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EFFECT OF SULPHUR DIOXIDE ON PLANT BIOCHEMICALS IN RAPHANUS SATIVUS

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ABSTRACT

In exposed seedlings, SO2 concentration ranging (653, 1306, 2612 & 3918 µg m-3 SO2) resulted in accumulation of metabolites especially ascorbic acid. It is the adaptive mechanisms that operate in plants, when exposed to SO2 stress. Carbohydrate level in plant is also influenced by SO2 exposures, the possible reason for decreased sugar content in plants under SO2 stress. All four concentrations of SO2 caused reduction in carbohydrate in both crops except at 653 µg 2m–3 of SO2 where a rise in carbohydrate level was seen. Minerals like Nitrogen and Phosphorus in leaves of Raphanus sativus were recorded to be substantially reduced following the long-term exposures of 653, 1306, 2612 and 3918 µg m–3 of SO2. SO2 caused more decline in leaf nitrogen content of Raphanus sativus. The experimental crop on exposure to SO2 had shown decline in Phosphorus content also. The reductions in carbohydrate, protein and mineral content were directly related to decline in chlorophyll content of treated seedlings.

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

plants producing visual symptoms. The effect of SO_2 was studied on the following aspects of plant biochemicals :

(a) Effect on changes in antioxidant level (Ascorbic acid)

(b) Effect on alterations in carbohydrate and protein metabolism,

(c) Effect on Changes in mineral content (Nitrogen, Phosphorus)

Experimental plant:

The experiment was carried on *Raphanus sativus (*Radish) which belongs to family Brassicaceae. *Raphanus sativus* is a cosmopolitan crop plant which is cultivated and consumed throughout the world. *Raphanus* is also an ancient kitchen garden vegetable. It can be grown from its roots, leaves or seeds depending upon its cultivar. Its habitat is terrestrial as well as wetlands. The flowers are blue to purple or pink to red or white in color

MATERIAL AND METHOD

Growth Conditions

Raphanus sativus 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 hours 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 (carried out in fumigation chamber).

SO_2 *Treatment*

$$Na_2SO_3 + H_2SO_4 \longrightarrow SO_2 \uparrow + Na_2SO_4 + H_2O$$

(126 mg) (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.

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.

a) Phenological analysis

B. 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 in boiling water 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.

C. 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 homogenized in 5 ml chilled tris maleate buffer and centrifuged at 2000 rpm for ten minutes. To 1 ml of supernatant 1 ml of trichloroacetic acid (TCA) 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 minutes 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).

D. Nitrogen Extraction & Determination

Nitrogen content in various plant parts (roots/leaves/seeds) 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. Total amount of nitrogen was calculated by preparing a calibration curve of ammonium sulphate and expressed as mg N_2 g^{-1} dry weight.

E. Phosphorus Extraction & Determination

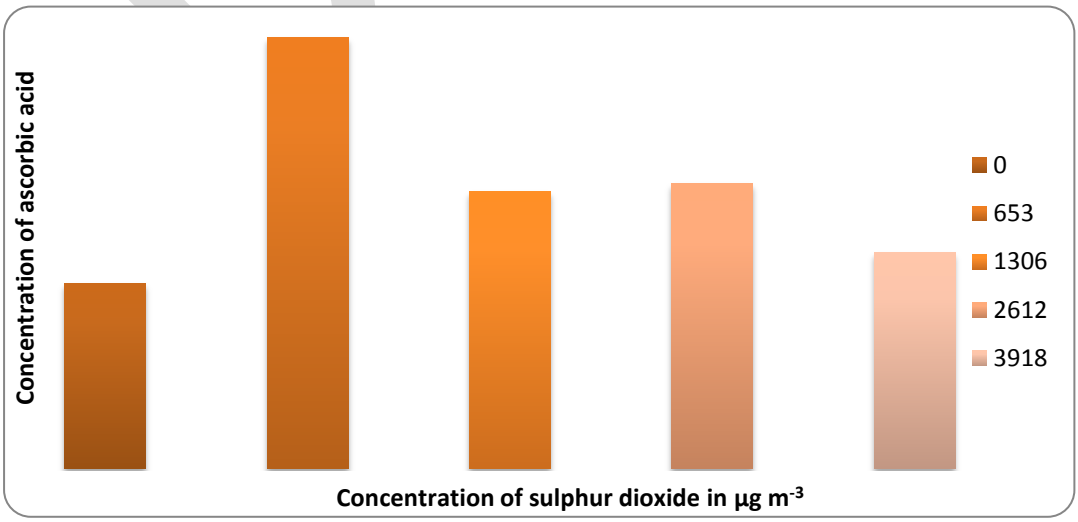
Olsen's (1954) method.

50 mg dry material (root/shoot/leaves) was homogenized in 10 ml of $NaHCO_3$ (4.2 g/l) and

Concentration of SO_2 (μgm^{-3})	Ascorbic acid (mg g^{-1} f.wt)
0	0.514
653	**0.546**
1306	0.526
2612	0.527
3918	0.518

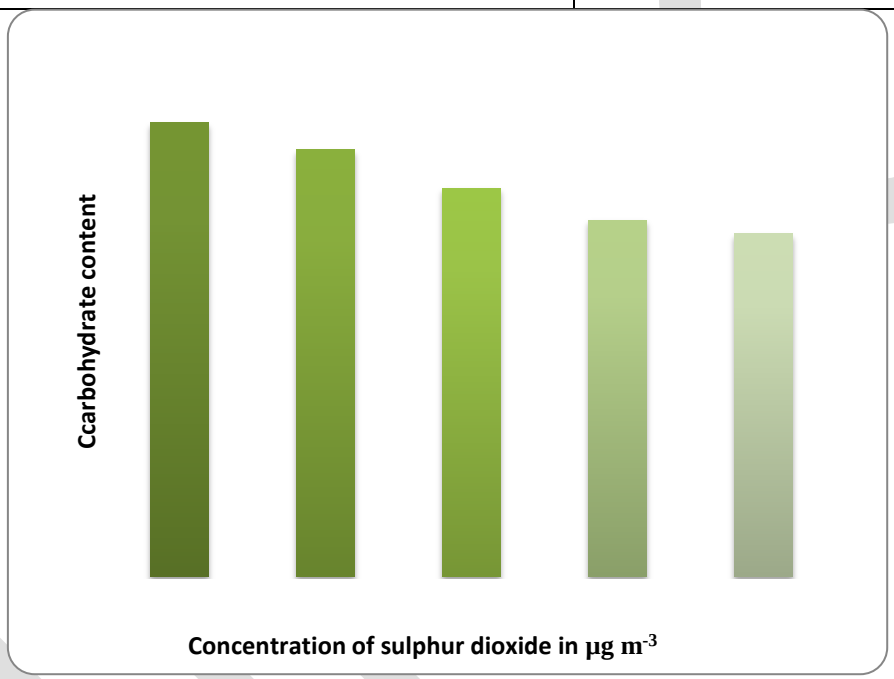
pinch of activated charcoal was added to this solution. It was kept on a shaker for 30 min. and then centrifuged at (2000 rpm) for 10 min. In a test tube 5 ml of filtrate and 5 ml molybdate reagent (15 g ammonium molybdate + 30 ml distilled water + 348 ml conc. HCl + add distilled water to make 1 liter) 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.

A. Table & Graph representing the concentration of Ascorbic acid (AA) at different concentrations of Sulphur dioxide at day 15



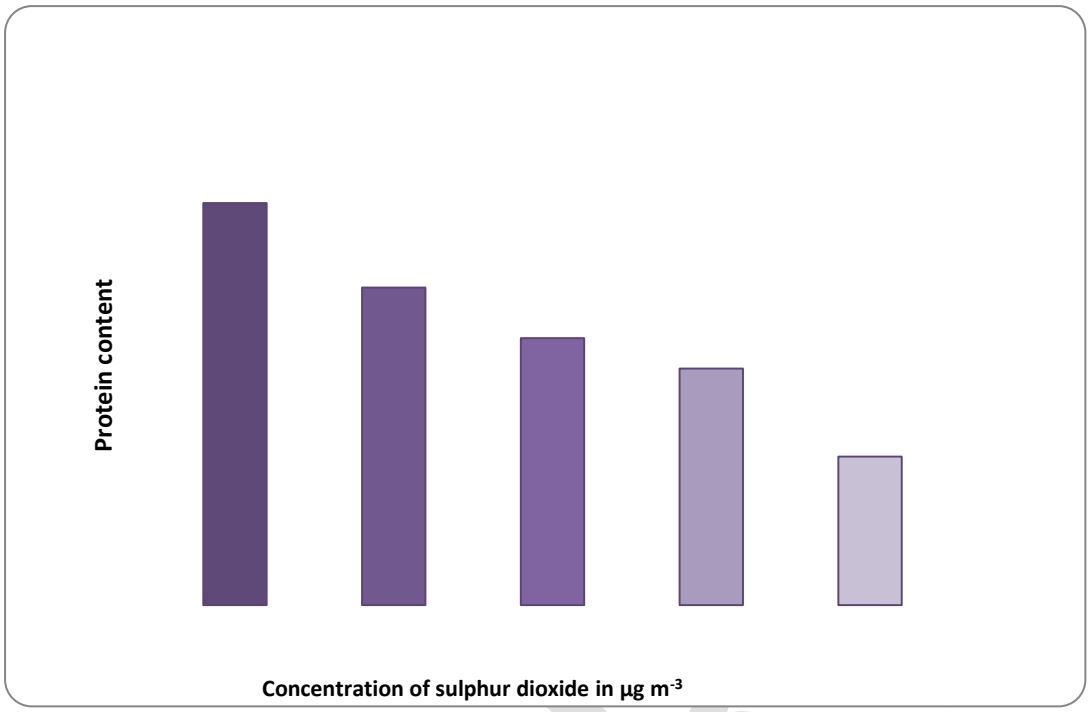
B. Table & Graph representing the concentration of Carbohydrates at different concentrations of Sulphur dioxide at day 15

Concentration of SO_2 (μgm^{-3})	Carbohydrate content (mgg^{-1}d.wt)
0	31.069
653	**29.208**
1306	26.582
2612	24.390
3918	23.443



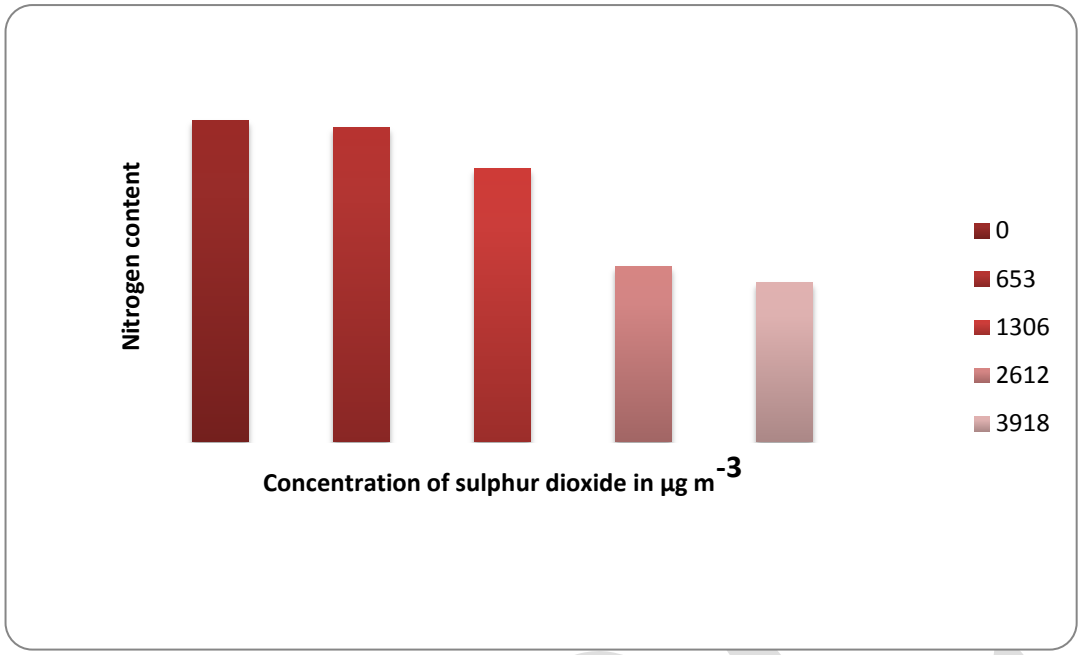
C. Table & Graph representing the concentration of Protein at different concentrations of Sulphur dioxide at day 15

Concentration of SO_2 (μgm^{-3})	**Protein content**
0	25.120
653	19.843
1306	16.676
2612	14.776
3918	9.287

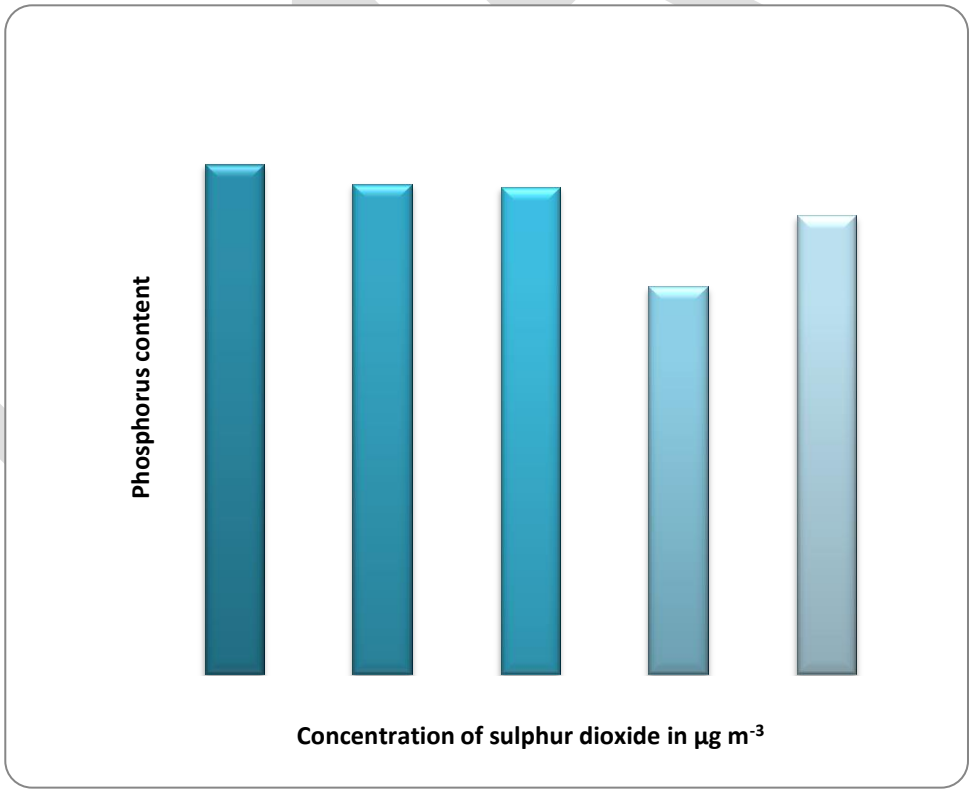


D. Table & Graph representing the concentration of Nitrogen at different concentrations of Sulphur dioxide at day 15

Concentration of SO_2 (µgm^{-3})	Nitrogen content
0	2.602
653	2.547
1306	2.210
2612	1.414
3918	1.278



E. Table & Graph representing the concentration of Phosphorous at different concentrations of Sulphur dioxide at day 15



DISSCUSSION

The reductions in carbohydrate, protein and mineral content were directly related to decline in chlorophyll content of treated seedlings. All four concentrations of SO_2 caused reduction in

carbohydrate content except at 653 µg m^{-3} of SO_2 where a rise in carbohydrate level was seen. On the contrary ascorbic acid contents of treated seedlings increased than those of control ones. Ascorbic acid concentration rose to maximum at 653µg m^{-3} of SO_2. 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

CONCLUSION:

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.**

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