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Effect of SO_2 on Ascorbic Acid Content in Crop Plants --First Line of Defence against Oxidative Stress

Aprajita Chauhan
Associate Professor, Department of Chemistry, Sri Aurobindo College (University of Delhi) New Delhi, India

Abstract:
Effect of different SO_2 concentrations on the ascorbic acid content in the leaves of three economically important plant species, viz., Vigna radiata (Mung bean), Solanum esculentum (= Lycopersicon esculentum)[Tomato] 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. Whereas SO_2 fumigation resulted in a progressive decrease in ascorbate content in both mung bean and tomato, there was in fact, a marginal increase in ascorbate in T-1 of Z.mays after 30 days followed by negligible reduction in the subsequent treatments. These studies highlight a positive correlation between ascorbic acid content and SO_2-sensitivity of plants and indicate that Z.mays exhibits a relatively greater resistance to SO_2-stress.

Keywords: *Antioxidant, Ascorbic acid, Maize, Mung bean, Tomato, Pollution tolerance, SO_2-fumigation*

1. Introduction

Huge amounts of toxic contaminants are being constantly emitted into the atmosphere as a result of various industrial and anthropogenic activities. Sulphur dioxide, in particular, has since long been recognized as a potent phytotoxicant with well documented detrimental effects on agricultural production. On one hand, where stringent regulatory control on SO_2 emissions has resulted in a marked decline in SO_2 pollution over Europe (WHO, 2000), ironically last decade and a half has witnessed a steady increase of this pollutant by upto 70% in India .

Plants form a sink for atmospheric SO_2. Solubility of SO_2 is very high and the foliar uptake is also very rapid. So a series of physiological and/or biochemical changes in the SO_2-exposed plants occur at the cellular level, much prior to the emergence of visible injury symptoms. These changes include alterations in the activities of free-radical scavenger enzymes, amino acid, reducing sugars and chlorophyll contents, as well as changes in the pattern of volatile emissions (Chauhan 1989a, 1989b, 1990, 2015a,2015b,2015c, Rai et al. 2011, Singh et al. 2012). In addition, ascorbic acid content constitutes a reliable parameter to assess SO_2-tolerance in plants. Being a natural antioxidant, ascorbate plays an important role in preventing uncontrolled radical chain reactions in the apoplastic space of cells (Takahama et al. 1992). Present investigations on three economically important crop plants were carried out to study the endogenous levels of ascorbate vis-a vis their tolerance to SO_2-stress in order to highlight the role of ascorbate as the first line of defence, against oxidative stress.

With the help of a statistical regression model, the significance of interaction and individual effects of SO_2 concentration and exposure upon ascorbic acid contents have been analyzed.

2. Material and Methods

Three economically important cultivated plant species viz., *Vigna radiata* (L.) Wilczek [Mung bean], *Solanum esculentum* [Tomato], 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=1^{m3}) 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(268.0 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, 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. Ascorbic acid determination

Ascorbic acid content of leaves was determined by using the modified colorimetric 2,6-dichlorophenol indophenol blue (DCPIP) method given by Keller and Schwager(1977). Fresh leaf tissue (0.2g) was homogenized with ice-chilled mortar and pestle using 5ml of 0.5% oxalic acid solution. The homogenate was centrifuged at 18000 x g for 15 minutes. The final volume was made to 5.0 ml with the existing solution.

5.0 ml of DCPIP dye solution was added to 1.0 ml of extract. After vigorous mixing the concentration of ascorbic acid was computed by measuring the extinction *E* at 520nm of blank solution (E_{0}) , of DCPIP solution with sample (E_s), and extinction due to turbidity on addition of 50µl of 1.0% ascorbic acid solution (E_t), by the equation (E_0-E_s-E_t) x factor f = ppm ascorbic acid. Concentration of ascorbic acid was evaluated from the standard curve of ascorbic acid prepared by using different concentrations of ascorbic acid.

3. Observations

The endogenous ascorbate content was seen to be maximum in the control plants of *Z. mays*, followed by that in *V. radiata* and *S. esculentum*. Following SO_2 fumigation, reduction in ascorbic acid content was observed to be maximum in *V.radiata,* followed by that in *S. esculentum* and *Z. mays*. In *Z. mays*, however, a slight increase in ascorbic acid content was noted initially up to 30 days of SO_2 exposure in plants with treatment T-1.

In *V. radiata*, all the three treatments T-1, T-2 and T-3 following SO_2 fumigation resulted in considerable reduction in ascorbic acid content .Maximum loss of ascorbic acid was recorded (14.78%) after 45 days of T-3, which was much more than the corresponding T-2 (7.51%) and T-1 (7.10%) treatments (Table 1;Fig.1).

In *S. esculentum, reduction in* ascorbic acid after fumigation treatments exhibited a similar pattern. Ascorbate decreased by 9.50% over the controls after 60 days in T-1. The percentage reduction for T-2 and T-3 was recorded at 9.75 and 13.52 respectively over a corresponding period of time (Table 2; Fig. 2).

Treatment T-1 showed an increase (5.8%) in ascorbic acid content following 30 days of fumigation. Subsequent fumigation, however, resulted in a decrease by 0.19 and 9.09% after 45 and 60 days respectively. Treatments T-2 and T-3 exhibited a reduction of 4.04 and 6.43% respectively after 60-day fumigation cycle (Table3; Fig. 3).

Statistical analysis on *V. radiata* revealed that SO_2 treatments had pronounced effects (P=0.05) on the ascorbic acid content in 45-day old plants (Table 1). The effect of fumigation period was, however, significant only after 30 days of SO_2 exposure (P= 0.005). The combined effect of SO_2 concentration X fumigation period increased with the age of the plant (P= 0.25-0.001). Ascorbic acid content in *S.esculentum* was more sensitive (P=0.05) to SO_2 concentration than to fumigation period (P= 0.25). However, the interactive effect (SO_2 treatment X fumigation period) was significant (P=0.001) in plants of all ages (Table 2).

SO_2 treatments significantly affected the ascorbic acid content in *Z.mays* (P=0.25-0.001) at all the plant ages (Table 3). However, the fumigation period did not show any significant effect. There was significant interaction between the two variables (SO_2 concentration X fumigation period) and ascorbic acid content (P=0.05-0.001).

Period of Fumigation (Days)	15		30		45	
TREATMENT Conc. (ppm) Time (h)	Ascorbic acid Content (mg/g f wt.)	Percent Reduction	Ascorbic acid Content (mg/g f wt.)	Percent Reduction	Ascorbic acid Content (mg/g f wt.)	Percent Reduction
C–1 (0x4)	1.409±0.0387		1.581±0.0239		1.658±0.0485	
T–1 (0.05x4)	1.0365±0.0077	3.12	1.477±0.237	7.1	1.540±0.252	7.1
C–2 (0x2)	1.305±0.0578		1.481±0.0200		1.703±0.0802	
T–2 (0.10x2)	1.230±0.827	5.74	1.396±0.042	9.45	1.575±0.612	7.51
C–3 (0x1)	1.414±0.0286		1.564±0.0037		1.758±0.0074	
T–3 (0.20x1)	1.275±0.050	9.33	1.401±0.032	10.42	1.498±0.0227	14.78

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)]

Table 1: Effect of So_2 on Ascorbic Acid in V.Radiata

→ 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)	9	55.78	0.09	0.66	9	66.60	0.050	2.5**	9	79.93	0.020	0.54
Exposure Time (h)	14	55.69	0.0	0.0	14	66.62	0.070	3.5**	14	79.10	0.190	1.46
SO_2 Conc. Exposure Time	29	55.92	0.2264	1.66**	29	66.69	0.0140	7.0**	29	79.24	0.3394	2.62
Error	6		0.1362		6		0.020		6		0.1294	
Levels of significance : ** P < 0.025 ; * P < 0.50												

Period of Fumigation (Days)	15		30		45		60	
Treatment Conc. (ppm) Time (h)	Ascorbic acid Content (mg/g f wt.)	Percent Reduction	Ascorbic acidContent (mg/g f wt.)	Percent Reduction	Ascorbic acid Content (mg/g f wt.)	Percent Reduction	Ascorbic acid Content (mg/g f wt.)	Percent Reduction
C–1 (0x4)	0.7670±0.25		0.970±0.24		0.990±0.083		1.205±0.014	
T–1 (0.05x4)	0.7540±0.12	1.69	0.895±0.866	7.7	0.910±0.734	8.08	1.090±0.116	9.5
C–2 (0x2)	0.813±0.112		0.8550±0.167		0.856±0.004		0.902±0.007	
T–2 (0.10x2)	0.763±0.186	6.10	0.782±0.075	8.5	0.777±0.028	9.2	0.814±0.014	9.75
C–3 (0x1)	0.813±0.112		0.8550±0.167		0.856±0.004		0.902±0.007	
T–3 (0.20x1)	0.752±0.245	7.50	0.776±0.049	10.10	0.760±0	11.20	0.780±0	13.52

Table 2: Effect of SO_2 on Ascorbic acid 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)]

→ Significance of Factorial Effects

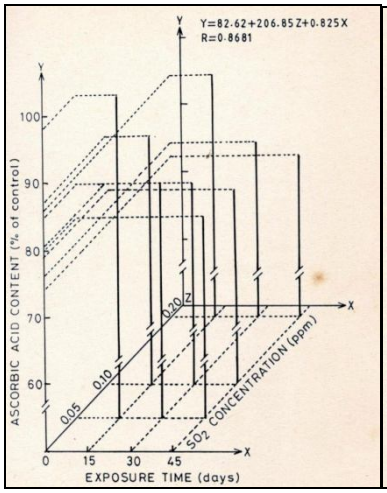
Period of fumigation (Days)	15				30				45				60			
Source of Variation	df	Sum of Squares	Mean Source	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F
SO_2 Conc. (ppm)	9	18.299	0.7506	0.77	9	22.045	0.090	2.43**	9	21.752	0.162	2.718	9	27.66	0.5864	3.58**
Exposure Time (h)	14	18.300	0.8487	0.87	14	21.999	0.043	1.16*	14	21.672	0.082	1.37*	14	27.11	0.083	0.527
SO_2 Conc. Exposure Time	29	18.317	2.547	2.647	29	22.133	0.177	4.78**	29	21.894	0.304	5.08**	29	27.86	0.833	5.091**
Error	6		0.9721		6		0.037		6		0.0597		6		0.1636	
Levels of significance :** P<0.05; * P < 0.50																

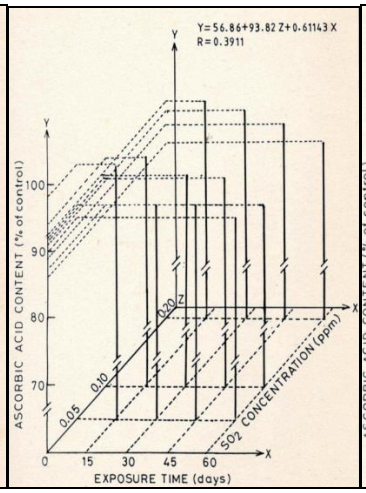
Period of Fumigation (Days)	15		30		45		60	
TREATMENT Conc. (ppm) Time (h)	Ascorbic acid Content (mg/g f wt.)	Percent Reduction	Ascorbic acid Content (mg/g f wt.)	Percent Reduction	Ascorbic acid Content (mg/g f wt.)	Percent Reduction	Ascorbic acid Content (mg/g f wt.)	Percent Reduction
C–1 (0□4)	1.024±0.1818		1.298±0.280		1.598±0.1861		1.1±0.229	
T–1 (0.05□4)	1.0242±0.1818	0	1.372±0.237	5.8	1.595±0.1798	-0.187	1.0±0.193	0.91
C–2 (0□2)	1.782±0.007		1.801±0.042		1.848±0.0074		1.829±0.035	
T–2 (0.10□2)	1.752±0.022	-1.6	1.768±0.039	-1.6	1.829±0.035	-2.05	1.755±0.01	-4.04
C–3 (0□1)	1.710±0.037		1.780±0.016		1.814±0.0185		1.817±0.183	
T–3 (0.20□1)	1.670±0.063	-2.3	1.742±0.043	-2.24	1.720±0.030	-5.18	1.700±0.178	-6.43

Table 3: Effect of SO_2 on Ascorbic acid Content in Z.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)]

→ 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)	9	70.30	3.34	9.82**	9	80.66	1.28	1.75**	9	90.20	0.28	1.22	9	74.26	3.38	10.56**
Exposure Time (h)	14	66.96	0	0	14	79.38	0	0	14	90.00	0.08	0.35	14	70.93	0.05	0.961
SO_2 Conc. Exposure Time	29	70.965	3.685	10.83**	29	81.39	2.01	2.75	29	90.51	0.59	2.56	29	74.83	3.95	10.56**
Error	6		0.34		6		0.73		6		0.23		6		0.032	
Levels of significance : ** P < 0.001 ; * P < 0.50																





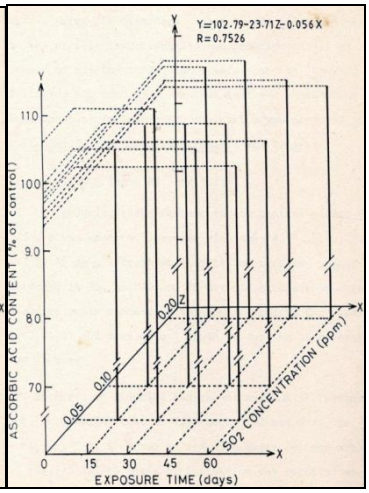


Fig. 1 Vigna radiata *Fig. 2 Solanum esculentum* *Fig. 3 Zea mays*

Figure 1-3: Significance of Factorial Effects

4. Discussion

There was a progressive decrease in ascorbate content in *S.esculentum* and *V.radiata* in response to SO_2 fumigation. However, in *Z.mays*, after SO_2 fumigation with 0.05 ppm (4h) for 30 days, there was a marginal initial increase in ascorbic acid content. On the other hand, fumigation with 0.1ppm SO_2 recorded a reduction in ascorbate content after 60 days. Treatment with 0.2ppm SO_2 also produced a negligible change in ascorbic acid content. Reduction in ascorbate content in response to low levels of SO_2 has also been reported in the young spruce plants (Keller and Schwager 1977), and in some crop plants (Varshney and Varshney 1984). In addition, the role of ascorbic acid content has been of considerable significance in all investigations involving Air Pollution Tolerance Index (APTI) of tree species growing either at the crossroads of cities or in highly polluted industrial towns for their use as potential bioindicators of SO_2 pollution (Tiwari and Tiwari 2008, Avinash Chauhan 2010, Chandawat et al. 2011, Deepalakshmi et al. 2013, Randhi and Anji Reddy 2013, Rai and Panda 2015).

Ascorbate is essential for plant metabolism and inhibition of its synthesis prevents growth (Arrigoni and De Tullio 2002).Ascorbic acid is concentrated mainly in the chloroplasts (Franke and Huber, 1964). It occurs in plant tissues combined with protein as ascorbigen. Cells normally maintain most of the ascorbic acid in the reduced form (Mapson 1958), whereas the oxidized form, DHA (Dehydroxyascorbate) is present is in small amounts. Under stress conditions there is a rapid conversion of ascorbic acid to DHA. The ascorbate system is known to activate many physiological and defence mechanisms, both in plants and animals (Lewin 1976) and thus plays a significant role in resistance of plants to all kinds of stresses (Keller and Schwager 1977).

Ascorbic acid is a natural antioxidant in plants and plays an important role in pollution tolerance (Chen et al. 1990). Ascorbate detoxifies the phytotoxic superoxide radical ($\cdot O_2^-$) and H_2O_2, generated as a result of SO_2 exposure. This protective mechanism involves the formation of DHA (Halliwell 1984),and this conversion is catalysed by the enzyme ascorbate peroxidase (Halliwell and Getteridge 1985).Being unstable, DHA eventually produces oxalic acid and threonic acids (Yang and Loewus 1975).Thus, by scavenging free radicals, ascorbic acid inhibits the chain initiation and sulphite oxidation thereby preventing uncontrolled free radical chain reactions(Takahama et al. 1992).

Plants containing high ascorbic acid are considered to be tolerant to air pollution. In the present studies, the endogenous level of ascorbic acid in *Z.mays* was much higher as compared to the other two plant species indicating its relative resistance to SO_2 pollution.

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5. References

i. Arrigoni, O. and De Tullio, M.C. 2002. Ascorbic acid: much more than just an antioxidant. Biochim.Biophys.Acta. 1569(1-3): 1-9.
ii. Chandawat, D.K. Verma, P.U. and Solanki, T.A. 2011. Air pollution tolerance index (APTI) of tree species at crossroads of Ahmedabad City. Life Sci. Leaflets, 20: 935-943.
iii. Chauhan,A. 1989a. Effect of sulphur dioxide on plants at biochemical and physiological levels. PhD. Thesis, University of Garhwal, India.
iv. 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). Izmir, Turkey:Ege University.
v. Chauhan, A. 1990. Early diagnosis of SO_2-stress by volatile emissions in some crop plants. Oecologia 84: 289-294.
vi. Chauhan, A. 2015a. Phytotoxic effects of SO_2 on crop plants: total chlorophyll content. Int. J. Chem.and App. 7: 51-61.
vii. Chauhan, A. 2015b. Phytotoxic effects of SO_2 on crop plants—total leaf protein content. Int.Adv. Jour.Sci. 9:5-16.
viii. Chauhan, A. 2015c. Phytotoxic effects of SO_2 on crop plants—abiotic stress and reducing sugars. Int.J.Sci. & Technol. 3: 25-29.
ix. Chauhan, Avinash. 2010. Tree as bio-indicator of automobile pollution in Dehradun city: A case study. N.Y.Sci.J. 3(6): 88-95.
x. Chen, Y.M., Lucas, P.W. and Wellburn, A.R. 1990. Relative relationship between foliar injury and change in antioxidants level in red and Norway spruce exposed to acidic mists. Environ. Pollut. 69: 1-15.
xi. Deepalakshmi, A.P. , Ramakrishnaiah, H. , Ramachdra, Y.L. and Radhika, R.N. 2013. Roadside plants as indicators of urban air pollution. IOSR J Environ. Toxicol. Food Technol. 3(3):10-14.
xii. Franke, W. and Heber, U. 1964. Über die quantitative verteilund der ascorbinsäure innerhalb der Planzenzelle. Z. Naturf. Teil C, 196: 1146-1149.
xiii. Halliwell,B.(1984).Chloroplast metabolism: the structure and function of chloroplasts in green leaf cells. Oxford: Clarendon Press.
xiv. Halliwell,B. and Gutteridge,J.M.C. (1985). Free radicals in biology and medicine. Oxford: Clarendon Press.
xv. Keller,T. and Schwager,H. 1977. Air pollution and ascorbic acid. Eur. J. Forest Pathol. 7: 338-350.
xvi. Lewin,S.(1976). Vitamin C: its molecular biology and medical potential . San Francisco:Academic Press.
xvii. Mapson, L.W. 1958. Metabolism of ascorbic acid in plants: Part I. Ann. Rev. Plant Physiol. 9: 119-150.
xviii. Rai, P.K. and Panda,Lalita L.S. 2015. Roadside plants as bio indicators of air pollution in an industrial region, Rourkela, India. Int. J. Adv. Res. & Technol. 4(1):14-36.

xix. Rai,R., Rajput, M., Agrawal ,M. and Agrawal, S.B. 2011. Gaseous air pollutants: a review on current and future trends of emissions and impact on agriculture. J. Sci. Res. B.H.U. 55: 77-102

xx. Randhi,U. and Anji Reddy, M. 2013. Air pollution tolerance levels of selected urban plant species in industrial areas of Hyderabad (A.P.), India. Int. J. Sci. Res. 2(6): 294-296.

xxi. Singh,L.P. ,Gill,S.S., Gill,R. and Tuteja,N. (2012). Mechanism of sulphur dioxide toxicity and tolerance in crop plants . In N. Tuteja, A.F.Tiburcio, S.S. Gill and R. Tuteja (Eds.), Improving crop resistance to abiotic stress (pp. 133-163). Berlin: Wiley-VCH Verlag GmBh & KGaA.

xxii. Takahama,U., Veljovic-lovanovic,S. and Heber,U. 1992. Effect of air pollutant SO_2 on leaves. Plant Physiol. 100 : 261-266.

xxiii. Tiwari,S. and Tiwari,M. 2006. Air pollution tolerance indices of few plants growing near Raigarh (India). J.Environ. Res. Dev. 1(2): 129-135.

xxiv. Varshney, S.R.K. and Varshney,C.K. 1984. Effects of SO_2 on ascorbic acid in crop plants. Environ.Pollut. (Serial A) 35: 285-290.

xxv. Yang, J.C. and Loewus, F.A. 1975. Metabolic conversion of L-ascorbic acid in oxalate-accumulating plants. Plant Physiol. 56(2): 283-285.

xxvi. WHO Regional Office in Europe. Copenhagen, Denmark.(2000). Chapter 10. Effect of sulphur dioxide on vegetation, critical levels, In Air quality guidelines. Second Edition. 1-17.