Sulphite Toxicity on spinach (*Spinacea oleracea*)

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ABSTRACT

Spinach (*Spinacea oleracea*) was screened for its resistance against sulphur dioxide. On treatment of plant leaf discs with different concentrations of aqueous sulphur dioxide under illumination for 4 hours, the plant showed a decrease in total chlorophyll content. Similar pattern was obtained for total phaeophytin and total carotenoids. In case of carbohydrate and starch content, a general increase was observed at all concentrations. Total phenolics showed a decrease. Total protein content showed an increase at low levels of sulphur dioxide and a decrease at high concentrations. On the other hand, in case of total amino acid content, a decrease was observed.

***Key words*:** Effect, *Spinacea oleracea,* sulphur dioxide.

INTRODUCTION

Air pollution is a problem being aggravated since 1950 in all parts of the world. The major sources of air pollution are industries, thermal power generations and fossil fuel driven vehicles. Examples of traditional air pollutants include sulphur dioxide, nitrogen oxides, carbon monoxide, hydrocarbons, volatile organic compounds, hydrogen sulphide, Peroxy acetyl nitrate and particulate matter. Sulphur dioxide is reported as the most widespread phytotoxic air pollutant both in India (Kumar and Prakash, 1977; Pavgi *et al*., 1991) as well as in other parts of the world (Ali, 1993; Vesala *et al*., 1995; Noji *et al*., 2001; Hao *et al.,* 2002). SO_2 is a colourless, corrosive, non-flammable gas with irritating and pungent odour. It is highly soluble in water (22% by weight at 0°C) and is ionized in a manner depending upon the pH of the solution. At pH 7.0, it exists largely in the form of bisulphite and sulphite ions (Puckett *et al*., 1973). Sulphur dioxide has direct effects on vegetation. These effects cause temporary and permanent injury to the plant/vegetation. The effect of SO_2 on vegetation have been well reviewed in terms of foliar injury and physiological and biochemical alterations (Malhotra and Khan, 1984; Anuradha *et al*., 1999; Krupa *et al*., 2001; Vorobeichik, 2002; Agrawal *et al*., 2003; Yi *et al*., 2005). Sulphur dioxide is reported to interfere with the structure and permeability of cellular membranes and with enzyme activity, which affect many biochemical processes in the cell. (Thomas *et*

al., 1950; Wellburn *et al*., 1972). In this paper we present the response of spinach (*Spinacea oleracea*) to SO_2 stress.

MATERIAL AND METHODS

Fresh young leaves of Spinach (*Spinacea oleracea*) grown during the 2005-growing season were used as the starting material for chemical evaluation of vegetation for SO2 resistance.

(i) Sampling of Tissue: Fresh young leaves of the plant were collected, washed, and patted dry, cut into piece, weighed & used for the preparation of homogenate. 10% (w/v) homogenate was prepared by homogenizing a weighed sample of plant leaves in a measured volume of distilled water. The homogenate was centrifuged at 5000 rpm for 10 min. The supernatant was carefully decanted & subsequently used for various estimations.

(ii) Treatment of Leaf Discs with Aqueous Sulphur Dioxide: 1g of leaf discs of equal dimensions (1cm dia) from Spinach (Spinacea oleracea), were treated with different concentrations of aqueous sulphur dioxide for 4 hours' in glass petridishes under light provided by a 100 W tungsten electric bulb. Different concentrations of aqueous SO_2 used were 250ppm, 500ppm, 750ppm and 1000ppm Parallel control was also run. After 4 hours, the experimental and control leaf discs were separated, washed, patted dry, weighed and 10% (w/v) homogenate was prepared.

(iii) Estimation of Pigments: Chlorophyll, phaeophytin and carotenoids were extracted in 80% acetone and estimated spectrophotometrically according to the methods of Strain *et a*l. (1971), Vernon (1960) and Duxbury and Yentech (1956) respectively.

(iv) Estimation of Proteins: Protein concentration was determined by the method of Lowry et al. (1951). Absorbance change was monitored on ELICO SL-27 spectrophotometer.

(v) Estimation of Carbohydrates: The estimation of carbohydrates was done by Phenol-sulphuric acid method given by Montgomery (1982).

(vi) Estimation of Amino acids: Estimation of amino acids was carried out by the method of Lee and Takahashi (1966).

(vii) Estimation of Starch: Estimation of starch was done by the method of Agarwal *et al*. (1982).

(viii) Estimation of Phenolics: The estimation of phenolics was carried out by the method given by Malick and Singh (1980).

RESULTS

The effect of sulphur dioxide on leaf discs of spinach (*Spinacea oleracea*) was evaluated in terms of effect on its biochemical parameters.

(i) Effect on Biochemical Parameters and Photosynthetic Pigments of the Plant.

Tables 1 and 2 show the effect of different concentrations of aqueous sulphur dioxide on total chlorophyll, total phaeophytin and total carotenoids. A general decrease of chlorophyll content was observed in the plant at all concentrations. In case of total chlorophyll, a decrease of 45.13%, 66.21%, 50.54%, and 39.45% was observed at 250ppm, 500ppm, 750ppm and 1000ppm respectively as compared with control.

Similar pattern was observed for total phaeophytin and total carotenoids. At 250 ppm, 500 ppm, 750 ppm and 1000ppm, a decrease of 47.16%, 65.55%, 51.07% and 41.87% respectively was observed, as compared with control, in phaeophytin content. In case of total carotenoids, values again showed a decrease of 41.54%, 57.39%, 44.71% and 41.90% at 250ppm, 500ppm, 750ppm and 1000ppm respectively, as compared with control.

(ii) Effect on Total Carbohydrates, Starch and Phenolics.

Table 3 represents the information about the total carbohydrates, starch and phenolics in treated and control leaf discs of *Spinacea oleracea*.

In case of carbohydrate and starch content, a general increase was observed at all concentrations. At 250ppm, 500ppm, 750ppm and 1000ppm, an increase of 1.12%, 6.74%, 15.73% and 26.96% respectively was observed, as compared with control, in carbohydrate content. In case of starch content, an increase of 69.47%, 71.57%, 73.68% and 94.73% was observed at 250ppm, 500ppm, 750ppm and 1000ppm respectively, as compared with control. Total phenolics showed a decrease of 9.52%, 28.57%, 23.80% and 16.66% at 250ppm, 500ppm, 750ppm and 1000ppm respectively, as compared with control.

(iii) Effect on Proteins and Amino acids

The effects of different concentrations of aqueous sulphur dioxide on protein content and free amino acids of leaf discs of *Spinaceae oleracea* are shown in Table 4. Total protein content showed an increase at low levels of sulphur dioxide and a decrease at high concentrations .At 250ppm and 500ppm, an increase of 2.34% and 16.40% respectively was observed. While as a decrease of 3.90% and 18.75% was observed respectively at 750ppm and 1000ppm, as compared with control, in protein content. On the other hand, in case of total free amino acid content, a decrease of 13.33%, 16.66%, 25% and 45% was observed respectively at 250ppm, 500ppm, 750ppm and 1000ppm, as compared with control.

DISCUSSION

Sulphur dioxide is highly phytotoxic causing much damage to plants and hence much loss of productivity. The multiple effects of sulphur dioxide/ sulphite on metabolism include chlorophyll destruction i.e. photosynthetic pigments (Vogelmann and

Borret, 1988) and interference with various enzyme activities (Niewiadomska *et al*., 1997). These initiate peroxidative processes lead to destruction of plant components. In our studies chlorophyll content got decreased which could be due to the formation of sulphurous acid (H_2SO_3), formed by the combination of sulphur dioxide with water in the plant tissue. This H_2SO_3 dissociates into H^+ and HSO_3^- ions, which cause degradation of chlorophyll by displacing Mg^{2+} ions by free H^+ ions (Rao and Le Blanc, 1966). Further, decrease in both phaeophytin and chlorophyll content could be due to decrease in overall enzymatic process and destruction of chlorophyll.

Sulphur dioxide/ sulphite exposure affected the starch content and carbohydrate content of plant leaf discs under study. There was a general increase in starch content and carbohydrate content, usually more marked at 1000ppm which could be due to the wider opening of stomatal apparatus for a longer duration in presence of sulphur dioxide as also reported by many workers (Mansfield and Freer-Smith, 1984; Bytnerowicz and Andrzej, 1996). The increase in carbohydrate content could be the result of breakdown of polysaccharides (Farooq and Beg, 1982).

Studies on changes in protein contents as a result of sulphur dioxide exposure revealed that the protein content increased at low levels and decreased at high concentrations (Spruged *et al*., 1980, Prasad and Rao, 1981a; Saxe, 1983), which was corroborated by our studies on *Spinacea oleracea*. It is earlier reported that a continuous short-term fumigation of plants with higher concentration of sulphur dioxide leads to a decrease in the protein content of leaf (Godzik and Linsken, 1974; Malhotra and Sarkar, 1979). Higher concentration of sulphur dioxide may break enzymes and proteins with disulphide bonds into thiosulphonates and thiols. Changes in amino acid content, and inactivation of enzyme in the sulphur dioxide treated plant leaf discs was held responsible for the decrease in protein synthesis (Prasad and Rao, 1982). The decrease could also be due to loss of ultrastructural organization of leaf cell and destruction of ribosomes (Mlodzinowski and Bialobok, 1977; Soikkeli and Tuovinnen, 1979). The increase in protein content at lower concentrations could be due to the utilization of sulphur for synthesis of more amino acids.

Changes in the level of amino acid after sulphur dioxide exposure was related to the disturbances in the synthesis of amino acids or proteins, as sulphur dioxide affects the whole nitrogen metabolism of plants (Godzik and Linskens, 1974). The decrease in total amino acid content as observed in our study may be due to the utilization of more and more amino acids content for protein synthesis. The decrease in amino acid pool has been attributed to the destruction of various amino acids like methionine and tryptophan during aerobic oxidation of sulphite (Yang, 1973).

Phenolics play an important role in the defense of plants against infection and their contents may vary during injury. In our studies, a decrease in total phenolic content was

observed. Practically in all higher plants, phenolics are formed from shikimate via shikimic acid pathway (Kainulainen, 1995). The amount of shikimic acid has been observed to diminish by sulphur dioxide treatment (Katoch *et al*., 1989 b; Katzel and Moller, 1993). This might be a reason for a decrease in phenolic content.

TABLE 1: Effect of different concentrations of aqueous sulphur dioxide on chlorophyll content in leaf discs of spinach

Parameter	Concentration (ppm)				
	Control	250	500	750	1000
Chlorophyll 'a' (µg/ml)	2.30±0.04	1.37±0.03 (-40.43)	0.90±0.02 (-60.86)	1.32±0.03 (-42.60)	1.36±0.03 (-40.86)
Chlorophyll 'b' (µg/ ml)	1.40±0.03	0.66±0.008 (-52.85)	0.34±0.005 (-75.71)	0.50±0.007 (-64.28)	0.87±0.02 (-37.85)
Total Chlorophyll (µg/ml)	3.70±0.06	2.03±0.04 (-45.13)	1.25±0.03 (-66.21)	1.83±0.035 (-50.54)	2.24±0.04 (-39.45)

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

TABLE 2: Effect of different concentrations of aqueous sulphur dioxide on phaeophytin and carotenoid content in leaf discs of spinach

Parameter	Concentration (ppm)				
	Control	250	500	750	1000
Phaeophytin'a' (µg/ml)	3.04±0.06	1.68±0.025 (-44.73)	1.14±0.02 (-62.5)	1.54±0.035 (-49.34)	1.71±0.035 (-43.75)
Phaeophytin 'b' (µg/ml)	4.20±0.07	2.53±0.045 (-39.76)	1.62±0.03 (-61.42)	2.47±0.04 (-41.19)	2.57±0.045 (-38.80)
Total phaeophytin (µg/ml)	5.11±0.08	2.70±0.05 (-47.16)	1.76±0.035 (-65.55)	2.50±0.045 (-51.07)	2.97±0.06 (-41.87)
Carotenoids (µg/ml)	2.84±0.055	1.66±0.03 (-41.54)	1.21±0.02 (-57.39)	1.57±0.03 (-44.71)	1.65±0.03 (-41.90)

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

TABLE 3: Effect of different concentrations of aqueous sulphur dioxide on carbohydrate content, starch and phenolics in leaf discs of spinach

Parameter	Concentration (ppm)				
	Control	250	500	750	1000
Carbohydrates (mg/ml)	0.089±0.005	0.09±0.005 (+1.12)	0.095±0.006 (+6.74)	0.103±0.006 (+15.73)	0.113±0.007 (+26.96)
Starch (mg/ ml)	0.95±0.05	1.61±0.11 (+69.47)	1.63±0.011 (+71.57)	1.65±0.11 (+73.68)	1.85±0.13 (+94.73)
Phenolics (mg/ml)	0.042±0.004	0.038±0.0025 (-9.52)	0.030±0.002 (-28.57)	0.032±0.0023 (-23.80)	0.035±0.0025 (-16.66)

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

TABLE 4: Effect of different concentrations of aqueous sulphur dioxide on the protein content and amino acids in leaf discs of spinach

Parameter	Concentration (ppm)				
	Control	250	500	750	1000
Proteins (mg/ ml)	1.28±0.08	1.31±0.08 (+2.34)	1.49±0.09 (+16.40)	1.23±0.08 (-3.90)	1.04±0.04 (-18.75)
Amino acids (mg/ ml)	0.12±0.007	0.104±0.007 (-13.33)	0.10±0.007 (-16.66)	0.09±0.005 (-25)	0.066±0.004 (-45)

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

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