Effect of Sulphur Dioxide on Biochemical Parameters of *Brassica oleracea*

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ABSTRACT

Haak (*Brassica 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 but a general increase in carotenoid and phaeophytin was observed. In case of free sugars and starch content, sugar content showed a decrease and there was increase in starch content with maximum increase at lower concentrations of sulphur dioxide. Total phenolics showed a slight increase. Total protein content showed a marked decrease and similar trend was observed for amino acids with maximum decrease at lower concentrations of sulphur dioxide.

Key words: *Brassica oleracea* , sulphur dioxide, sulphite

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; Ganai *et al*., 2007 and Dar *et al*., 2008). 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; Amer *et al.*, 1989, 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 Haak (*Brassica oleracea*) to SO_2 stress.

MATERIAL AND METHODS

Fresh young leaves of Haak (*Brassica oleracea*) grown during the 2005-2006 growing season were used as the starting material for chemical evaluation of vegetation for SO_2 resistance.

(i) **Sampling of tissue**

Fresh young leaves of the plant were collected, washed, and patted dry, cut into pieces, weighed and 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 and subsequently used for various estimations.

(ii) **Treatment of leaf discs with aqueous sulphur dioxide**

1g of leaf discs of equal dimensions (1cm dia) from Haak (*Brassica 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 al.* (1971);Vernon (1960) ; 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 free sugars 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 Haak (*Brassica oleracea*) was evaluated in terms of effect on its biochemical parameters.

I. **Effect of different concentrations of aqueous sulphur dioxide on photosynthetic pigments of the** (*Brassica oleracea*)

Table 1 shows 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 5.22%, 12.62%, 1.83 %, and 5.29% was observed at 250ppm, 500ppm, 750ppm and 1000ppm respectively as compared with control.

Table 1. Effect of different concentrations of aqueous sulphur dioxide on pigment content in leaf discs of *Brassica oleracea*

Parameter	Concentration (ppm)				
	Control	250	500	750	1000
Total Chlorophyll (µg/ml)	1.19±0.01	1.13±0.05 (-5.22)	1.039±0.002 (-12.62)	1.167±0.025 (-1.83)	1.126±0.025 (-5.29)
Total phaeophytin (µg/ml)	1.329±0.048	1.487±0.056 (+11.88)	1.50±0.055 (+12.52)	1.71±0.067 (+28.53)	1.72±0.06 (+28.61)
Carotenoids (µg/ml)	0.81±0.022	0.891±0.03 (+10.03)	0.89±0.027 (+9.8)	1.08±0.035 (+33.24)	1.075±0.03 (+33.2)

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

A general increase was observed for total phaeophytin and total carotenoids. At 250 ppm, 500 ppm, 750 ppm and 1000ppm, an increase of 11.88 %, 12.52 %, 28.53 % and 25.25 % respectively was observed, as compared with control, in phaeophytin content. In case of total carotenoids, values again showed an increase of 10.03%, 9.8%, 33.24% and 31.30% at 250ppm, 500ppm, 750ppm and 1000ppm respectively, as compared with control.

II **Effect of different concentrations of aqueous sulphur dioxide on total free sugars, starch and phenolics.**

Table 2 represents the information about the total free sugars, starch and phenolics in treated and control leaf discs of (*Brassica oleracea*).

Table 2. Effect of different concentrations of aqueous sulphur dioxide on free sugar content, starch and phenolics in leaf discs of *Brassica oleracea*

Parameter	Concentration (ppm)				
	Control	250	500	750	1000
Free sugars (mg/ml)	0.143±0.007	0.135±0.005 (-5.59)	0.142±0.007 (-0.69)	0.11±0.004 (-20.27)	0.120±0.0045 (-16.08)
Starch (mg/ml)	0.146±0.07	0.198±0.03 (+35.61)	0.184±0.004 (+26.02)	0.166±0.006 (+13.68)	0.156±0.008 (+6.84)
Phenolics (mg/ml)	0.058±0.004	0.059±0.0035 (+1.72)	0.059±0.004 (+1.72)	0.062±0.005 (+6.89)	0.061±0.005 (+5.17)

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

In case of free sugars, a decrease of order as 5.59%,0.69%,20.27% and 16.08 %was observed at 250ppm, 500ppm, 750ppm and 1000ppm, and an increase of 35.61%, 26.02%, 13.69% and 6.84% respectively was observed for starch content, as compared with control. Total phenolics showed a slight increase of 1.72%, 1.72%, 6.89% and 5.17% at 250ppm, 500ppm, 750ppm and 1000ppm respectively, as compared with control.

III. **Effect of different concentrations of aqueous sulphur dioxide 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 (*Brassica oleracea*) are shown in Table 3. Total protein content showed a decrease and was of order 29.38%, 35.10%,39.08% and 35.51%. On the other hand, in case of total free amino acid content, a decrease of 68.65%, 7.46%, 16.41% and 93.97% was observed respectively at 250ppm, 500ppm, 750ppm and 1000ppm, as compared with control.

Table 3. Effect of different concentrations of aqueous sulphur dioxide on the protein content and amino acids in leaf discs of *Brassica oleracea*

Parameter	Concentration (ppm)				
	Control	250	500	750	1000
Proteins (mg/ml)	2.45±0.115	1.73±0.07 (-29.38)	1.59±0.063 (-35.10)	1.49±0.053 (-39.08)	1.58±0.063 (-35.51)
Amino acids (mg/ ml)	0.134±0.06	0.042±0.004 (-68.65)	0.124±0.05 (-7.46)	0.112±0.004 (-16.41)	0.084±0.06 (-93.97)

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

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 leading 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). And opposite trend for phaeophtin and carotenoids was observed.

Sulphur dioxide/ sulphite exposure affected the starch content and free sugar content of plant leaf discs under study. There have been found to be a general increase in starch content and decrease in free sugar content. This could be due to the wider opening of stomatal apparatus for a longer duration in presence of sulphur dioxide as reported by many workers (Mansfield and Freer-Smith, 1984; Bytnerowicz and Andrzej, 1996). The decrease in free sugars could be due to the destruction of chlorophyll which adversely affects the rate of photosynthesis because of the competition between CO_2 and SO_2 for the carboxylase enzyme (Ziegler, 1973).

Phenolics play an important role in the defense of plants against infection and their contents may vary during injury. In our studies, a slight increase in total phenolic content was observed. Practically in all higher plants, phenolics are formed from shikimate via shikimic acid pathway (Kainulainen *et al.*, 1995). SO_2 might may be stimulating the enzymes necessary for the production of secondary metabolites (Raza and Gouri, 1996; Wu *et al.*, 1999).

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, 1981; Saxe, 1983). 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, inactivation of enzyme in the sulphur dioxide treated plant leaf discs may be 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).

Changes in the level of amino acid after sulphur dioxide exposure may be due 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 could be due to the destruction of various amino acids like methionine and tryptophan during aerobic oxidation of sulphite (Yang, 1973).

REFERENCES

Agarwal, M., Nandi, P.K. and Rao, D.N. 1982. Effect of ozone and sulphur pollutants separately and in mixture on chlorophyll and cartenoid pigments of *Oryza sativa*. *Water, Air and Soil Pollutant*, **18**:449-454.

Agarwal, M., Singh, B., Rajput, M., Marshal, F. and Bell, J.N. 2003. Effect of air pollution on peri-urban agriculture: a case study. *Environ Pollut.*, **126**(3): 323-9.

Ali, A.E. 1993. Damage to plants due to industrial pollution and their use as bio indicators in Egypt. *Env. Pollut.*, **81**: 251-255.

Amer, S.M., Mikhael, E. and El-Ashry, Z.M. 1989. Cytogenetic effect of sulfur dioxide on *Vicia faba* plant. I. *Cytologia*, **54**: 211-221.

Anuradha, D., Siddiqui, S.A., Dubey, B.C. and Dube, A. 1999. Studies on effect of sulphur dioxide on bio chemical aspects of groundnut. *Advances in Plant Science Research in India*, **10**:75-79.

Bytnerowicz and Andrzej. 1996. Physiological aspects of air pollution stress in forests. *Phyton (Horn.)*, **36**:15-22.

Dar, A.A., Ganai, B.A. and Sheikh, Q.A. 2008. Effect of aqueous sulphur dioxide on *Rumex hestata*. *J. Ind. Poll. Contrl.*, **1**: 1-8.

Duxbury, A.C. and Yentsch, C.S. 1956. Plankton pigment monographs. *J. Mar. Res.*, **15**:19-101.

Ganai, B.A., Aliya, A., Masood, A., Zargar, M.A. and Ganie, S.A. 2007. Sulphite toxicity on spinach (*Spinacea oleracea*). *J. Res. Dev.*, **7**:109-118.

Ganai, B.A., Nowsheen, Q., Masood, A., Zargar, M.A. and Javid, I.B. 2007. Effect of sulfur dioxide on *Malva syslvestris*. *Journal of Research and Development*, **7**: 19-24.

Godzik, S. and Linskens, H.F. 1974. Concentration changes of free amino acids in primary bean leaves after continuous and interrupted sulphur dioxide fumigation and recovery. *Environ. Pollut.*, **7**: 25-38.

Hao, J., Li, J., Duan, L., He, K. and Dai, W. 2002. Valuation of forest damage cost from SO_2 emission: a case study in Hunan Province, China. *Huan Jing Ke Xue*, **23**(6): 1-5.

Kainu, Flainen, P., Holopainen, J.K. and Okasanea, J. 1995. Effects of sulphur dioxide on the concentration of carbohydrates and secondary compounds in Scots pine and Norway spruce seedlings. *New Phytol.*, **130**: 231-238.

Krupa, S.V. and Legge, A.H. 2001. Saskatoon serviceberry and ambient SO_2 exposures: study sites revisited. *Environ. Pollut.*, **111**(3): 363-5.

Kumar, S. and Prakash, C.B. 1977. Air pollution for sulphur dioxide emission in India. *Chemical Age of India*, **28**:465-472.

Lee, Y. P. and Takahashi, T. 1966. An improved colorimetric determination of amino acid with the use of ninhydrin. *Anal. Biochem.*, **14**: 71-77.

Lowry, O.H., Rose Brough, N. J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275.

Malhotra, S.S. and Khan, A.A. 1984. Biochemical and physiological impact of major pollutants. p. 113-157. In: *Air Pollution and Plant Life (M. Treshow, ed): John Wiley, Chichester.*

Malhotra, S.S. and Sarkar, S. K. 1979. Effect of sulphur dioxide on sugar and free amino acid content of pine seedlings. *Physiol. Plant,* **47**: 223-228.

Malick, C.P. and Singh, M.B. 1980. *Plants Enzymology and Histo-enzymology. Kalyani Publisher, New Delhi.*

Mansfield, T.A. and Freer-Smith, P.H. 1984. The role of stomata in resistance mechanism. In: *Gaseous Air Pollutants and Plant Metabolics.* Koziol and Whatley, eds. Worths, London.

Mlodzionowski, F. and Bialobok, S. 1977. The effect of sulphur dioxide on ultra structural organization of larch needles. *Acta Societatis Botani Corum Po-loniae,* **46**: 629-634.

Montgomery, R. 1982. Determination of gylcogen by Phenol- sulphuric acid method. *Arch. Biochem. Biophys.*, **67**: 378-386.

Niewiadomska, E. and Miszalski, Z. 1997. Determination of some oxidative stress parameters in variegated leaves of *Chorophytum cosmosum* (thunb) Bak. *Acta Physiol. Planta*, **19**: 33-39.

Noji, M., Saito, M., Nakamura, M., Aono, M., Saji, H. and Saito, K. 2001. Cysteine – synthase over expression in tobacco confers tolerance to sulphur containing environmental pollutants. *Plant Physiology,* **126**: 973-980.

Pavgi, S., Farooq, M., Venkateshwar, C. and Beg, M.U. 1991. Physiological and Biochemical effects of sulphur dioxide on wheat varieties. *Environment and Ecology,* 9:760-765.

Prasad, B.J. and Rao, D.N. 1981. Growth responses of *Phaseolus aureus* to petrocoke pollution. *J. Exp. Bot.*, **32**:1343.

Prasad, B. J. and Rao, D.N. 1982. Relative sensitivity of a leguminous and a cereal crop to SO_2 pollution. Environ. *Pollution,* **29**(Ser.A) 57-70.

Puckett, K., Nieboer, E., Flora, W.P. and Richardson, D.H.S. 1973. Sulphur dioxide. Its effect on photosynthetic, 14 C fixation and suggested mechanism of phytotoxicity. *New Phytologist.*, **72**:141-154.

Raza, S.H. and Gouri, K. 1996. Effect of sulphur dioxide toxicity (pollutant) and ascorbic acid (antioxidant) on Vikas and Prasanna varietes of paddy. *J. Indian Bot. Soc.*, **75**:279-282.

Rao, D.N. and Le- Blanc, F. 1966. Effect of sulphur dioxide on the lichen algae with special reference to chlorophyll. *Bryologist,* **69**:69.

Saxe, H. 1983. Long term effects of low levels of SO_2 on bean plants *(Phaseolus vulgaris)* II. Immission response effects on biomass production: Quantity and Quality. *Physiol. Plant,* **57**: 108.

Soikkeli, S. and Tuovinnein, T. 1979. Damage in mesophyll ultrastructure of needle of Norway spruce in two industrial environments in Central Finland. *Annales Botanici*

Fennici, **16**:50-64.

Spruged, D.G., Muller, J.E., Smith, H.J and Xerikos, P.B. 1980. Sulphur dioxide effects on yield and seed quality in field grown soybeans *(Glycine max cv. Wells). Phytopathol.* **70**: 1129.

Strain, H.H., Bengavin, T.C. and Walter, A.S. 1971. Analytical procedure for isolation, identification, estimation, investigation of chlorophyll. *In: Methods in Enzymology (A.S.Pietro ed.), New York, and Academic Press*, **23**:452-476.

Thomas, M.D., Hendricks, R.H. and Hill, G.R. 1950. Sulphur metabolism on plants: Effect of sulphur dioxide on vegetation. *Industrial Engineering Chemistry*, **42**: 2231-2235.

Vernon, L.P. 1960. Spectrophotometeric determination of chlorophylls and phaeophytins in plant extracts. *Anal. Chem.*, **32**: 1144-1150.

Vesala, T., Hameri, K., Ahonen, T. and Kulmels, M. 1995. Experimental and numerical analysis of stomatal absorption of sulphur dioxide and transport by pine needles. *Atmospheric Environ.*, **29**: 825-830.

Vogelmann, F. A. and Borret, N. 1988. Effect of SO_2 on photosynthetic pigments. *Environmental Experimental Botany*, **28**: 19-25.

Vorobeichik, E.L. 2002. Changes in spatial structure of the destruction process under the conditions of atmospheric pollution of forest ecosystems. *Izv Akad Nauk Ser Biol.*, **3**: 368-79.

Wellburn, A.R., Magernie, O. and Wellburn, F.A.M. 1972. Effects of sulphur dioxide and nitrogen dioxide polluted air upon the ultra structure of chloroplasts. *Environ. Pollut.*, **3**:37.

Wu, Z. X., Han, D. M., Ji, Z. L. and Chen, W. X. 1999. Effect of sulphur dioxide treatment on enzymatic browning of logan pericarp during storage.*Acta-Horticulture-Sinica*, **26**:91-95.

Yang, S.F. 1973. Destruction of tryptophan during the aerobic oxidation of sulphite ions. *Environ. Res.*, **6**: 395-402.

Yi, H., Liu, J. and Zheng, K. 2005. Effect of sulphur dioxide hydrates on cell cycle, sister chromatid exchange and micronuclei in barley. *Ecotoxicol Environ Saf.*, **62** (3): 421-26.

Ziegler, I. 1973. Effect of sulfite on PEP-carboxylase and malate formation in extracts of *Zea mays*. *Phytochemistry*, **12**: 1027-1030.