatica* to a considerable extent.

5. Synthesis of many new stress induced proteins is not evident by PAGE studies and this observation reveals the intolerance of *C. asiatica* plants towards mercury.

6. Guaiacol peroxidase activity increase is found to be effective tolerance mechanisms to some extent adopted by this plant, at least up to 24 days of growth.

7. Bioaccumulation study shows accumulation of mercury in the root, runner and leaf in the order - root > stem > leaf. But additional amount of mercury given to nutrient medium do not affect the accumulation pattern of the metal.

8. The triazole triadimefon is moderately effective on *Centella asiatica* as a plant growth regulator whereas the phytotoxicity of mercury is antagonised to some extent by modification in the distribution pattern of metabolites. However, complete recovery of plants from phytotoxic effects of mercury is not possible by triadimefon treatment.

9. During growth about 60 % of the mercury is accumulated in the plant body retaining almost 30% in the residual solution and only less than 10% of mercury is lost from the plants, hence phytovolatalization is very low. The accumulation potential of *C. asiatica* is an important concern because the plant is widely consumed as it is an integral ingredient of many ayurvedic medicinal preparations.

10. Plants treated with mercury + triadimefon show significant reduction of mercury accumulation and consequently the quantity retained in the residual solution is more. This observation reveals the antagonistic effects of triadimefon on the absorption/ accumulation of mercury.

11. It seems that given the high sensitivity of *Centella asiatica* plants towards mercury, this plant accumulates significant amount of mercury even at low concentration (15 μM) present in the growth media.

12. Hence the bioaccumulation potential of *Centella asiatica* towards mercury indicates that cultivation of this plant in soil contaminated with low concentrations of mercury may result in considerable accumulation leading to health hazards to mankind.

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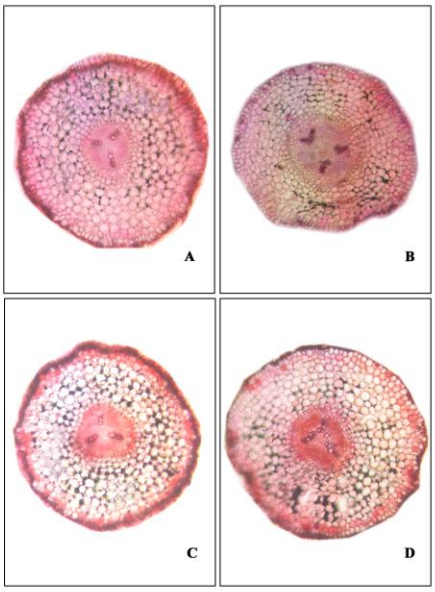


Fig. 7. Histochemical staining of *Centella asiatica* roots treated with Mercury and Triadimefon on 4th day.
A. Control; **B**. Mercury; **C**. Triadimefon; **D**. Mercury+Triadimefon

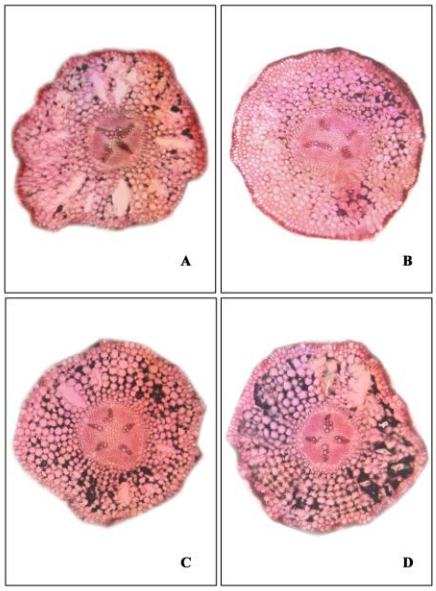


Fig. 8. Histochemical staining of *Centella asiatica* roots treated with Mercury and Triadimefon on 12th day.
A. Control; **B**. Mercury; **C**. Triadimefon; **D**. Mercury+Triadimefon

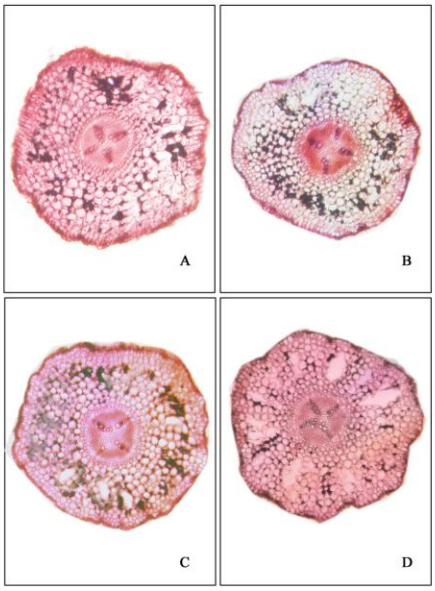


Fig. 9. Histochemical staining of *Centella asiatica* roots treated with Mercury and Triadimefon on 24th day.
A. Control; **B**. Mercury; **C**. Triadimefon; **D**. Mercury+Triadimefon

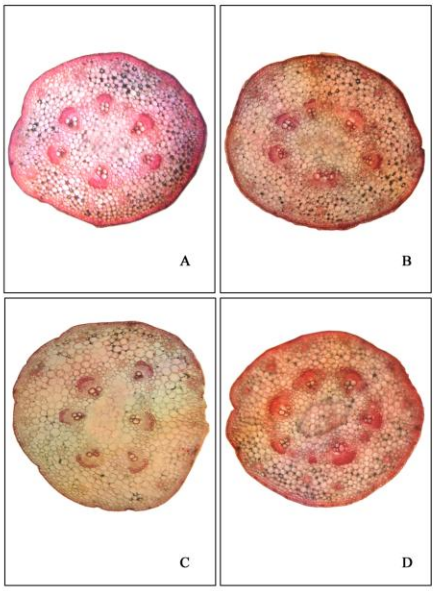


Fig. 10. Histochemical staining of *Centella asiatica* stem treated with Mercury and Triadimefon on 4th day.
A. Control; **B**. Mercury; **C**. Triadimefon; **D**. Mercury+Triadimefon

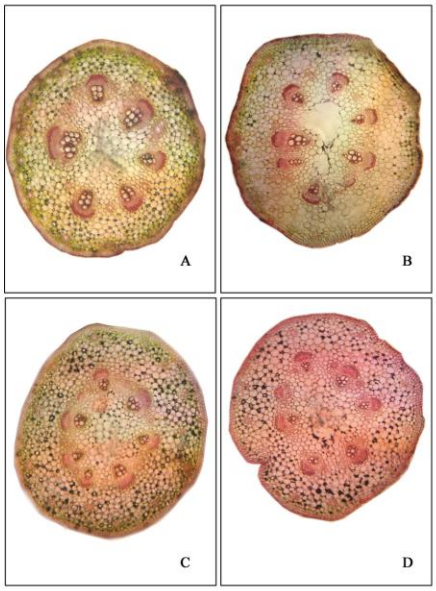


Fig. 11. Histochemical staining of *Centella asiatica* stem treated with Mercury and Triadimefon on 12th day.
A. Control; **B**. Mercury; **C**. Triadimefon; **D**. Mercury+Triadimefon

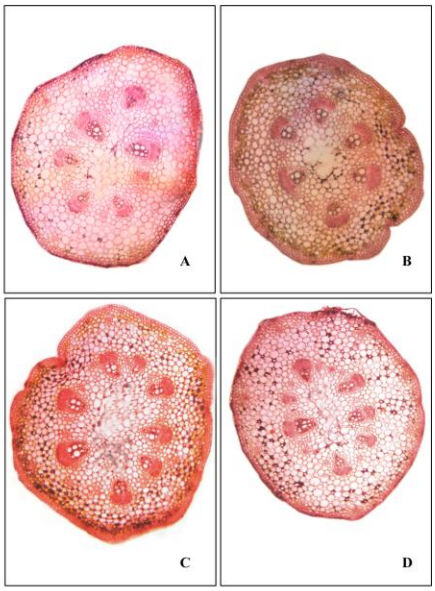


Fig. 12. Histochemical staining of *Centella asiatica* stem treated with Mercury and Triadimefon on 24th day.
A. Control; **B**. Mercury; **C**. Triadimefon; **D**. Mercury+Triadimefon

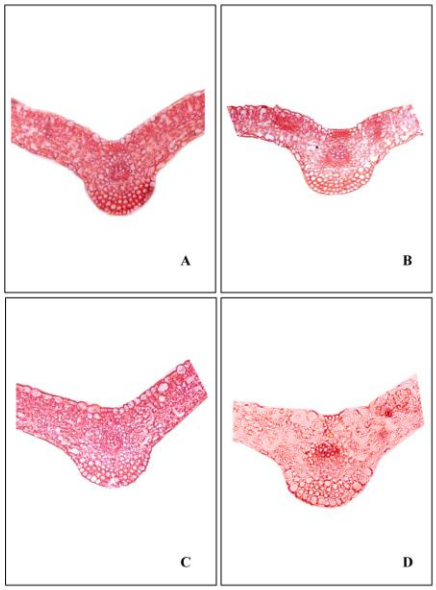


Fig. 13. Histochemical staining of *Centella asiatica* leaf treated with Mercury and Triadimefon on 4th day.
A. Control; **B**. Mercury; **C**. Triadimefon; **D**. Mercury+Triadimefon

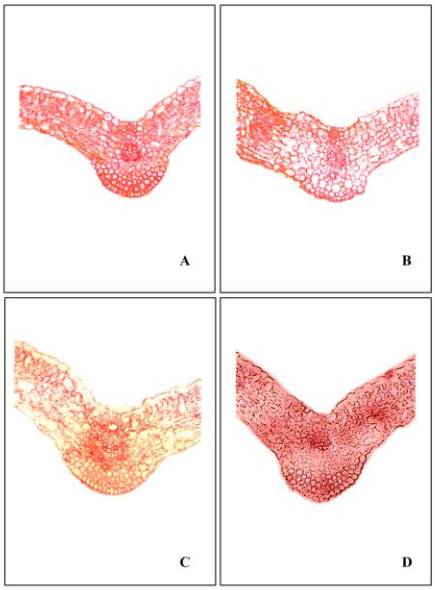


Fig. 14. Histochemical staining of *Centella asiatica* leaf treated with Mercury and Triadimefon on 12th day.
A. Control; **B**. Mercury; **C**. Triadimefon; **D**. Mercury+Triadimefon

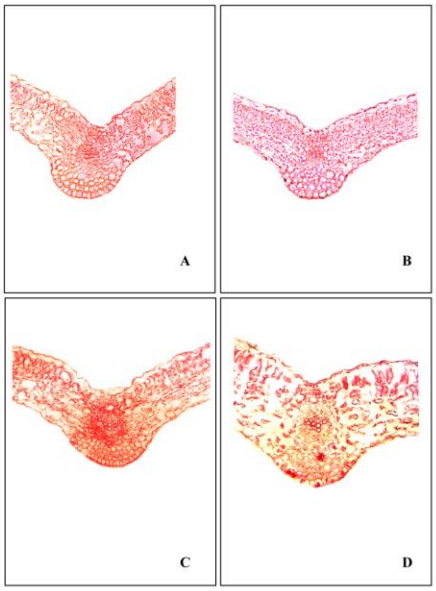
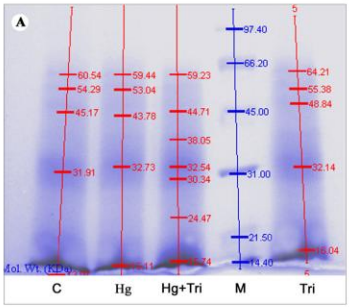


Fig. 15. Histochemical staining of *Centella asiatica* leaf treated with Mercury and Triadimefon on 24th day.
A. Control; **B**. Mercury; **C**. Triadimefon; **D**. Mercury + Triadimefon



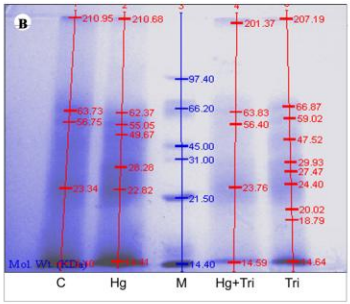


Fig. 23. Protein profile of *Centella asiatica* root treated with Mercury and Triadimefon. **A**. 4th day; **B**. 24th day
C - Control **Hg+Tri** - Mercury+Triadimefon
Hg - Mercury **Tri** - Triadimefon **M** - Protein Marker

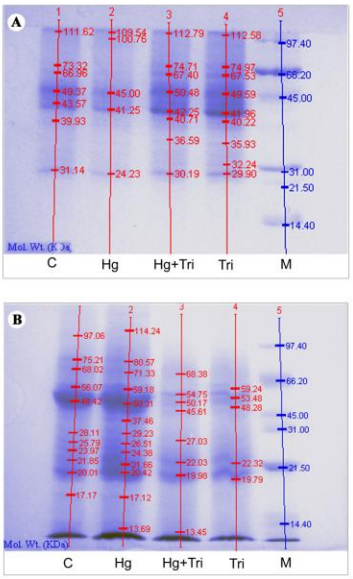


Fig. 24. Protein profile of *Centella asiatica* stem treated with Mercury and Triadimefon. **A**. 4th day; **B**. 24th day
C - Control **Hg+Tri** - Mercury +Triadimefon
Hg - Mercury **Tri** - Triadimefon M- Protein Marker

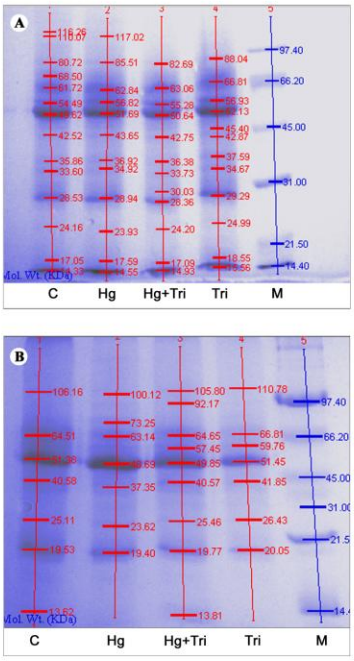


Fig. 25. Protein profile of *Centella asiatica* leaf treated with Mercury and Triadimefon. **A**. 4th day; **B**. 24th day
C - Control **Hg+Tri** - Mercury+Triadimefon
Hg - Mercury **Tri** - Triadimefon M - Protein Marker