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Metabolic Effects of Sulfur Dioxide Fumigation on *Mangifera indica* Plants

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Sulphur dioxide is a major industrial air pollutant well studied for deleterious effects on different components of the environment including physiological and biochemical changes (Malhotra and Khan, 1984; Beg, 1988). These metabolic alterations may change the quality and quantity of the crop produce. Most studies with sulphur dioxide have been focused on lichens, bryophytes, crop plants and forest species but little information exists regarding horticultural species of economic importance. Sulphur dioxide is reported to reduce the quality and quantity of mangoes in orchards near coal fired bricks klins (Rao, 1972), thermal power station (Beg et al., 1990) and industrial complexes (Annonymous, 1997). In this study the response of Mango, a popular fruit tree of India, was assessed towards sulphur dioxide for determining threshold concentration producing mild and severe injury, sulphur dioxide sorption and metabolic alterations. Williams and Banerjee (1995) observed considerable reduction in chlorophyll, carotenoids, ascorbic acid and protein and an increase in sugar and sulphur content in pollution affected leaves of mango plants growing around a coal based thermal power station.

MATERIALS AND METHODS

Mangifera indica (Variety-Lucknow Safeda) is an important commercial fruit crop grown in orchards, field borders and road sides. Experimental plants were raised from the seeds in earthen pots containing garden soil, composted farmyard manure and NPK. One year old (20 cm height) plants were shifted to the laboratory and acclimatized for one month in a well illuminated chamber (30,000 Lux) with 12 hour light and dark periods. Three pots, containing two plants each, were subjected to exposure at 0.0, 0.8, 2.5, 6.5 and 8.0 ppm initial sulphur dioxide for 4 h in a continuous gas flow exposure chamber as per EPA protocols of phytotoxicity testing (Rubinstein et al., 1975). The chamber (dimensions 28x32x45 cm), SO_2 generation system

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and estimation, air speed (0.54 ms^{-1}) and boundry layer resistance (negligible-0.007S cm^{-1}) were the same as reported earlier (Farooq et al., 1985). The humidity in the exposure chamber was controlled through a trap of anhydrous silica gel at the entry of air in the chamber. Average humidity was around 70% during the exposure. Sulphur dioxide sorption by plants was measured at specified intervals (Omasa and Abo, 1980). The concentration of SO2 gets reduced during plant exposure becuase of foliar uptake and hence steady concentration could not be strictly maintained. The plants were observed for a 48 h post exposure period for the development of visible foliar injuries if any. Using fresh batches of plants exposed to higher concentrations, levels needed for mild injury (MI) and severe injury (SI) symptoms were determined.

Each of the six plants was analysed separately. The top 5 leaves from exposed and unexposed plants were plucked separately, cut into pieces, divided into two samples and weighed. One sample was used for dry weight and total sulphur content by turbidimetric method (Chan, 1975). The other sample was used for biochemical analysis. Chlorophyll, protein, free amino acids, starch, total free sugar, reducing sugar, peroxidase and acid phosphatase were estimated by methods described earlier (Farooq et al., 1985). Pheophytin and total phenolics were estimated following the methods of Vernon (1960) and Farkas and Kiralay (1962), respectively. Sulphite oxidase (Cohen and Fridovich 1971) and pH was estimated in the leaf extract of unexposed plants. For the assay of peroxidase and acid phosphatase a 2.0% homogenate was prepared in chilled water, for sulphite oxidase, a 10.0% homogenate was prepared in chilled, 0.lM tris-HCl buffer pH 8.5, mixed with 10^{-4} M EDTA. The data were assessed by Student's 't' test (Baily, 1959).

RESULTS AND DISCUSSION

Table 1 lists the symptoms observed on the foliage of mango plants within 48 h after a 4h exposure to sulphur dioxide. No visible symptoms were observed at 0.8 ppm while at 2.5 ppm interveinal necrosis was clear. The necrosis was progressive in nature, spreading from margin towards midrib. At 6.5 ppm symptoms were more severe with, almost all the upper leaves having turned brown and desiccated. At 8.0 ppm chlorotic symptoms developed immediately covering almost 90% of the leaf area. Exposure to sulphur dioxide caused gradual accumulation in sulphur content. The contents of sulphur in the tissue at 0.8, 2.5, 6.5 and 8.0 ppm Sulphur dioxide were 0.15, 0.46, 0.86 and 1.27 mg/g dry weight respectively. This showed that the absorption of sulphur dioxide by plants increased with dose of

Table 1. Injury symptoms and total sulphur content in *M. indica* saplings after sulphur dioxide exposure.

Exposure Concentration (ppm)	Total sulphur Content in Plants (mg/g dry wt)	Absolute Increase in sulphur Content (mg/g dry wt.)	Development of Injury Symptoms
0.0	1.87±0.34	-	-
0.8	2.02±0.30 (+8.0)	0.15	No injury
2.5*	2.33±0.57 (+24.6)	0.46	Mild injury: plants showed interveinal necrosis.
6.5**	2.73±0.42b (+46.0)	0.86	Severe injury:Newer leaves turned brown and desiccated while old leaves remained unaffected,
8.0	3.14±0.15d (+68.5)	1.27	Severe injury: symptoms appeared immediately after exposure both in new and old leaves. After 48h injured area of the leaves become dry.

* = MI concentration, ** = SI concentration.
Each value is the mean of six samples ± S.D., Significance b = $p<0.02$, d = $p<0.01$ as determined by Student's 't' test. Figures in paranthesis indicate percentage increase over control.

exposure along with more severe injury. The concentration of sulphur dioxide measured in the chamber with and without plants is plotted in Figure 1. The plants caused a drop in chamber concentration of sulphur dioxide. The quantity of sulphur dioxide taken up per unit weight was proportional to exposure concentration.

Metabolic changes in exposed plants are reported in table 2. Protein content was higher in exposed plants showing significant increase both at 2.5 and 6.5 ppm sulphur dioxide. Amino acid content was not significantly altered except at 8.0 ppm where it was decreased. Starch, total free sugar, reducing sugar contents were significantly higher in exposed plants at

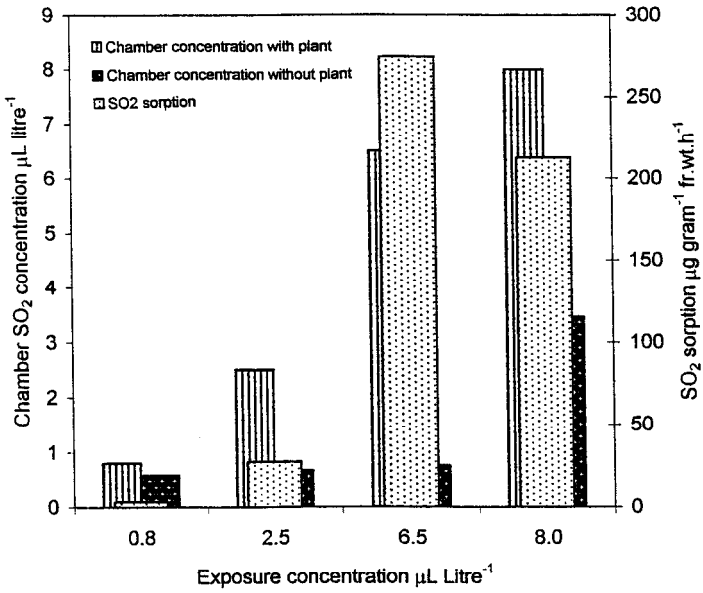

Figure 1. Sulphur dioxide absorption by *M. indica* plants

all the exposure concentrations. Content of phenolics increased gradually with exposure concentrations. The maximum value was observed at MI and was significant both at MI and SI concentrations. Chlorophyll contents in sulphur dioxide exposed plants increased while no change in the pheophytin content was observed. The activity of peroxidase increased with exposure concentrations in sulphur dioxide exposed plants. The increase at 0.8 and 2.5 ppm was 1.7 and 2.7 fold while at 6.5 and 8.0 ppm exposure concentration it was 86 and 165 fold with respect to control. Acid phosphatase activity decreased significantly at 2.5 and 6.5 ppm sulphur dioxide concentrations in comparison of control. The pH of control leaf extract was 5.2 while sulphite oxidase activity was 1.20 n moles oxygen consumed/min/g fr. wt. Sulphur dioxide causes injury to plants mainly dependent on entry though stomata and solubility in cell sap. Increased rate of sulphur dioxide absorption may damage cellular membrane architecture resulting in permeability changes (Farooq and Beg, 1980). As the cellular contents begin to leak into the intracellular spaces mediated by damage to membranes, oxidative enzymes react with phenols converting than to quinones (Moran et al., 1997). o-Quinones polymerize with aminoacids, amines and sulfhydryl groups of proteins to form low molecular weight, reddish brown pigments in exposed leaves. This involvement of phenol appears to be the cause of visible necrotic lesions in injured leaves (Howell, 1974). Alterations in phynolics and there accumulation products are common indicators of stress in plants

Table 2. Effect of sulphur dioxide on the Metabolism of *M .indica* Plants

Parameters	Sulphur dioxide Concentration (ppm)				
	0.0	0.8	2.5*	6.5**	8.0
Protein	17.81 ±2.27	15.22 ±2.13	20.20 ±1.16a	23.45 ±4.15b	18.17 ±1.69
Amino acids	3.16 ±0.50	3.09 ±0.18	3.00 ±0.20	3.06 ±0.35	2.55 ±0.31a
Starch	51.18 ±5.37	60.26 ±5.35b	110.48 ±15.07d	96.43 ±13.09d	99.84 ±6.31d
Total free sugars	165.66 ±7.67	177.01 ±28.12	209.71 ±24.57c	192.22 ±23.95a	215.51 ±25.97d
Reducing sugars	57.85 ±6.35	68.94 ±8.71a	78.38 ±8.05d	71.54 ±4.68c	81.48 ±8.55d
Total phenolics	71.62 ±11.05	78.89 ±7.09	100.74 ±8.65d	83.54 ±5.45a	91.90 ±11.95b
Chlorophyll	3.39 ±0.19	3.64 ±0.30	3.74 ±0.30a	4.26 ±0.49c	3.59 ±0.51
Pheophytin	5.69 ±0.48	5.65 ±0.47	5.81 ±0.43	6.46 ±0.89	5.57 ±0.75
Peroxidase(1)	0.03 ±0.01	0.05 ±0.00a	0.08 ±0.01d	2.57 ±2.01a	5.05 ±3.48
Acid Phosphatase(2)	0.62 ±0.16	0.58 ±0.20	0.35 ±0.16b	0.38 ±0.18a	0.44 ±2.02
Dry Weight (%)	42.93 ±1.36	42.66 ±1.85	41.60 ±1.75	44.50 ±1.75	44.50 ±2.02

*MI concentration, **SI concentration.
The data are expressed as mg/g dry weight; (1) .OD change/min/mg protein; (2) mg phenol produced/mg protein/min. and are the average of six samples ± S.D. Significance as determined by student's 't' test a = $p<0.05$, b = $p<0.02$, c = $p<0.01$, d = $p<0.001$.

(Scalbert and Haslam, 1987). In the present study severe injury produced by the pollutant was indicated by the necrosis of large areas of leaf tissues which became dry and turned brown. Several investigators correlated the degree of foliar necrosis with the quantity of sulphur dioxide absorbed (Caput et al., 1978). Absorbed sulphur dioxide increase the sulphite concentration in leaf tissues, but the plants can

overcome its phytotoxic effects by readily coverting it to sulphate. Sulphite oxidase plays an important role in reducing sulphur dioxide toxicity and acts as a determining factor for plant tolerance to sulphur dioxide (Ayazaloo et al., 1982). The pH of cytoplasmic fluid also plays an important role in determining tolerance of a plant. Plants with acidic pH values were found to be more susceptible to sulphur dioxide than those with pH values 7.0 or above (Shuwen et al., 1982). It is also clear that metabolic alterations in plants are initiated at the concentrations much below those needed for visible injury. Enhanced synthesis occurs in sensitive plants while in resistant plants synthesis is retarded on exposure to sulphur dioxide even at sub-lethal concentration. In most susceptible plants anabolism is activated and several biochemical constituents accumulate (Pavgi et al., 1991). Jagar et a1.(1985) found that protein content increased in sensitive cultivar as a result of gaseous exposure. Starch and protein are also affected by sulphur dioxide fumigation . In the present study also starch, total soluble sugar specially reducing sugars increased as a result of sulphur dioxide exposure as reported by Koziol and Jordan, (1978).

Peroxidase and acid phosphatase were also determined in exposed plants. In our study significant inhibition in acid phosphatase and activation of peroxidase in the exposed plant are found which are in confirmation with the findings of Keller et al., (1976) and Malhotra and Khan) (1980). These are stress mediated metabolic adjustments, as defensive mechanisms.

The appearance of visible symptoms, acidic pH, low sulfite oxidase activity, accumulation of biochemical constituents and decrease in hydrolytic enzyme-acid phosphatase suggest that mango may be categorized as pollution sensitive plant. Effects to protect this important commercial crop from gaseous emissions are essential.

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