Journal of Environmental Biology **April 2007,** 28(2) 303-306 (2007)

Leaf biochemistry of *Lycopersicon esculentum* Mill. at different stages of plant development as affected by mercury treatment

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(Received: January 22, 2005 ; Revised received: August 25, 2005 ; Accepted: September 17, 2005)

Abstract: *The effect of mercury (Hg) on the biochemical parameters of Lycopersicon esculentum Mill leaf was studied. Application of mercuric chloride in varying concentrations (0.5, 1.0, 1.5 and 2.0 mM $HgCl_2$ kg^{-1} sand) caused significant reduction that went up to 89% and 72% chlorophyll a and chlorophyll b contents repectively (at flowering stage), 69% in carotenoid content, 64% in total soluble protein content and 91% in nitrate reductase activity (all at post-flowering stage). The amounts of nitrate and proline increased maximally (151% and 143% respectively) at the flowering stage, whereas total soluble sugar enhanced by 57% at the post-flowering stage. Changes observed in most of the parameters, were concentration dependent. Such studies seem to be able to discover suitable bioindicators of heavy metal pollution.*

Key words: *Mercury, Lycopersicon esculentum, Foliar biochemistry, Metal pollution*

Introduction

India has experienced a fast rate of industrial growth aimed at attaining high economic and technological benefits. The lack of appropriate legislative measures permits uncontrolled release of industrial effluents which contain both organic and inorganic wastes. The raw sewage water contains toxic elements and heavy metals such as iron, copper, zinc, manganese, lead, cadmium, mercury, nickel, cobalt and chromium (Dass and Kaul, 1992). The metals like cobalt, iron, manganese and zinc are essential for plant life but are required in a very small or trace amounts, and become toxic at higher concentrations. On the other hand, metals like mercury, cadmium, chromium, nickel and lead are not needed by plants and animals.

Methyl mercury is a highly dangerous pollutant, which accumulates in the food chain as does D.D.T. Mercury compounds (both organic and inorganic) can also become a powerful pollutant in the terrestrial environments. Tomatoes are particularly sensitive to mercury pollution, whereas bean and corn are less sensitive (Kumar and Häder, 1999; Gauba *et al.*, 2004).

Tomato (*Lycopersicon esculentum* Mill.), an important vegetable crop, produces fruits that are refreshing and appetizing, and are consumed raw in salads. These are also consumed in the form of puree, paste, ketchup, sauce, soup and powder.

The study was undertaken to determine the effects of mercury on some biochemical parameters of tomato leaf at different stages of plant development in Tomato. The biochemical attributes related to C and N metabolism constitute the primary requirements for plant growth. Alteration in these parameters is expected to affect biomass accumulation and fruit yield (Gauba *et al.,* 2004)

Materials and Methods

The study was conducted on Pusa Ruby variety of *Lycopersicon esculentum* Mill. in 10 replicates. Seeds obtained from Indian Agricultural Research Institute, New Delhi, were grown up to seedling stage in the field, and then transplanted in pots containing 11 kg of acid washed Yamuna sand each. They were supplied with full strength Hoagland's solution containing desired nutrients after an interval of 7 days during the course of experiment. Standard irrigation practice was followed. The mercury treatment was given in the form of mercuric chloride in 4 concentrations *viz.* 0.5, 1.0, 1.5 and 2.0 mM $HgCl_2$/ kg of sand. Untreated 10 replicates were taken as control. Experiment was conducted in October, 2003. Sampling was done at the pre flowering (30 days), flowering (55 days) and post flowering (90 days) stages for biochemical studies.

The chlorophyll and carotenoid contents of fresh leaves were estimated by the method of Hiscox and Israelstam (1979), using the dimethyl sulphoxide (DMSO). The chlorophyll concentration in mg/gm of fresh leaves was calculated using the formulae given by MacLachlan and Zalik (1963) and Duxbury and Yentsch (1956), whereas estimation of total soluble proteins was done by the method of Bradford (1976). The protein concentrations were determined by using bovine serum albumin as standard. The nitrate reductase activity was estimated by the method of Klepper *et al*. (1971), whereas concentration of nitrate was determined against the standard curve using sodium nitrate ($NaNO_2$) solution. Nitrate extraction was done by the method of Grover *et al*. (1978). The soluble sugar content was estimated by the method of Dey (1990), against a standard curve prepared by using a glucose solution. Proline was estimated following the method of Bates *et al*. (1973).

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Results and Discussion

Analysis of the leaf tissue of *L. esculentum* plants treated with mercuric chloride exhibited inhibitory effect with respect to chlorophyll a, chlorophyll b and carotenoid contents at all concentrations of mercury as compared with controls. The inhibitory effects gradually increased with increasing degree of concentration. Most of the differences observed were significant at 5% level. The highest reduction in chl a (89%) and chl b (72%) was recorded at the post flowering stage. The amount of carotenoid in the control plants increased with growing plant age. It further declined in case of treated plants, the concentration-dependent decline being the maximum (69%) at the post-flowering stage (Tables 1-3).

A concentration dependent decrease in total soluble protein content, over the control, was observed in the leaves, although a continuous increase from pre flowering to post flowering stage was observed in the controls. Maximum decrease (64%) was noted at the post-flowering stage with 2.0 mM Hg concentration. Most of the differences were significant at 5% level (Table 4).

A gradual increase in nitrate content with increasing concentration of mercury was observed over control. Maximum increase (151%) was observed at 2.0 mM of mercury concentration at pre flowering stage. Differences were significant in most of the cases at 5% level of significance (Table 5).

Nitrate reductase activity in the leaves of the treated plants decreased over the control with increasing concentrations of $HgCl_2$. The effect was more pronounced (91%) at post flowering stage (Table 6).

Proline content of leaves increased with plant age and mercury concentration. The enhancement in the proline content

Table - 1: Variations in chlorophyll a content (mg/g fresh wt.) of *Lycopersicon esculentum* Mill. during various stages of development as affected by mercury. Values are a mean of three observations

Stage / Treatment	Pre flowering	Flowering	Post flowering
0	0.73	0.19	0.15
0.5	0.55	0.12	0.12
1.0	0.47	0.09	0.11
1.5	0.26	0.04	0.09
2.0	0.26	0.02	0.07
LSD at 5%	0.036	0.04	0.032

Table - 2: Variations in chlorophyll b (mg/g fresh wt.) of *Lycopersicon esculentum* Mill. during various stages of development as affected by mercury. Values are a mean of three observations

Stage / Treatment	Pre flowering	Flowering	Post flowering
0	0.64	0.58	0.32
0.5	0.48	0.47	0.30
1.0	0.43	0.34	0.28
1.5	0.29	0.27	0.21
2.0	0.29	0.16	0.14
LSD at 5%	0.047	0.03	0.04

Table - 3: Variations in carotenoid content (mg/g fresh wt.) of *Lycopersicon esculentum* Mill. during various stages of development as affected by mercury. Values are a mean of three observations

Stage / Treatment	Pre flowering	Flowering	Post flowering
0	0.84	0.54	0.16
0.5	0.64	0.44	0.15
1.0	0.48	0.42	0.13
1.5	0.28	0.21	0.12
2.0	0.27	0.20	0.05
LSD at 5%	0.115	0.0037	0.041

Table - 4: Variations in total soluble protein (µg/g fresh wt.) of *Lycopersicon esculentum* Mill. during various stages of development as affected by mercury. Values are a mean of three observations

Stage / Treatment	Pre flowering	Flowering	Post flowering
0	328.0	396.0	896.0
0.5	245.3	359.2	751.6
1.0	231.2	321.2	688.8
1.5	197.6	316.2	549.6
2.0	192.6	314.8	319.2
LSD at 5%	49.747	28.116	21.193

Table - 5: Variations in nitrate content (µ moles/g fresh wt.) of *Lycopersicon esculentum* Mill. during various stages of development as affected by mercury. Values are a mean of three observations

Stage / Treatment	Pre flowering	Flowering	Post flowering
0	2580.0	2220.2	2581.1
0.5	2840.0	2400.3	2640.0
1.0	3333.3	3400.5	3380.7
1.5	3800.0	3700.0	3820.1
2.0	6466.6	3820.0	4460.1
LSD at 5%	2.294	7.361	6.399

Table - 6: Variations in nitrate reductase activity (µ moles NO_2^-/hr/g fresh wt.) of *Lycopersicon esculentum* Mill. at various stages of plant development as affected by mercury. Values are a mean of three observations

Stage / Treatment	Pre flowering	Flowering	Post flowering
0	17.66	50.55	37.12
0.5	13.42	21.54	11.92
1.0	12.7	21.07	7.83
1.5	10.35	20.25	3.52
2.0	10.12	10.12	3.45
LSD at 5%	0.686	0.763	0.403

Table - 7: Variations in proline content (μ mole/g fresh wt.) of *Lycopersicon esculentum* Mill. during various stages of development as affected by mercury. Values are a mean of three observations

Stage / Treatment	Pre flowering	Flowering	Post flowering
0	11.95	17.39	19.11
0.5	12.95	19.28	21.97
1.0	17.42	24.57	23.91
1.5	19.43	27.99	26.89
2.0	29.08	31.22	30.17
LSD at 5%	2.638	3.682	1.96

Table - 8: Variations in total soluble sugar (μg/g fresh wt.) of *Lycopersicon esculentum* Mill. during various stages of development as affected by mercury. Values are a mean of three observations

Stage / Treatment	Pre flowering	Flowering	Post flowering
0	266.5	622.3	1410.1
0.5	328.7	642.2	1444.3
1.0	366.6	692.2	1766.2
1.5	372.1	696.9	1958.5
2.0	386.7	888.8	2208.2
LSD at 5%	0.686	0.763	0.403

was highest (143%) at pre flowering stage with 2.0 mM of $HgCl_2$ concentration (Table 7).

Similarly, the total sugar content showed a significant increase with plant age and with mercury level. The amount of soluble sugar was estimated to be the highest at the post flowering stage with 2.0 mM Hg, showing an increase of 57% over the control. Differences were significant either at 1% or 5% level of significance (Table 8).

These results indicate that mercury has a concentration dependent inhibitory effect on chlorophyll a, b and carotenoid contents in *Lycopersicon esculentum* Mill. leaves. The decline in chlorophyll content may be due to interference of the metal with chlorophyll synthesis (Prasad and Prasad, 1987; Polaco, 1977; Puranik *et al*., 1990). Protochlorophyll accumulation due to strong inhibition of chlorophyll synthesis has been observed in *Euglena gracilis*, grown in mercury containing medium (De Fillips *et al*., 1981). Chlorophyll level may also decline due to enzymatic degradation as a high activity of chlorophyllase has been observed in mercury treated plants (De *et al*., 1985). The decline may also be attributed to an impaired supply of Mg and Fe to the leaves (Gregor and Lindberg, 1986). Similar effect of mercury on chlorophyll content has been observed in *Albizia lebbek* (Tripathi and Tripathi, 1999) and in *Morus alba* (Mohapatra and Panigrahi, 1991).

Heavy metals reduce the soluble protein in agricultural crops (Hemlatha *et al*., 1997) as observed in this study too. The decrease is caused either by a reduced *de novo* synthesis or by an increased decomposition of proteins to amino acids (Todd *and Arnold*, 1961). Vallee and Ulmer (1972), have emphasized that heavy metal ions have strong affinities for side chain ligands of proteins, indicating that enzyme activities and other functions are affected by heavy metal ions. A reduction in the amount of proteins was observed in seedlings of *Zea mays* at all concentrations of mercury employed by Kalimuthu and Sivasubramanian (1990). Similarly, a concentration dependent decrease in soluble protein content over the control was observed in the leaves of *Albizia lebbek* (Tripathi and Tripathi, 1999).

Low nitrate reductase activity may result in the accumulation of nitrate, as nitrogen is absorbed from the soil in the form of nitrate. The *in vivo* nitrate reduction depends upon the supply of NADH as a source of electrons. Klepper *et al*. (1971) reported that glycolysis supplies NADH for nitrate reduction. Swahney *et al.* (1978) presented evidence that Krebs cycle plays an important role in supplying NADH for nitrate reduction. Thus, an increase in the amount of nitrate could be related to a decreased NR activity and reduced photosynthesis (due to decrease in amount of chlorophyll pigments), which is the source of substrate for Krebs cycle.

The present study indicates a decrease in NR activity with increasing concentration of mercury. Heavy metals like Hg (II) are known to interact with -SS- and -SH groups of proteins and -COOH and $-NH_2$ groups of enzyme and decrease the enzyme activity forming proteins -Hg (II) complex, changing its conformation and solubility (Siegel, 1974). NR inhibition *(in vivo)* by mercury has been observed earlier also (Vyas and Puranik, 1993). According to Vallee and Ulmer (1972), inhibition of nitrate uptake by heavy metals is another possibility for the observed inhibition of NR activity, as nitrate is one of the inducers of NR activity.

Proline, a free amino acid accumulates in plants when they experience moisture stress conditions and decline on release of the stress (Pokhriyal *et al*., 1991). Proline accumulation and high stability index are suggested as a selection criterion for drought resistance. Our results indicate that accumulation of proline increases with increasing mercury concentration. Besides, the untreated plants also maintain a certain level of proline content.

Sugar is manufactured during photosynthesis and breaks down during respiration by plants. Metals decrease the sugar content with their increasing concentration in agricultural crops (Bazzaz *et al*., 1975; Hemlatha *et al*., 1997). The low sugar level may be due to lowered synthesis or a diversion of the metabolites to other synthesis processes.

However, in the present study the consistent increase in the sugar content of leaves of the Hg-treated tomato plants, despite a decrease in chlorophyll content which could reduce the rate of photosynthesis, is indicative of the fact that the process of translocation of photosynthate from leaves to other parts of the plant is badly affected in this species. In consequence, whatever amount of photosynthate is produced, seemingly keeps

accumulating in the leaf tissues thus raising the sugar level in the leaves pretty high.

Our observations suggest that the presence of Hg in the soil may result in a rapid physiological disturbance as is obvious from changes in the biochemical status of the leaves. The green pigments and soluble protein contents as well as the NR activity in the leaf tissue were adversely affected, whereas proline accumulation was enhanced. Interestingly, sugar content of the leaf also increased indicating a significant damage to the phloem transport system.

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