Effect of Sulphur Dioxide on *Malva sylvestris*

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ABSTRACT

Leaves of *Malva sylvestris* were evaluated for the effect of sulphur dioxide on different parameters like pigments, proteins, amino acids, starch, free sugars and phenolics. On treatment with different concentrations of aqueous sulphur dioxide under illumination for 4h, all the biochemical parameters like pigment content, protein, amino acid, starch and phenolic content got decreased, while free sugar content increased in the plant under study.

Key words: *Malva sylvestris*, sulphur dioxide, sulphite.

INTRODUCTION

Sulphur dioxide is reported to be the most widespread phytotoxic air pollutant, both in India as well as in other parts of the world (Pavgi *et al*., 1991; Julkunen *et al*., 1995; Noji *et al*., 2001). Its effects on vegetation have been well reviewed in terms of foliar injury (Malhotra and Khan, 1984; Venkateshwar *et al*., 1992) and physiological and biochemical alterations (Holopainen *et al*., 1993 and Kailunainen *et al*., 1995). The effects of sulphur dioxide on microbes and animals are also well established (Chan *et al*., 2002; Wu and Meng, 2003). Among plants, conifers appear to be most sensitive to the effect of sulphur dioxide (Ozolincius *et al*., 2005). Reports of sulphur dioxide affecting DNA suggest that sulphur dioxide is a genotoxic agent which influences the mitotic activity of plants and retards the plant growth. One of the prominent modes of action of sulphur dioxide on plants is free-radical mechanism which leads to peroxidative damage of plant-cell constituents (Niewiadomska *et al.,* 1997). In this paper, the results of exposure of plants to sulphur dioxide have been highlighted.

MATERIAL AND METHODS

Sampling of Tissues

Fresh young leaves of Sunchul *(Malva sylvestris)* were collected, washed and patted dry. 1g of leaf discs of equal dimensions were treated with different concentrations of aqueous sulphur dioxide (prepared by dissolving sodium sulphite in water) for 4h in glass petridishes, under light provided by a 100W tungsten electric bulb. Aqueous sulphur dioxide of 1000 ppm concentration was prepared in the laboratory by dissolving 1.8g of

sodium sulphite in 500ml of distilled water. From this solution, different concentrations of sulphur dioxide like 250ppm, 500ppm and 750ppm were prepared by appropriate dilution. Parallel control was also run. After 4h, the leaf discs were washed, patted dry, weighed and homogenized. 10% (w/v) homogenate was prepared. For parameters like amino acids, starch and phenolics, the homogenate as such was used for estimation. For estimation of pigments, proteins and free sugars, the homogenate was centrifuged at 5000rpm for 10min. The supernatant was carefully decanted and used for various estimations.

Estimations

Chlorophyll, phaeophytin and carotenoids were extracted in 80% acetone and estimated according to the methods of Strain *et al.* (1971), Vernon (1960) and Duxbury and Yentesch (1956) respectively by using ELICO SL-27 spectrophotometer. Protein content was determined by the method given by Lowry *et al* (1951). Amino acids were estimated according to the method of Lee and Takahashi (1966). Starch estimation was carried out by the method of Agarwal *et al.* (1982). The estimation of total phenolics was carried out according to the method given by Malick and Singh (1980). Free sugars were estimated according to the method given by Montgomery (1982).

RESULTS

Results revealed that on sulphur dioxide treatment, almost all the parameters showed a considerable decrease, except the carbohydrate content of plant, which increased on exposure to sulphur dioxide. Table 1 shows the effect of sulphur dioxide on total chlorophyll, phaeophytin and carotenoid content of leaf discs of *M. sylvestris*. General decrease was observed in chlorophyll content with 33.7%, 51.7%, 54.1% and 55.4% decrease at 250ppm, 500ppm, 750ppm and 1000ppm sulphur dioxide concentration respectively. In case of total phaeophytin, 41%, 59.7%, 63.4% and 53.5% decrease was observed at 250ppm, 500ppm, 750ppm and 1000ppm sulphur dioxide concentration respectively. Total carotenoids got decreased by 9.3%, 18.5%, 24.4% and 8.17% at 250ppm, 500ppm, 750ppm and 1000ppm concentration of sulphur dioxide respectively.

Table 1: Effect of different concentrations of aqueous SO_2 on pigment content of *Malva sylvestris*

PARAMETER	CONCENTRATION (ppm)				
	Control	250	500	750	1000
Chlorophyll (µg/ml)	5.469±0.08	3.627±0.04 (-33.7)	2.638±0.05 (-51.7)	2.507±0.05 (-54.1)	2.438±0.06 (-55.4)
Phaeophytin (µg/ml)	7.333±0.18	4.3±0.06 (1-4.3)	2.95±0.12 (-59.7)	2.684±0.12 (-63.4)	3.403±0.04 (-53.5)
Carotenoids (µg/ml)	2.506±0.05	2.273±0.06 (-9.3)	1.797±0.05 (-18.5)	1.667±0.05 (-24.4)	2.026±0.10 (-8.17)

Data represent the average of three samples analysed separately ± S.D.

Values in brackets represent % decrease (-) compared to the control.

Table 2 shows the effect of different concentrations of aqueous sulphur dioxide on carbohydrate, starch and phenolic content of *M. sylvestris*. Carbohydrate content showed a general increase on sulphur dioxide treatment. As compared to control, 10.7%, 28.7%, 29.4% and 23.4% increase was observed at 250ppm, 500ppm, 750ppm and 1000ppm sulphur dioxide concentrations respectively. Starch content in *M. sylvestris* was found to decrease by 7%, 15.6%, 32% and 5.8% at 250ppm, 500ppm, 750ppm and 1000ppm sulphur dioxide respectively. Phenolic content was also found to decrease on exposure to sulphur dioxide. At 250ppm concentration of SO_2, 13.65% decrease was observed in the phenolic content of 25%, 40.9% and 63.6% decrease was observed at 500ppm, 750ppm and 1000ppm aqueous sulphur dioxide concentration respectively.

Table 2: Effect of different concentrations of aqueous SO_2 on carbohydrate, starch and phenolic content of *Malva sylvestris*

PARAMETER	CONCENTRATION (ppm)				
	Control	250	500	750	1000
Carbohydrates (mg/ml)	0.144±0.007	0.160±0.007 (+10.7)	0.186±0.008 (+28.7)	0.187±0.008 (+29.4)	0.179±0.08 (+23.4)
Starch (mg/ml)	2.55±0.04	2.37±0.04 (-7)	2.15±0.02 (-15.6)	1.71±0.01 (-32)	2.40±0.03 (-5.8)
Phenolics (mg/ml)	0.044±0.002	0.038±0.001 (-13.6)	0.033±0.001 (-25)	0.026±0.007 (-40.9)	0.016±0.006 (-63.6)

Data represent the average of three samples analysed separately ± S.D.

Values in brackets represent % increase (+) or % decrease (-), compared to control.

Table 3 shows the effect of different concentrations of aqueous sulphur dioxide on protein and amino acid content of *M. sylvestris.* As compared to the control, 0.44%, 17.4%, 11.6% and 4.9% decrease was observed in the protein content at 250 ppm, 500 ppm, 750 ppm and 1000 ppm aqueous sulphur dioxide respectively. General decrease was observed in amino acid cotent of *M. sylvestris,* i.e., 72 %, 10.1 %, 15.2 % and 39.1 % at 250 ppm, 500 ppm, 750 ppm and 1000 ppm respectively.

Table 3: Effect of different concentrations of aqueous SO_2 on protein and amino acid content of *Malva sylvestris*

PARAMETER	CONCENTRATION (ppm)				
	Control	250	500	750	1000
Proteins (mg/ml)	2.23±0.108	2.22±0.108 (-0.44)	1.84±0.05 (-17.4)	1.97±0.105 (-11.6)	2.12±0.106 (-4.9)
Amino acids (mg/ml)	0.138±0.014	0.128±0.010 (-7.2)	0.124±0.010 (-10.1)	0.117±0.006 (-15.2)	0.084±0.004 (-39.1)

Data represent the average of three samples analyzed separately ± S.D.
Values in brackets represent % decrease (-), compared to control.

DISCUSSION

Sulphur dioxide is highly phytotoxic and causes much damage to plants leading to loss of productivity. Sulphur dioxide has multiple effects like destruction of chlorophyll at higher concentration (Vogelman and Borett, 1988) and interference with various enzymes (Niewiadomska *et al*., 1997), leading to the destruction of various plant components. Sulphur dioxide influences the chlorophyll content by various mechanisms like increased acidity, bleaching, conversion to phaeophytin by splitting Mg^{2+} and forming complexes with proteins (Rao and Le-Blanc, 1966). Different concentrations of aqueous sulphur dioxide were used to evaluate the effect of sulphur dioxide on *M. sylvestris*. Decrease in starch content could be attributed to the destruction of photosynthetic pigments on exposure to sulphur dioxide. The increase in content of free sugars might be due to the breakdown of polysaccharides by the action of hydrolytic enzymes that get activated by sulphur dioxide treatment.

In the present study, a decrease was observed in the phenolic content of leaf discs, on exposure to sulphur dioxide. This might be due to the reduction in the amount of shikimic acid by sulphur dioxide treatment (Katzel and Moller, 1993). Phenolics are formed from shikimate via shikimic acid pathway (Kailunainen, 1995).

Higher concentrations of sulphur dioxide may break enzymes and protein disulphide bonds into thiosulphonates and thiols. This might be the reason for observed decrease in protein content of the plant. Changes in amino acid content could be due to the disturbance in synthesis of amino acids, as sulphur dioxide affects whole nitrogen metabolism of plants (Godzik and Linsken, 1974). The decrease in protein and amino acid content might also be due to the loss of ultrastructural organization of the cells and destruction of ribosomes on exposure to sulphur dioxide (Soikkeli and Tuovinnen, 1979).

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