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Phytotoxic Effects of So_2 on Crop Plants --Abiotic Stress and Reducing Sugars

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Abstract:
Effect of different SO_2 concentrations on the reducing sugar content in the leaves of three economically important plant species, viz., Solanum esculentum (= Lycopersicon esculentum)[Tomato], Vigna radiata (Mung bean) and Zea mays (Maize) was studied . Controlled fumigation experiments were carried out using three different treatments of SO_2 : T-1 = 0.05 ppm(134.0µg m^{-3} SO_2) [x 4h], T-2 = 0.1 ppm(268.0 µg m^{-3} SO_2) [x 2h] and T-3 = 0.2 ppm(536.0 µg m^{-3} SO_2) [x 1h] for 60 days. In Maize, the exposure period was extended to 75 days. All the three plant species recorded an increase in sugar content following SO_2 exposure. Maximum increase in reducing sugar content was observed in S.esculentum, followed by V.radiata and Z.mays . Changes in reducing sugar content seems to point towards a shift in the energy budget in order to make energy readily available for repair/replacement of tissue damaged by SO_2 toxicity. Reducing sugar content can be used as a reliable indicator of the metabolic stress state of plants in the absence of any visible injury symptoms.

Keywords: *SO_2, Controlled-fumigation , Reducing sugar content, Tomato, Mung bean, Maize, Energy budget, Statistical regression model*

1. Introduction

Sulphur dioxide has been recognized as one of the most potent phytotoxicants, capable of causing extensive damage to vegetation. Despite a decline in global SO_2-emissions in the past decade [i], India has recorded and increase of this pollutant by over 70% during the same period.

Plant responses to SO_2-exposure are complex and involve a series of physiological and/or biochemical changes occurring at the cellular level. Such changes may well serve as primary indicators of latent plant injury and include gross alterations in enzyme activity, lipid biosynthesis, amino acid and chlorophyll content, inhibition of photosynthetic processes, volatile emissions, and energy translocation[ii-viii]. Among others, reducing sugars seem to be a useful parameter to assess metabolic disorders caused by SO_2-stress. Present investigations on three economically important plants were made to study the levels of soluble vis-à-vis the mechanisms of plant tolerance to SO_2 -stress.Significance of individual and interactive effects of SO_2concentration and exposure time upon the reducing sugar contents has been analyzed by statistical regression model.

2. Material and Methods

Three economically important cultivated plant species viz., *Solanumesculentum* [Tomato],*Vignaradiata* (L.) Wilczek [Mung bean], and *Zea mays* L. [Maize] were grown from seeds in the nursery. Fifteen-day-old seedlings of these plants were subjected to different SO_2 treatments through an artificial fumigation system. Sulfur dioxide was generated by bubbling $Na_2S_2O_5$ in water and circulated in closed-top fumigation chambers (1 x 1 x1m= 1m3) at temperatures ranging between 25-29^{0}C ± 1^{0}C and at a RH of 60 ± 5%. Two 200W metal halide lamps were used for illumination with a light/dark cycle of 12/12 hours.

2.1. Treatment protocols of SO_2

T-1 = 0.05 ppm (134.0µg m^{-3} SO_2) [x 4h], T-2 = 0.1 ppm(268.0µg m^{-3} SO_2) [x 2h] and T-3 = 0.2 ppm(536.0µg m^{-3} SO_2) [x 1h] for 60 days, thus keeping the SO_2 dose constant. *V. radiata* was fumigated for only 45 days. Controls(C) were maintained simultaneously by exposing the plants to air alone.

2.2. Estimation of Reducing Sugars

Fresh leaf tissue (0.2g) was homogenized with 80% aqueous ice cold ethanol. The homogenate was centrifuged at 1500 x g for 15 min in a K-24 refrigerated centrifuge. The final volume of the supernatant was made up to 10.0 ml with ethanol. Reducing sugars in the leaf tissue were estimated by the anthrone reaction. A blue-green complex was formed after treatment of the samples (5.0ml) with 10.0ml of 0.2% chilled anthrone reagent. The extinction E was measured at 620nm with a Spectronic 20 spectrophotometer.

2.3. Statistical Analysis

Analysis of variance (ANOVA) and multiple regression analysis were employed to test the significance of individual as well as interactive effects of SO_2 concentration (ppm) and the exposure time (h) upon total chlorophyll content. The relationship between these variables was calculated with the help of an empirical (statistical regression) model and correlation coefficient (R).

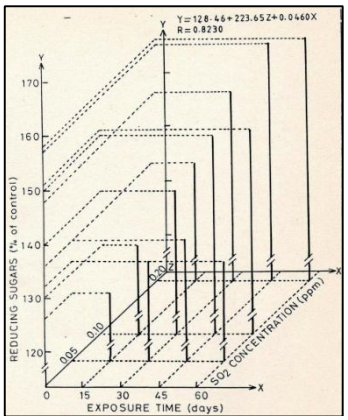

Figure 1: S.esculentum

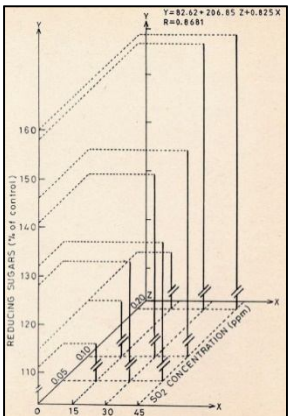

Figure 2: V.radiata

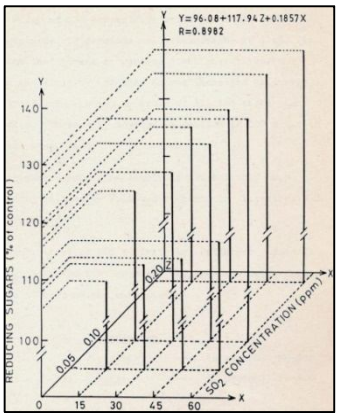

Figure 3: Z.mays

Figures 1-3: Significance of Factorial Effects

3. Observations

Reducing sugars of leaves of all the three plant species were found to increase following SO_2exposure. However, maximum increase in reducing sugar content following SO_2 fumigation was recorded in *S.esculentum*, followed by that in *V.radiata* and *Z.mays* respectively.

In*S.esculentum* increase in reducing sugars was maximum in Treatment T- 3 followed by that in Treatments T-2 and T-1. Maximum increase in sugar contents after 60 days of fumigation were calculated at 57.35%,49.62% and 31.60% for the treatments T-3,T-2 and T-1 respectively (Table 1; Fig.1). *V.radiata*was subjected to SO_2–fumigation for 45 days only. The treatment T-3 showed maximum increase in reducing sugar content (40.02%) after 45 days. Treatments 2 and T-1 recorded a maximum increment of 31.63% and 24.30% respectively for the time period. (Table 3; Fig.2). In plants of *Z. mays* reducing sugar content in T-1 increased by 5.38,6.53,9.34 and 12.12 percent over the controls following 15, 30, 45 and 60 days of SO_2 fumigation respectively. There was some increment in the sugar content in plants subjected to T-2 (maximum of 29.24% after 60-day fumigation). The T-3 treatment showed a 30.09% increase in the reducing sugars over 60 days of SO_2 exposure (Table 5; Fig 3).

Statistical analysis reveals that in *S.esculentum*, all factors viz., SO_2- ,fumigation period , and their combination (SO_2 treatment x fumigation period) exerted significant effects (P=0.25-0.001) on the reducing sugar content(Table 1). Reducing sugars in *V. radiata* were significantly affected by different SO_2treatments (P=0.001). The fumigation period was of significance only till 30 days of fumigation (P> 0.25). The combined action of factors (SO_2x time) resulted in a significant increase (P= 0.001) in reducing sugars (Table 3). All the SO_2treatments, singly as well in combination with fumigation period exerted significant effect (P=0.001) on reducing sugars in *Z.mays*. However, the effect was not significant by the fumigation period acting alone (Table 5).

Period of Fumigation (Days)	15		30		45		60	
Treatment Conc. (ppm) Time (h)	**Reducing Sugar Content (mg/g f wt.)**	**Percent Increase**	**Reducing Sugar Content (mg/g f wt.)**	**Percent Increase**	**Reducing Sugar Content (mg/g f wt.)**	**Percent Increase**	**Reducing Sugar Content (mg/g f wt.)**	**Percent Increase**
C–1 (0×4)	4.97±0.846		6.80±0.326		8.80±0.95		14.51±1.40	
T–1 (0.05×4)	6.75±0.349	26.37	10.0±1.115	32.00	13.66±1.24	35.87	21.22±0.785	31.60
C–2 (0×2)	2.17±0.628		2.97±0.38		5.377±0.38		8.88±0.6983	
T–2 (0.10×2)	3.111±0.251	30.20	5.7±0.537	47.89	10.66±0.68	49.60	18.0±0.8498	50.60
C–3 (0×1)	4.97±0.349		6.8±0.326		8.8±0		14.51±1.408	
T–3 (0.20×1)	7.50±0. 397	34.60	11.33±0.725	40.00	20.4±0	56.86	34.0±5.02	57.35

Table 1: Effect Of So_2 Treatments Onreducing Sugar Content In S. esculentum
Mean (±SD) of 5 replicates C–1, C–2, C–3 : Controls [air × time (h)];
T–1, T–2, T–3 : Treatments [Conc. of SO_2 (ppm) × Exposure time (h)]

3.1. Significance of Factorial Effects

Period of fumigation (Days)	15				30				45				60			
Source of Variation	df	Sum of Squares	Mean Source	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F
SO_2 Conc. (ppm)	17	1452.27	141.98	** 5.54	17	3029.5	166.5	** 8.05	17	7623.68	701.28	* 2.29	17	20298.7	1847.65	** 2.77
Exposure Time (h)	26	1352.25	41.96	* 1.64	26	3094.73	231.73	** 5.08	26	7319.79	397.39	** 1.29	26	19492.6	1041.65	** 1.53
SO_2 Conc. Exposure Time	53	1519.84	209.55	** 8.16	53	3290.08	427.00	** 14.84	53	8327.48	1405.08	** 4.58	53	22019.0	3568.0	** 5.25
Error	10		25.61		10		28.77		10		306.41		10		678.65	

*Table 2: Levels of significance : ** P < 0.1 ;* P < 0.25*

Period of Fumigation (Days)	15		30		45	
TREATMENT Conc. (ppm) Time (h)	Reducing Sugar Content (mg/g f wt.)	Percent Increase	Reducing Sugar Content (mg/g f wt.)	Percent Increase	Reducing Sugar Content (mg/g f wt.)	Percent Increase
C–1 (0×4)	8.22±0.4365		8.35±1.40		15.20±2.16	
T–1 (0.05×4)	9.11±1.14	9.76	10.57±1.16	21.00	20.08±5.09	24.30
C–2 (0×2)	5.66±0.533		5.42±0.92		7.11±0.74	
T–2 (0.10×2)	6.53±0.961	13.32	7.64±0.81	29.00	10.4±1.83	31.63
C–3 (0×1)	3.2±0.326		4.4±0.46		4.93±0.507	
T–3 (0.20×1)	3.68±0.412	13.04	7.2±0.65	38.80	8.22±1.22	40.02

Table 3: Effect Of So_2 Treatments On Reducing Sugar Content In V.radiata
Mean (±SD) of 5 replicates C–1, C–2, C–3 : Controls [air × time (h)];
T–1, T–2, T–3 : Treatments [Conc. of SO_2 (ppm) × Exposure time (h)]

3.2. Significance of Factorial Effects

Period of fumigation (Days)	15				30				45			
Source of Variation	df	Sum of Squares	Mean Source	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F
SO_2 Conc. (ppm)	17	1556.23	76.83	0.87	17	2986.0	135.53	2.69**	17	7913.74	1153.42	3.2**
Exposure Time (h)	26	1592.57	103.17	* 1.17	26	2930.0	78.72	** 1.56	26	6921.87	161.51	0.46
SO_2 Conc. Exposure Time	53	1752.16	267.76	** 3.05	53	3116.0	264.56	** 5.25	53	8425.96	1665.63	** 4.61
Error	10		0.0997		10		50.31		10		360.65	

*Table 4: Levels of significance : ** P < 0.1 ; * P < 0.25-0.50*

Period of Fumigation (Days)	15		30		45		60	
TREATMENT Conc. (ppm) Time (h)	Reducing Sugar Content (mg/g f wt.)	Percent Increase	Reducing Sugar Content (mg/g f wt.)	Percent Increase	Reducing Sugar Content (mg/g f wt.)	Percent Increase	Reducing Sugar Content (mg/g f wt.)	Percent Increase
C–1 (0×4)	8.66±0.249		10.26±1.13		12.31±1.33		13.77±1.05	
T–1 (0.05×4)	9.13±0.0821	5.38	10.93±0.67	6.53	13.46±1.48	9.34	15.44±1.73	12.12
C–2 (0×2)	2.68±0.136		5.60±0.533		6.17±0.503		8.511±0.674	
T–2 (0.10×2)	3.10±0.251	15.67	6.60±0.461	18.92	7.64±0.397	23.82	10.93±0.067	29.24
C–3 (0×1)	7.02±0.8350		10.31±0.806		13.46±1.48		15.13±2.33	
T–3 (0.20×1)	8.22±1.13	17.06	12.4±0.65	20.27	16.80±0.730	25.90	19.68±1.97	30.09

Table 5: Effect of So_2 Treatments on Reducing Sugar Content INZ. mays
Mean (±SD) of 5 replicates C–1, C–2, C–3 : Controls [air × time (h)]'
T–1, T–2, T–3 : Treatments [Conc. of SO_2 (ppm) × Exposure time (h)]

3.3. Significance of Factorial Effects

Period of fumigation (Days)	15				30				45				60			
Source of Variation	df	Sum of Squares	Mean Source	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F
SO_2 Conc. (ppm)	17	2598.68	356.02	16.49**	17	5020.67	286.76	7,60**	17	7643.16	577.36	7.41**	17	11147.68	485.14	3.35**
Exposure Time (h)	26	2250.37	7.71	0.397**	26	4755.82	21.91	0.585	26	7143. 32	77.52	0.995	26	10796.97	134.40	0.93*
SO_2 Conc. Exposure Time	53	2627.97	385.31	17.85**	53	5077.32	343.41	9.09**	53	7798.57	732.77	9,40**	53	11427.04	764.5	5.2**
Error	10		21.58		10		37.74		10				10		144'96	

*Table 6: Levels of significance : ** P < 0.1- 0.001 ; * P< 0.50*

4. Discussion

Reducing sugar content of plants increased following SO_2 fumigation in all the three plant species investigated. Leaves of *S.esculentum*, however, showed maximum increase in reducing sugars, followed by *V.radiata* and *Z.mays* Increment in reducing sugar content in plants exposed to SO_2 has been reported by earlier investigators [ix-xi]. At the same time non-reducing sugars and non-structural total carbohydrates and starch get reduces in response to SO_2 exposure[x, xii-xv]. Increase in reducing sugar content in response to SO_2 -stress may be due to the breakdown of polysaccharides rich in reducing sugars. This trend is also indicative of the functional changes in the energy budget of the plant as chemical energy needs to be made readily available for repair or replacement of damaged plant tissue. This can be made possible either by keeping the products of photosynthesis within the leaves or by translocating sugars from storage in stems and roots towards the leaves, thereby changing the sugar-starch ratio [x]. Increased respiratory rate also reflects use of such energy during SO_2-stress. Energy generated by enhanced respiration is used for the detoxification of sulphite to sulphate or in repairing the tissue damage due to SO_2-stress[xvi,xvii]. In addition, such plants also emit volatiles, acetaldehyde and ethanol [iv].

Diversion of energy resources from building of new tissue to repair/replacement of damaged tissue logically causes a reduction in the net productivity of plants. In the present study, plants of *S.esculentum* show maximum reduction in growth and productivity followed by that in *V.radiata* and *Z.mays* as evidenced by biochemical studies, viz., chlorophyll content [vii,xviii] and total proteins[viii,xix].

In addition to providing respiratory substrates, changes in reducing sugar levels also have some protective role. Polyhydric sugars are known to act as scavengers of the hydroxyl ($OH^{\bullet}$) and superoxide ($\bullet O_2^-$) free radicals, thereby helping a plant to cope with increasing abiotic stress [xx]. This is in addition to the enhanced activities of free-radical scavenger enzymes like peroxidases [ii] and Superoxide dismutase –SOD [iii].Multiple regression analysis indicates a highly significant correlation between the damage caused by different

SO_2 concentrations and the reducing sugar content in plants of all ages. The extent of increase in reducing sugars in SO_2-fumigated plants can thus be an indicator of the metabolic stress state of plants in the absence of any visible injury symptoms.

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6. References

i. WHO Regional Office in Europe. Copenhagen, Denmark.(2000). Chapter 10. Effect of sulphur dioxide on vegetation, critical levels, In Air qualityguidelines. Second Edition. 1-17.
ii. Chauhan, A. (1989a). Effect of sulphur dioxide on plants at biochemical and physiological levels. PhD. Thesis, University of Garhwal, India.
iii. Chauhan, A. (1989b). Superoxide dismutase—a bioindicator of plant response to SO_2-stress. In M.A. Öztürk (Ed.),Plants and Pollutants in Developed and Developing Countries(pp.549-568). International Symposium ,Izmir-Turkey (22-28 August,1988). Ege University, Izmir,Turkey.
iv. Chauhan A. (1990). Early diagnosis of SO_2-stress by volatile emissions in some crop plants. Oecologia 84, 289-294.
v. Rai, R., Rajput, M., Agrawal M., & Agrawal, S.B. (2011). Gaseous air pollutants: a review on current and future trends of emissions and impact on agriculture. Journal of Scientific ResearchB.H.U.55, 77-102.
vi. Singh L.P., Gill S.S., Gill R. & Tuteja N. (2012). Mechanism of sulphur dioxide toxicity and tolerance in crop plants. In N. Tuteja, A.F.Tiburcio, S.S. Gill & R. Tuteja (Eds.),Improving Crop Resistance to Abiotic Stress (pp. 133-163). Berlin:Wiley-VCH Verlag GmBh & KGaA.
vii. Chauhan, A. (2015a). Phytotoxic effects of SO_2 on crop plants: Total chlorophyll content.International Journal of Chemistry and Applications 7, 51-61.
viii. Chauhan , A. (2015b). Phytotoxic effects of SO_2 on crop plants—Total leaf protein content. International Advanced Journal of Science 9,5-16.
ix. Khan,A.R., & Malhotra, S.S. (1977). Effects of aqueous sulphur dioxide on pine needle glycolipids. Phytochemistry 16, 539-543.
x. Koziol, M.J., & Jordan, C.F. (1978). Changes in carbohydrate levels in red kidney bean (Phaseolus vulgaris L.) exposed to sulphur dioxide. Journal of Experimental Botany 29, 1037-1043.
xi. Malhotra, S.S., & Sarkar, S.K. (1979). Effects of sulphur dioxide on sugar and free amino acid content of pine seedlings. Physiologia Plantarum 47, 223-228.
xii. Ziegler, I.(1975). The effect of SO2 pollution on plant metabolism. Residue Reviews 56, 79-105.
xiii. Constantinidou, H.A.,& Kozlowski, T.T. (1979). Effect of SO_2 and O_2 on Ulnus americana seedling. 1. Visible injury and growth 2. Carbohydrate, proteins and lipids. Canadian Journal of Botany 57, 170-184.
xiv. Tripathi, A.K.,& Gautam, M. (2007). Biochemical parameters in plants as indicators of air pollution. Journal of Environmental Biology 28(1),127-132.
xv. Hamid, N.,& Jawaid, F. (2009). Effect of short-term exposure of two different concentrations of sulphur dioxide and nitrogen dioxide mixture on some biochemical parameters of Soybean (Glycine max (L.)Merr.). Pakistan Journal of Botany 41(5) , 2223-2228.
xvi. Shoman, R.E. (1972). Residual effects of sulphur dioxide on the net photosynthetic and respiratory rates of lichen thalli and cultured lichen symbionts. Bryologist 75, 335-341.
xvii. LeBlanc, B.F., & Rao, D.N. (1975). Effects of air pollutants on lichens and bryophytes. InJ.B.Mudd&T.T.Kozlowski (Eds.), Responses of Plants to Air Pollution.(pp.237-272). New York: Academic Press.
xviii. Koziol, M.J. (1980). Effects of prolonged exposure to SO_2 on the growth and carbohydrate metabolism of soyabean and ryegrass. DPhil Thesis. University of Oxford, U.K.
xix. Malhotra, S.S., & Khan, A.A. (1984). Biochemical and physiological impact of major pollutants. In M. Treshow (Ed.), Air Pollution and Plant Life (pp. 113-157). Chichester: Joyn Wiley & Sons
xx. Asada,K. (1980). Formation and scavenging of superoxide in chloroplasts, with relation to injury by sulphur dioxide. Research Report, National Institute for Environmental Studies, Japan 11, 165-179.