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EFFECT OF SULPHUR DIOXIDE ON PLANT BIOCHEMICALS

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Abstract

The effect of SO_2 on biochemical in (*Brassica juncea [L.] Czern. and Coss. cv. Pusa Bold; Raphanus sativus L Mino Early Long White*).In exposed seedlings SO_2 concentration ranging (653, 1306, 2612&3918 µg m^{-3} S Resulted accumulation of metabolites especially ascorbic acid is the adaptive mechanisms that operate in pla when exposed to SO_2 stress. Appreciable decrease due to SO_2 was observed in ascorbic acid content of *Rapha sativus* plants. Carbohydrate level in plant is also influenced by SO_2 exposures, the possible reason for decre sugar content in plants under SO_2 stress. All four concentrations of SO_2 caused reduction in carbohydrate in crops except at 653 µg m^{-3} of SO_2 where a rise in carbohydrate level was seen. A perusal of literature suggests SO_2 had a marked impact on protein content of leaves and seeds. They reported that protein degradation under stress was either due to decreased photosynthesis or due to inhibition of protein synthesis. Appreciable decrease to 653 µg m^{-3} and 3918 µg m^{-3} of SO_2 was observed in the protein content of leaves as well as seeds. A ma decline found in the nitrogen& phosphorus content of SO_2 fumigated plants. Minerals like nitrogen and phosph in leaves of *Brassica juncea* and *Raphanus sativus* were recorded to be substantially reduced following the lo term exposures of 653, 1306, 2612 and 3918 µg m^{-3} of SO_2. Out of the two crops, SO_2 caused more decline in nitrogen content of *Raphanus sativus* than that of *Brassica juncea*. The experimental crops, on exposure to SO_2 shown decline in their phosphorus content. The reductions in carbohydrate, protein and mineral content were dire related to decline in chlorophyll content of treated seedlings. Results indicate that calcium hydroxide treatm afforded protective effects against SO_2 as evidenced by enhancement in plant length, biomass accumulation, leaf and yield of both crops. When SO_2 exposed plants were periodically sprayed with calcium hydroxide their gro performance was improved. The effect of SO_2 on biochemical breakdown and rate of photosynthesis was due mo to the specific direct action of SO_2 and was not a function of increased acidity.

Keywords: - Sulphur Dioxide, Plant Biochemicals

Introduction

SO_2 is one of the major air pollutants in industrialized areas that can damage vegetation, and it is one of the most prevalent phytotoxic air pollutants and causes substantial damages to green plant. An accompanying effect of SO_2 toxicity is the appearance of reactive oxygen species by transferring the electron to oxygen *via* the photosynthetic electron transport chain, radicals can be generated (Elstner, 1982).

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This is favoured by inhibition of key enzymes of the Calvin cycle, which has been observed after SO_2 fumigation (Jager *et al.*, 1986). The resulting superoxide reacts with either spontaneously or is enzymatically catalyzed to H_2O_2.

The enzyme needed for this reaction is superoxide dismutase, which plays an important role in defense against SO_2 toxicity (Tanaka and Sugahara, 1980).The present study was initiated to determine if low concentration of SO_2 have any effect on the process of photosynthesis without producing visual symptoms. The effect of SO_2 was studied on the following aspects of plant biochemical: (a) Effect on changes in antioxidant level, (b) Effect on alterations in carbohydrate, protein and metabolism, (c) Effect on Changes in mineral content.

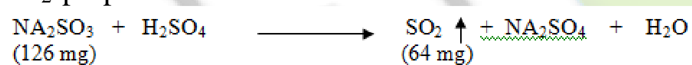
MATERIAL AND METHOD

Growth Conditions

Plants from the family Brassicaceae (*Brassica juncea (L.) Czern.and Coss.cv.Pusa Bold; Raphanus sativus L.cv.Mino Early Long White*) seeds were washed with sterile distilled water and then treated with 0.1% mercuric chloride for 5 minutes and finally washed with sterile distilled water for 15 minutes. Surface sterilized seeds were allowed to imbibe water for 6 hrs.and thereafter sown on petriplates lined with cotton over which Whatman no.40 filter paper was placed. Seeds were placed on filter paper. For each variety, five sets each having 150 seeds were maintained. Then seeds were sown in polythene bags/earthen ware containing garden soil. The soil was sandy loam and homogenously mixed with farmyard manure. All experiment will carried out in a fumigation chamber (1m*1m*1m) made of iron rods. Each chamber was portable and covered with transparent polythene sheets. Then Plants were exposed to SO_2 from 5th day onwards. Plants of each species were divided into five sets. Out of five four sets were exposed to four different concentration of SO_2 (653, 1306, 2612, 3918 µg m^{-3}) while the fifth one was used as control.

SO_2 ***Treatment***

SO_2 was prepared by allowing a reaction of dilute sulphuric acid (10% H_2SO_4) and sodium sulphite (NA_2SO_3) under controlled condition of temperature and humidity. Complete reaction of 1M NA_2SO_3 with 10% H_2SO_4 produces 1M SO_2 or 126 mg of NA_2SO_3 yields 64 mg SO_2 .The chemical reaction for SO_2 preparation is as follows-


NA_2SO_3 + H_2SO_4 (126 mg) ⟶ SO_2 ↑ + NA_2SO_4 + H_2O (64 mg)

Hence, 1.968 of NA_2SO_3 is required to produce 1 mg (1000 µg m^{-3}) of SO_2. Therefore, on the basis of this equation 1.285, 2.571, 5.142, 7.713 mg of NA_2SO_3 were used to obtain 653, 1306, 2612, 3918 µg m^{-3} of SO_2,respectively,inside the exposure system. The plants were given the treatment of SO_2 on alternate day for 2 hrs.

Calcium hydroxide treatment

Calcium hydroxide (0.5% aqueous solution) was used for amelioration studies. Results of germination studies showed that lower concentration of SO_2 (653 and 1306 µg m^{-3}) did not cause any appreciable reduction. On the contrary, higher concentration (2612 and 3918 µg m^{-3}) of SO_2 proved to be highly toxic. Plants treated with 2612 and 3918 µg m^{-3} of SO_2 were selected for amelioration studies and calcium hydroxide was used as an ameliorating agent.For ameliorating study set treated with 0.5% $Ca(OH)_2$, 0.5% $Ca(OH)_2$+2612 µg m^{-3} SO_2 , 2612 µg m^{-3} SO_2 , 0.5% $Ca(OH)_2$+3918 µg m^{-3} SO_2 and 3918 µg m^{-3} SO_2 were designated as C,C+T_1, T_1,C+ T_2 and T_2 sets, respectively. The calcium hydroxide solution was sprayed on SO_2 (2612 µg m^{-3} and 3918 µg m^{-3} SO_2) treated plants once in a week with the help of a sprayer.

The study on both plants was carried out till maturity (90 d in *Brassica juncea* and 120 d in *Raphanus sativus*).the observations for various attributes were recorded in 15 d, 30 d, 45 d, 60 d, 75 d, 90 d old plants of *Brassica juncea* and *Raphanus sativus* respectively.

Growth Analysis

Plants grown on polythene bags/pots were taken out and roots were rinsed several times in deionized water to remove unwanted nutrients from root surface. Excess moisture was removed using absorbent towels.

Phenological analysis

Carbohydrate Extraction &Determination

Total carbohydrate amount was estimated by anthrone colorimetric method (Yemm and Willis, 1954). For carbohydrate estimation, plant parts (root/leaf/seed) were placed in oven for 24 hrs at 80°C. Dried sample (50 mg) was crushed in 2.5 ml of 2.5 N HCl and then kept on boiling water both for 3 hours. After 3 hours sodium carbonate was added to it till effervescence ceases. Final volume was made 25 ml with distilled water. Again it was centrifuged and 4 ml of anthrone reagent (200mg anthrone in 100 ml HCl) was added to 5 ml of sample and placed in water bath for 8 min. It was cooled and optical density was recorded at 630 nm. Calibration curve of glucose was used for estimation of carbohydrate in mg g^{-1} dry weight.

Protein Extraction &Determination

Protein estimation in leaves/root/seed was carried out according to method developed by Lowry *et al.* (1951). Fresh tissue (50 mg) was homogenised in 5 ml chilled tris maleate buffer and centrifuged at 2000 rpm for ten minutes. To 1 ml of supernatant 1 ml of TCA (20%) was added and kept in refrigerator for overnight Next day, reaction mixture was again centrifuged and pellet was dissolved in 0.1 N NaOH (3 ml). After two hours, 1 ml of above solution was mixed with 5 ml alkaline copper tartarate solution. Ten min. later, 1 ml of folin ciocalteau reagent (double diluted) was added and solution was kept in dark for 30 min. Then optical density (OD) of sample was recorded at 660 nm and amount of protein (mg g^{-1} fresh weight) was determined with the help of standard curve of bovine serum albumin (BSA).

Nitrogen Extraction &Determination

Nitrogen content in various plant parts (roots/leave/seed) was determined according to method developed by Snell and Snell (1954). Dried plant sample (50 mg) was digested in 2 ml of H_2O_2 (30%) and 5 ml of conc. solution and placed on hot plate for 30 min. Again, 3 ml of H_2O_2 was added and kept on hot plate for another 60 min or till the digest become clear. After cooling, 1 ml of digest was taken out in a test tube and 3 ml of Nessler's reagent and 1 ml of distilled water was added to this. Optical density of coloured solution was recorded with the help of spectrophotometer at 425 nm. Total amount of nitrogen was calculated by preparing a calibration curve of ammonium sulphate and expressed as mg N_2 g^{-1} dry weight.

Phosphorus Extraction &Determination

Phosphorus content was estimated according to Olsen's (1954) method. 50 mg dry material (root/shoot/leaves) was homogenized in 10 ml of $NaHCO_3$ (4.2 g/l) and pinch of activated charcoal was added to this solution. It was kept on a shaker for 30 min. and then centrifuged at (2000 rpm) for 10 min. In a test tube 5 ml of filtrate and 5 ml molybdate reagent (15 g ammonium molybdate + 30 ml distilled water + 348 ml conc. HCl + add distilled water to make 1 liter) was added and swirled. After that, 1 ml of working $SnCl_2$ solution (prepared by mixing 1 ml stock $SnCl_2$ solution (40%) and 60 ml distilled water) was added to reaction mixture. Final volume was made upto 25 ml. Optical density of each sample was recorded at 660 nm. Calibration curve prepared by potassium hydrogen phosphate (KH_2PO_4) was used to calculate the amount of phosphorus in mg g^{-1} dry weight.

Ascorbic acid Extraction &Determination

Ascorbic acid was determined with help of titration method (Sadasivam and Manickam, 1992). The quantitative estimation of ascorbic acid was based on the fact that ascorbic acid reduces the blue coloured dye 2, 6 dichlorophenol indophenol (DCPIP) to a colourless form. Following reagents were prepared for ascorbic acid estimation–

1. 4% oxalic acid (4 g oxalic acid was dissolved in 100 ml distilled water)
2. DCPIP solution (53 mg DCPIP and 42 mg sodium bicarbonate was dissolved in 200 ml distilled water)
3. Stock solution of ascorbic acid (100 mg ascorbic acid was dissolved in 100 ml of 4% oxalic acid in a standard flask)
4. Working solution of ascorbic acid (It was prepared by diluting 10 ml of stock solution to 100 ml with the help of distilled water)

5 ml of working solution was taken out in 100 ml conical flask. 10 ml of oxalic acid (4%) was added to it and titrated against the dichlorophenol indophenol. Appearance of pink colour, which persisted for few minutes, was considered as end point. Volume of dye consumed (V_1) was considered as equivalent to amount of ascorbic acid. Fresh tissue (50 mg leaf/root) was homogenised in small volume of oxalic acid (5ml) and then filtered. Final volume was made upto 100 ml with oxalic acid. 5 ml of this solution was taken out and 10 ml of oxalic acid was added to it and titrated against dicholorophenol indophenol. The volume (V_2) of dye consumed was noticed. Amount of ascorbic was calculated with the help of following formula–

$$\text{Ascorbic acid (mg g}^{-1}\text{ fresh weight)} = \frac{0.5}{V_1} \times \frac{V_2}{5} \times \frac{100}{\text{wt. of sample}}$$

RESULT & DISSCUSION

The seeds of some cultivars (*Brassica juncea,*) exposed to 653 µg m^{-3} SO_2 show better germination and seedling growth than control ones. The reductions in carbohydrate, protein and mineral content were directly related to decline in chlorophyll content of treated seedlings. On the contrary ascorbic acid contents of treated seedlings increased than those of control ones. All four concentrations of SO_2 caused reduction in carbohydrate in both crops except at 653 µg m^{-3} of SO_2 where a rise in carbohydrate level was seen. SO_2 affects photosynthesis process adversely either by interfering with the electron flow in electron transport chain of chloroplast system (Puckett *et al.*, 1974) or by causing swelling of thylakoid in chloroplast (Wellburn *et al.*, 1972). Protein forms the structural and functional basis of the cell. Appreciable decrease due to 653 µg m^{-3} and 3918 µg m^{-3} of SO_2 was observed in the protein content of leaves as well as seeds. Inhibition of nitrogenous parameters such as protein and total nitrogen content upon exposure of high doses of SO_2 may be due to interaction at different sites to inhibit a large number of enzymes having functional sulfhydryl group resulting in disruption of protein synthesis pathways (Saxena and Saxena, 1999). Minerals like nitrogen and phosphorus in leaves of both test crops were recorded to be substantially reduced following exposures of 653, 1306, 2612 and 3918 µg m^{-3} of SO_2. Nitrogen and phosphorus actively participate in various physiological activities and any imbalance in the optima of these biochemical components ultimately perturbs the growth and yield. Out of the two crops, SO_2 caused more decline in leaf nitrogen content of *Raphanus sativus* than that of *Brassica juncea*.

Table 1a Estimation of some biochemical components in leaves of *Raphanus sativus* fumigated with different concentrations of sulphur dioxide at 15 d and 30 d plant age.

Attribute	Plant age (d)													
	15 d							30 d						
	Concentration of Sulphur dioxide (μg m^{-3})					CD		Concentration of Sulphur dioxide (μg m^{-3})					CD	
	0	653	1306	2612	3918	5%	1%	0	653	1306	2612	3918	5%	1%
Ascorbic acid (mg g^{-1} f. wt.)	0.514	0.546	0.526	0.527	0.518	0.135	0.144	0.754	0.749	0.654	0.598	0.624	0.135	0.292
Air pollution tolerance index (APTI)	9.224	9.357	8.798	8.745	8.776			9.307	9.110	8.943	8.790	8.6321		

Table 1b Estimation of some biochemical components in leaves of *Raphanus sativus* fumigated with different concentrations of sulphur dioxide at 15 d and 30 d plant age.

Attribute	Plant age (d)						
	30 d						
	Concentration of Sulphur dioxide (μg m^{-3})					CD	
	0	653	1306	2612	3918	5%	1%
Carbohydrate (mg g^{-1} d. wt.)	31.069 ± 0.232	29.208 ± 0.233	26.582** ± 1.182	24.390** ± 0.356	23.443** ± 0.704	2.532	2.713
Protein (mg g^{-1} f. wt.)	25.120 ± 1.318	19.843** ± 2.224	16.676** ± 0.967	14.776** ± 1.318	9.287** ± 0.967	5.253	5.627
Nitrogen (mg g^{-1} d. wt.)	2.602 ± 0.035	2.547 ± 0.027	2.210** ± 0.016	1.414** ± 0.034	1.278** ± 0.020	0.066	0.071
Phosphorus (mg g^{-1} d. wt.)	0.533 ± 0.006	0.512 ± 0.013	0.509 ± 0.009	0.405** ± 0.013	0.479** ± 0.020	0.033	0.035

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Phosphorus plays a vital role as energy carrier for important reaction of plant life like the oxidation of sugars with the aid of several enzymes and in the initial reaction in photosynthesis & SO_2 had shown decline in their phosphorus content. Phosphorus deficiency could be one of the reasons for inhibition of photosynthesis. In *Brassica juncea*, ascorbic acid accumulation was increasing with increase in concentration of SO_2 . On the contrary, appreciable decrease due to SO_2 was observed in ascorbic acid content of *Raphanus sativus* plants. Reduction in level of ascorbic acid in pollutant exposed plants has been ascribed to enzyme toxicity and sulphonation of -SH groups (Mapson, 1958). One of the significant findings of this study is that calcium hydroxide works as an antidote against SO_2 toxicity in test crops. A strong protective effect of calcium hydroxide against ill-effects of SO_2 was reported by

Table 2a Estimation of some biochemical components in leaves of Raphanus sativus treated with SO_2 and calcium hydroxide at 15 d plant age.

Attribute	Plant age (d) 15 d							
	Treatment						CD	
	Control	C	C+T_1	C+T_2	T_1	T_2	5%	1%
Ascorbic acid ($mg\,g^{-1}$ f. wt.)	0.514 ± 0.034	0.531 ± 0.019	0.539 ± 0.016	0.515 ± 0.004	0.527 ± 0.029	0.518 ± 0.044	0.068	0.095
Air pollution tolerance index (APTI)	9.224	8.923	8.830	8.753	8.745	8.776	□	□

C : 0.5% $Ca(OH)_2$, C + T_1 : 0.5% $Ca(OH)_2$ + 2612 μg m□3 SO_2, C + T_2 : 0.5% $Ca(OH)_2$ + 3918 μg m□3 SO_2, T_1 : 2612 μg m□3 SO_2, T_2 : 3918 μg m□3 SO_2

Table 2b Estimation of some biochemical components in leaves of Raphanus sativus treated with SO_2 and calcium hydroxide at 15 d plant age.

Attribute	Plant age (d) 15 days							
	Treatment						CD	
	Control	C	C+T_1	C+T_2	T_1	T_2	5%	1%
Carbohydrate ($mg\,g^{-1}$ d. wt.)	19.355 ± 0.441	18.230 ± 0.585	17.920 ± 0.116	17.454** ± 0.232	16.795** ± 0.242	15.980** ± 0.373	1.187	1.676
Protein ($mg\,g^{-1}$ f. wt.)	7.810 ± 0.967	6.499 ± 0.633	7.654 ± 0.967	6.021 ± 0.365	6.101 ± 1.00	4.432** ± 0.633	2.491	3.517
Nitrogen ($mg\,g^{-1}$ d. wt.)	0.840 ± 0.016	0.843 ± 0.024	0.817** ± 0.012	0.777** ± 0.019	0.705** ± 0.020	0.668** ± 0.011	0.060	0.085
Phosphorous ($mg\,g^{-1}$ d. wt.)	0.227 ± 0.024	0.248 ± 0.013	0.245 ± 0.008	0.243 ± 0.011	0.240 ± 0.019	0.232 ± 0.100	0.042	0.059

many workers (Rao *et al*., 1985; Kirk-Othmer, 1991; Budavari 1989). The growth improvement due to calcium hydroxide was ascribed to its acid neutralizing capacity. Calcium hydroxide ionizes in water to form calcium (Ca^{+2}) and hydroxyl (OH^-) ions, forming a strong base or alkali which effectively neutralizes the acidity of soil solution (NOSB, 2002).

Changes in antioxidant level

Noctor and Foyer (1998) investigated that ascorbic

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acid is one of the most extensively studied antioxidants and has been detected in majority of cell types, organelles and apoplast. Mapson (1958) and Materna (1972) observed that amount of ascorbic acid was decreased by SO_2 exposure. They also revealed the fact that species having higher level of ascorbic acid are more tolerant to SO_2 stress. Singh and Rao (1983) also found a direct relation between tolerance to pollution and amount of ascorbic acid. They derived a new measure of tolerance APTI (air pollution tolerance index) which represents tolerance level of plants to air pollution. They found that APTI values were dependent on ascorbic acid content of leaves.

Alterations in carbohydrate and protein metabolism

Carbohydrate level in plant is also influenced by SO_2 exposures. According to Nandi (1984) and Pande and Mansfield (1985), the possible reason for decreased sugar content in plants under SO_2 stress might be related to the increased respiration that deplete most of the food reserves.

A perusal of literature suggests that SO_2 had a marked impact on protein content of leaves and seeds. Earlier studies in this regard were made by Godzik and Linskens (1974), Sij and Swanson (1974), Constantinidou and Kozlowski (1979), Davies *et al*. (1981) and Singh *et al.* (1985). They reported that protein degradation under SO_2 stress was either due to decreased photosynthesis or due to inhibition of protein synthesis.

Changes in mineral content

Mishra (1980) reported a marked decline in the nitrogen content of SO_2 fumigated *Arachis hypogaea* plants. Such reduction in nitrogen and phosphorus content of SO_2 treated plants was also recorded by Agrawal *et al*. (1985). Peuke and Tischner (1994) exposed spruce seedlings to various doses of SO_2 and results of their study showed decrease in nitrate-nitrogen content of SO_2 fumigated seedlings.

CONCLUSION

Brassica juncea was most resistant cultivar to SO_2 while *Raphanus sativus* proved to be most sensitive cultivar. Accumulation of metabolites especially ascorbic acid and proline are the adaptive mechanisms that operate in plants, when exposed to SO_2 stress. Accumulation of ascorbic acid in plants exposed to SO_2, can be employed as sensitive biochemical indicator to SO_2 stress. Study reports that initial concentration of SO_2 (653 μg m^{-3}) caused slight stimulation in growth of *Brassica juncea*. This suggests that plants of *Brassica juncea* have the capacity to assimilate higher levels of sulphur as compared to other crops simply because of its higher requirement of the element. Thus *Brassica juncea* can tolerate or grow better up to a certain critical level of SO_2 , the level of which is higher than other crops. The present investigation led us to believe that calcium hydroxide acts as an antidote against SO_2 stress in two test crops i.e. *Brassica juncea* and *Raphanus sativus*.

References

1.Agrawal M, PK Nandi and DN Rao 1985. Effects of SO2 fumigation soil system and growth behaviour of *Vicia faba* plants. *Plant Soil Sci* 86 : 69 - 78.

2.*Budavari S (ed) 1989. *The Merck Index* : 1692 Merck and Co., Rahway New Zealand .

3.Constantinidou HA and TT Kozlowski 1979. Effects of SO2 and ozone on *Ulmus americana* seedlings II Carbohydrate, proteins and lipids. *Can J Bot* 57 : 176 - 184.

4.Davies JA, DD Davitt and SP Penny-Packer 1981. The influence of soil moisture on macroscopic SO2 injury to pinto bean foliage. *Phytopathology* 71 : 1208 - 1212.

5.Elstner EF 1982. Oxygen activation and oxygen toxicity. *Ann Rev Plant Physiol* 33 : 73 - 96.

6.Godzik S and HP Linsken 1974. Concentration changes of free amino acids in primary bean leaves after continuous and interrupted SO_2 fumigation and recovery. *Environ Pollut* 7 : 25 - 38.

7.*Jager HJ, HJ Weigel and L Grunhage 1986. Physiologische and biochemische aspekte der wirkung von immission auf Waldbaume. *Eur J Pathol* 16 : 98 - 109.

8.Kirk-Othmer 1991. In *Calcium Compounds* : 788 - 789 (eds) MM Kroschwitz and Howe-Grant. Wiley Interscience Publ.

9.Lowry OH, NJ Rosebrough, AL Farr and RJ Randall 1951. Protein measurement with folin-phenol reagent. *J Biol Chem* 193 : 265 - 275.

10.Mapson LW 1958. Metabolism of ascorbic acid in plant function. *Ann Rev Plant Physiol* 9 : 119 - 150.

11.*Materna J 1972. Ein fluss Miedriger Schwefeldioxydkozentionen auf die fichte. *MIH Forstt Bunds Vers Aust Wien* 497 : 219 - 231.

12.Mishra LC 1980. Effects of SO2 fumigation on groundnut (*Arachis hypogaea*). *Environ Exp Bot* 20 : 397 - 400.

13.Nandi PK 1984. *Phytotoxicity of soil and air pollution and its control*. A Ph.D. Thesis, Banaras Hindu University, Varanasi India.

14.Noctor G and C Foyer 1998. Ascorbate and

glutathione : keeping active oxygen under control. *Ann Rev Plant Physiol Plant Mol Biol* 49 : 249 - 279.
15.NOSB 2002. Calcium hydroxide : crops. *National Organic Standards Board Technical Advisory Panel Review*. Compiled by OMRI for the USDA National Organic Program.
16.Olsen SR, CV Cole, FS Watanable and LA Dean 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. US Department of Agriculture, Circular 939.
17.Pande PC and TA Mansfield 1985. Response of spring barley to SO_2 and NO_2 pollution. *Environ Pollut* 38 : 87 - 97.
18.Peuke AD and R Tischner 1994. The effects of SO_2 fumigation on the nitrogen metabolism of aseptically grown spruce seedlings. *Environ Pollut* 83 : 371 - 377.
19.Puckett KJ, DHS Richardson, WF Flora and E Nieboer 1974. Photosynthetic ^{14}C fixation by the lichen *Umbilicaria muttenbergii* tuck following short exposures to aqueous SO2 . *New Phytol* 73 : 1183 - 1192.
20.Rao DN, PK Nandi and M Agrawal 1985. Studies on the amelioration of air pollution effects. *Trends Plant Res* : 437 - 445.
21.Sadasivam S and A Manickam 1992. In *Biochemical Methods for Agricultural Sciences* : 178 - 180 Wiley Eastern Limited, New Delhi India.
22.Saxena DK and A Saxena 1999. Biomonitoring of SO_2 phytotoxicity on *Sphagnum squarrosum* cram Samml. *J Indian Bot Soc* 78 (III and IV) : 367 - 374.
23.Sij SS and CA Swanson 1974. Short-term kinetic studies on inhibition of photosynthesis by SO2 . *J Environ Qual* 3 : 103 - 107.
24.Singh SK and DN Rao 1983. Evaluation of plants for their tolerance to air pollution. *Proc Symp on Air Pollution Control* : 218 - 224.
25.Singh SN, M Yunus, K Srivastava, K Kulshreshtha and KJ Ahmad 1985. Response of *Calendula officinalis* (L.) to long-term fumigation with SO_2. *Environ Pollut* 39 : 17 - 25.
26.Snell FD and CT Snell 1954. In *Colorimetric Methods of Analysis* Vol. 3 (ed) E Robert. Freiger Publ Company, Huntington, New York USA.
27.Tanaka K and K Sugahara 1980. Role of superoxide dismutase in defense against SO_2 toxicity and induction of superoxide dismutase with SO_2 fumigation. *Res Rep Natl Inst Environ Stud* 11 : 155 - 164.
28.Wellburn AR, O Majernik, FAM Wellburn 1972. Effects of SO_2- and NO_2-polluted air upon ultrastructure of chloroplasts. *Environ Pollut* 3 : 37 - 49.
29.Yemm EW and AJ Willis 1954. The estimation of carbohydrates in plant extracts by anthrone. *J Biochem* 57 : 508 - 514.

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