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Original Contribution

EFFECT OF SULPHUR DIOXIDE ON THE BIOCHEMICAL PARAMETERS OF SPINACH (*SPINACEA OLERACIA*)

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ABSTRACT

Significant decrease in photosynthetic pigments, phenolics and amino acid was observed by the treatment of leaves with aqueous SO_2 at 250, 500, 750 and 1000 µg ml^{-1} concentration as compared to the control. However, carbohydrate showed an increase at all the test concentrations. Total protein content exhibited an increase at lower concentration (250 and 500 µg ml^{-1}) and a decrease at higher concentration (750 and 1000 µg ml^{-1}).

Key words: Carbohydrate, phenolics, pigments, protein, SO_2, *Spinacea oleracea*

INTRODUCTION

Spinach (Spinacia oleracea) is an edible flowering plant in the family of Amaranthaceae. It is native to central and southwestern Asia. It is an annual plant (rarely biennial), which grows to a height of up to 30 cm. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, ovate to triangular-based, very variable in size from about 2-30 cm long and 1-15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3-4 mm diameter, maturing into a small hard dry lumpy fruit cluster 5-10 mm across containing several seeds.

Sulfur dioxide (also sulphur dioxide) is the chemical compound with the formula SO_2. It is produced by volcanoes and in various industrial processes. Since coal and petroleum often contain sulfur compounds, their combustion generates sulfur dioxide. Further oxidation of SO_2, usually in the presence of a catalyst such as NO_2, forms H_2SO_4, and thus acid rain. This is one of the causes for concern over the environmental impact of the use of these fuels as power sources.

It is a colourless, corrosive, non-flammable gas with irritating and pungent odour and has direct effect on vegetation. These effects cause temporary and permanent injury to plant/vegetation (1) .Changes in the physical appearance of vegetation is an indication that the plant metabolism is impaired. The SO_2 enters plants by penetrating through stomatal openings and influx is mainly a diffusive flux dependent on stomatal aperture (2).SO_2 directly alter the ability of mesophyll cells to fix CO_2 (3) and thus influence the photosynthetic capacity of the plant. Toxicity is due to the reducing property of the gas. SO_2 is reported to interfere with the structure and permeability of cellular membranes and with enzymatic activity which affects many biochemical processes in the cell.

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MATERIALS AND METHODS

Preparation of aqueous SO_2 – Aqueous SO_2 of 1000 µg ml^{-1} conc. was prepared by dissolving 1.02 g of sodium sulphite in 1000 ml of distilled water. From this stock solution, different concentrations viz; 250, 500 and 750µg ml^{-1} were prepared.

Treatment of leaf discs with aqueous SO_2 and extraction- 1g of leaf discs of equal dimensions (1 cm dia) from spinach were treated with different concentrations of aqueous SO_2 for 4hrs. in glass petridishes under light provided by a 100w tungsten lamp. Different concentrations of aqueous SO_2 used were 250, 500, 750 and 1000µg ml^{-1}. Parallel control was also run. After 4 hrs. the experimental and control leaf discs were separated, washed, dried, weighed and 10% (w/v) homogenate was prepared. The homogenate was centrifuged at 5000rpm for 10 minutes. The supernatant was carefully decanted and subsequently used for various estimations.

Biochemical Estimations

Chlorophyll, phaeophytin, carotenoid, protein, carbohydrate, amino acid and phenolics content were estimated by standard methods (4).

RESULTS AND DISCUSSION

Table 1 shows the effect of different concentrations of aqueous sulphur dioxide on total chlorophyll, total phaeophytin and total carotenoid content. In case of total chlorophyll, a decrease of 45.13, 66.21, 50.45 and 39.45 percent was observed at 250, 500, 750 and 1000 µg ml^{-1} respectively, as compared to the control. Similar pattern was observed for total phaeophytin and total carotenoid content at all the test concentrations. A decrease of 47.16, 65.55, 51.07 and 41.87 percent at 250, 500, 750 and 1000 µ ml^{-1} concentration respectively was observed in phaeophytin content as compared to the control, while as total carotenoids showed a decrease of 41.54, 57.39, 44.71 and 41.90 percent respectively. Decrease in photosynthetic pigments could be due to the formation of sulphuric acid (H_2SO_4), formed by the combination of SO_2 with water in the plant tissues and subsequent dissociation into H^+ and HSO_3 ions which cause degradation of pigments (5). It is evident from **table 2** that at 250, 500, 750 and 1000 µg ml^{-1} concentration of aqueous SO_2 treatment, carbohydrate content showed an increase of 1.12, 6.74, 15.73 and 26.96 percent respectively as compared to the control. In case of starch content, an increase of 69.47, 71.57, 73.68 and 94.73 percent was observed. This could be due to the wider opening of stomatal apparatus for a longer duration in presence of SO_2 (2) .Total phenolics showed a decrease of 9.25, 28.57, 23.80 and 16.66 percent at all the test concentrations. Phenolics which are formed from shikimic acid have been observed to diminish by SO_2 treatment (6). Total protein content at 250 and 500 µg ml^{-1} concentrations showed an increase of 2.34 and 16.40 percent respectively, while a decrease of 3.90 and 18.75 percent was observed at 750 and 1000 µg ml^{-1} as compared to the control. The increase in protein content at lower concentration could be due to the utilization of sulphur for synthesis of more amino acids, while a decrease could be due to the loss of ultrastructural organization of leaf cell and destruction of ribosomes (7). In case of total amino acid content, a decrease of 13.33, 16.66, 25 and 45 percent was observed respectively, at 250, 500, 750 and 1000 µg ml^{-1}, as compared to the control. 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 (8).

Table 1. *Effect of different concentrations of aqueous SO_2 on photosynthetic pigments in leaf discs of spinach (Spinacea oleracia)*

Photosynthetic pigments	**Concentration (µg ml^{-1})**				
	Control	**250**	**500**	**750**	**1000**
Chlorophyll ‘a’ (µg ml^{-1})	2.30 ± 0.04	1.37± 0.03 (-40.43)	0.90 ± 0.02 (-60.86)	1.32±0.03 (-42.60)	1.36 ± 0.03 (-40.86)
Chlorophyll ‘b’ ((µg ml^{-1})	1.40 ± 0.03	0.66±0.008 (-52.85)	0.34±0.005 (-75.71)	0.50±0.007 (-64.28)	0.87±0.02 (-37.85)
Total chlorophyll (µg ml^{-1})	3.70 ±0.06	2.03±0.04 (-45.13)	1.25±0.03 (-66.21)	1.83±0.035 (-50.54)	2.24± 0.04 (-39.45)
Phaeophytin ‘a’ (µg ml^{-1})	3.04 ± 0.06	1.68±0.025 (-44.73)	1.14±0.02 (-62.5)	1.54 ± 0.035 (-49.34)	1.71 ± 0.035 (-43.75)
Phaeophytin ‘b’ (µg ml^{-1})	4.20 ± 0.07	2.53±0.045 (-39.76)	1.62±0.03 (-61.42)	2.47±0.04 (-41.19)	2.57 ± 0.045 (-38.80)
Total phaeophytin (µg ml^{-1})	5.11 ± 0.08	2.70±0.05 (-47.16)	1.76±0.035 (-65.55)	2.50 ± 0.045 (-51.07)	2.97±0.06 (-41.87)
Carotenoids (µg ml^{-1})	2.83 ± 0.055	1.66 ± 0.03 (-41.54)	1.21±0.02 (-57.39)	1.57 ± 0.03 (-44.71)	1.65±0.03 (-41.90)

Data represent the average of four samples analyzed separately ± S.D. values
Figures in parenthesis % increase (+) or % decrease (-) as compared to the control

Table 2.*Effect of different concentrations of aqueous SO_2 on carbohydrate, starch, phenolics, protein and amino acid content in leaf discs of spinach (Spinacea oleracia)*

Compounds	Concentration (μg ml^{-1})				
	Control	250	500	750	1000
Carbohydrate (mg ml^{-1})	0.089 ± 0.005	0.09±0.005 (+1.12)	0.095±0.006 (+6.74)	0.103±0.006 (+15.73)	0.113±0.007 (+26.96)
Starch (mg ml^{-1})	0.95 ± 0.05	1.61±0.11 (+69.47)	1.63±0.011 (+71.57)	1.65±0.11 (+73.68)	1.85±0.13 (+94.73)
Phenolics (mg ml^{-1})	0.042 ± 0.004	0.038±0.0025 (-9.52)	0.030±0.002 (-28.57)	0.032±0.0023 (-23.80)	0.035±0.0025 (-16.66)
Protein (mg ml^{-1})	1.28 ± 0.08	1.31± 0.08 (+2.34)	1.49±0.09 (+16.40)	1.23± 0.08 (-3.90)	1.04 ± 0.04 (-18.75)
Amino acid (mg ml^{-1})	0.12 ± 0.007	0.104±0.007 (-13.33)	0.10±0.007 (-16.66)	0.09±0.005 (-25)	0.066±0.004 (-45)

Data represent the average of four samples analyzed separately ± S.D. values
Figures in parenthesis % increase (+) or % decrease (-) as compared to the control

CONCLUSION

90% of sulphur dioxide emissions released into the air are from man made sources. It is believed that around 50% of the acid rain that occurs in Canada is due to pollution caused in the United States of America, and the effect of polluting industries in England can be felt in Norway.

Both natural vegetation and crops are affected by acid rain. The roots are damaged by acidic rainfall, causing the growth of the plant to be stunted, or even in its death. Nutrients present in the soil, are destroyed by the acidity. Useful micro organisms which release nutrients from decaying organic matter, into the soil are killed off, resulting in less nutrients being available for the plants. The acid rain, falling on the plants damages the waxy layer on the leaves and makes the plant vulnerable to diseases. The cumulative effect means that even if the plant survives it will be very weak and unable to survive climatic conditions like strong winds, heavy rainfall, or a short dry period. Plant germination and reproduction is also inhibited by the effects of acid rain.

Other effects

All living things, whether plants or animals, whether living on land or in the water or trees, are affected either directly or indirectly by acid rain. One effect of SO_2 is on respiratory health. Even buildings, bridges and other structures are affected. In cities, paint from buildings has peeled off and colours of cars have faded due to the effects of acid rain. From the Taj Mahal in India to the Washington Monument great buildings all over the world have been affected by the acid rainfall which causes corrosion, fracturing, and discoloration in the structures. In Europe, structures like The Acropolis in Greece and Renaissance buildings in Italy, as well as several churches and cathedrals have suffered visible damage. Even books, manuscripts, paintings, and sculpture are being affected in museums and libraries, where the ventilation system cannot eliminate the acid particles from the air which circulates in the building. In some parts of Poland, trains are required to run slowly, as the tracks are badly damaged due to corrosion caused by acid rainfall.

Solutions

The bottom line is that all things on earth are being affected by this problem and the good news is that something is being done to solve it. Pressure from the environmental groups and public has increased as the effects of the havoc caused by acid rain become more apparent. Governments all over the world have drawn up plans to tackle this problem.

Lakes that have become highly acidic can be treated by adding large quantities of alkaline substances like quicklime, in a process called liming.

The best approach seems to be in prevention. To this end, environmental regulations have been enacted to limit the quantity of emissions released in the atmosphere. Several industries have added scrubbers to their smoke stacks to reduce the amount of sulphur dioxide dumped in the atmosphere. Specially designed catalytic converters are used to ensure that the gases coming out from exhaust pipes of automobiles are rendered harmless. Several industries which use coal as fuel have begun to wash the coal before using it thereby reducing the amount of Sulphur present in it, and consequently the amount of emissions. Usage of coal with a low Sulphur content also reduces the problem.

We as individuals can take several steps to alleviate the effects of this problem. A reduction in use of vehicles will reduce the amount of emission caused by our vehicles. So do not use the car unless it is absolutely required. For going short distances, walk or try to use a bicycle. This will not only protect the environment but also improve your health. If the distance is greater, try using public transportation. If you must use your vehicle try forming a car pool and share your vehicle with someone else. Ensure that your vehicle is properly tuned, and fitted with a catalytic converter, to reduce the emissions.

Reducing power consumption will reduce the amount of coal burnt to produce electricity, and thus reduce the amount of pollution. This is true even if your electricity company does not use coal for producing electricity, but some other more environmentally friendly way. This is because the electricity you have saved can now be used elsewhere, thus benefiting nature.
Speak to others about this problem. Increasing awareness is one way of ensuring that things are done to solve this global problem.

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