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RESEARCH ARTICLE

Impact of sulphur dioxide concentration on growth and biochemical attributes of *Vicia faba* (L.)

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SUMMARY

Air Pollution has become the major problem for the world among other pollutions. The main sources of air pollutions are rapid economic growth, urbanization and industrialization. Sulphur dioxide SO_2 formerly viewed as the most valuable pollutant around the world. SO_2 enters into leaves primarily in gaseous form through the stoamata, although there is evidence for lilmited pathway via cuticle. High concentration of sulphur dioxide can produce acute injury in the form of foliar necrosis, even after relatively short duration exposure. *Vicia faba* is one of the most important winter crop of high nutritive value in the world. The present study was designed to ascertain the impacts of sulphur dioxide SO_2 pollution on *Vicia faba* plant. The present experiments was performed on the crop plant *Vicia faba* L. The monitoring of ambient air of selected sites of the Meerut city was done and the monthly mean values of sulphur dioxide ranged between 150 to 800 μgm^{-3} in winter was observed. The entire experiment was conducted with keeping the sulphur dioxide concentration below and high to the mean value of ambient air to ascertain the impacts on the selected crop plants. Ecophysiological parameters were measured and results show drastic changes especially on the higher sulphur dioxide concentrations. Plant were found to survive in moderate concentrations *i.e.*, 2612 μgm^{-3}. Biomass and root to shoot length of plants were observed to be in negative correlation with the SO_2 concentration. Chlorophyll a and b along with total chlorophyll content were found to reduce significantly on exposure of pollutant gas. Oxidative stress was also found severe in the extreme conditions in the plants which were indicated with the lipid peroxidation.

Key Words : RL, SL, LN, RB, SB, LA, *Vicia faba*

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Vicia faba plant is considered as one of the most popular and cheapest protein source (Spilsbury,2004). The response of stomata to SO_2 entry is largely dependent on leaf age, concentration and combination of pollutants (Pfanz *et al.*, 1987).

SO_2 readily dissolves in the apoplastic water to produce mainly sulphite (SO_3^{--}), bisulphite (HSO_3^-) and H+ ions, reducing the pH of the medium (Legge and Krupa, 2002). The phytotoxicity of SO_2 due to SO_3^{2-} and HSO_3^- ions (DeKok, 1990). Most leaves have the capacity to detoxify, sulphite and bisulphite, if the concentrations are not excessively high, by oxidizing them to less toxic sulphate ion (Rao, 1992). Several studies have shown that the disturbances caused by SO_2 to biochemical functions (Li *et al.*, 2007) and cell structure (Jutawong and Suwanwarer, 1997; Tripathi and Gautam, 2007) of plants appear before visual symptoms or growth reductions. The low concentrations of SO_2 have been shown to stimulate the growth and physiological responses, especially in plants growing in sulphur deficient soil (Darrall, 1989), where SO_2 might be metabolized to fulfill the demand of sulphur as nutrient (DeKok, 1990).

However, the higher uptake of SO_2 turns toxic and is reported to damage plants and reduce growth and productivity by interfering with different physiological and metabolic

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processes (Agrawal and Deepak, 2003; Agrawal *et al.*, 2006). The effects of SO_2 on physiological and biochemical characteristics of plants have been well documented (Darrall, 1989; Agrawal *et al.*, 2006; Chauhan and Joshi, 2010). Physiological processes such as photosynthesis, respiration, stomatal activity, transpiration and translocation are reported to be adversely affected by SO_2 (Darrall, 1989; Agrawal and Deepak, 2003; Li *et al.*, 2007). Alterations in various physiological functions were ascribed to changes in permeability of plasma membrane (Legge and Krupa, 2002), by interfering with enzymatic activities, and altering metabolic functions and nutrient uptake and water relations (Li *et al.*, 2007). Photosynthetic pigments and many enzymes are associated with the membranes of chloroplasts. Aqueous SO_2 can cause damage to plant metabolism by acting as an electron transport system.

In the present study, an attempt was made to study the impacts of sulphur dioxide on the eco-physiological attributes of *Vicia faba* (L.).

MATERIAL AND METHODS

Plant material (subject) and the growth performance:

For the present study the crop plant , *Vicia faba* L. (broad bean) was selected because of their availability and being the staple crop of the region. The plant species was grown in normal conditions in garden soil. The plants were 15 – 30 days old and had either four or five pairs of leaves. The plants were acclimatized by moving them into the control chamber at least 24 hours. Before experiment in the morning, each experiment was well watered to minimize stomatal response to water stress.

Samples of fully mature and healthy plant leaves of plants were collected from all the chambers. Plant material was analyzed for:

Leaf area:

Leaf area was measured with a Area Measurement System (Desta T Devices, UK).

Leaf Extract pH:

For determining the leaf extract pH, a 5 g fresh leaf was homogenized with 50 ml double distilled water and the homogenate was centrifuged at 5000 RPM for 10 minutes. The pH of the supernatant was measured with an expanded pH meter 331 (Thermo Orion Plus A).

Toxicity analysis:

The physical parameters were considered for the tolerance index where by the length of the longest root and of the longest shoot on each individual plant were measured. The tolerance indexes of the plants was quantified by comparing rates of root elongation and shoot elongation, and shoot and root dry weights in culture chambers with and without addition of SO_2 gas (a modified method of Wilkins, 1978). The formula for the tolerance index is:

$$\text{Tolerance index (\%)} = \frac{\text{Growth in gaseous exposure}}{\text{Growth in control}} \text{ x } 100$$

Chlorophyll estimation:

The chlorophyll was measured as a criterion to characterize and to select the most appropriate species for the Mesocosmic study. The values of Chlorophyll (a), (b) and carotenoids were also measured with a slight change in the method and absorbance spectra (Mc Lachlan and Zalic, 1963).

Protein estimation:

Protein in plant samples were measured prior to the extraction of protein from the tissues by Lowry *et al.* (1951) method.

Lipid peroxidation (LPO):

Malondialdehyde (MDA) concentration is a widely used method to analyze lipid peroxidation in biological material. Lipid peroxidation is a widely used stress indicator of plant membranes. The method described by Heath and Packer (1968) is the basic protocol used and has been adopted for the current study.

Accumulation of SO_4 in leaves:

Sulphate in dry leaves samples were estimated by the turbidity method given by Patterson (1958).

Statistical analysis:

Statistical analyses of data were performed using paired samples with SPSS (Ver. 12.0, SPSS, and Chicago, USA). The difference was compared for the same species between two sites. Significant differences between means were considered if $P < 0.05$. Pearson's Correlation matrix that indicate the dependency of the parameters precisely. The matrix was prepared after calculating the correlation value on Microsoft Excel.

RESULTS AND DISCUSSION

The experimental findings obtained from the present study have been discussed in following heads:

Exposure of SO_2 to the crop plant *Vicia faba* L. :

The given data represent variations in the plant growth at different time intervals (period of a fortnight *i.e.*, 15^{th}, 30^{th}, 45^{th} and 60^{th} day). The Table 1, 2, 3 and 4 represent the data of per cent variation among the prominent growth indicative parameters such as root (RL) and shoot length (SL), leaf number (LN) per plant, root (RB) and shoot biomass (SB) and leaf area (LA) (an averaged value per plant) of *Vicia faba* L. in control conditions (Table 1) and in exposed conditions.

Table 1 : Comparative data on morphological parameters obtained from the control samples of *Vicia faba* L.

Parameters	Per cent variation		
	Control		
	15-30d	30-45d	45-60d
Root length (cm)	16.45	22.25	15.13
Shoot length (cm)	25.01	22.15	8.21
Leaf number	44.89	47.89	33.33
Root biomass (g)	43.72	23.19	10.80
Shoot biomass (g)	6.35	3.90	10.79
Leaf area (cm^2)	12.00	9.17	8.35

Each table represent the % variation of the parameters mentioned earlier calculated on the basis of growth response at a different and set time interval of 15 days *i.e.*, (15 – 30), (30 – 45) and (45 – 60).

From the Table 1 control set of *Vicia faba* has all the positive values of % variation in each set of time scale for each parameters e.g., the variation in RL varies from 16.45 to 22.25 in 45 days however; this % was found to be dropped in the 60th day with a value 15.13. Similar patterns were observed in SL, LN and RB where by the earlier 45 days showed a vigor in growth response with a value about (25.01, 22.15, and 8.21); (44.89, 47.89 and 33.33); (43.72, 23.19 and 10.80), respectively whereby, reduced value of variation in the results are quite visible in the late growth period. Leaf area however; was observed to be reduced although in a random manner with a value 12.0, 9.17 and 8.35.

Table 2 represents the data of *Vicia faba* L. exposed to a concentration of 653 μgm^{-3} of SO_2. It is quite visible from the data including all the positive values showing plant ability to withstand the exposed concentrations of sulphur dioxide. Least variations in shoot biomass *viz*., 6.82, 3.69 and 4.03 at every after 15 days consecutively signifies almost a reduced growth or stunted growth further indicate the toxic manifestation of the SO_2.

Table 2 : Comparative data on morphological parameters obtained from the samples of *Vicia faba* L. exposed to 653 μgm^{-3} SO_2

Parameters	Per cent variation		
	Exposed to SO_2 (653 μgm^{-3})		
	15-30d	30-45d	45-60d
Root length (cm)	16.88	22.84	16.14
Shoot length (cm)	26.62	22.14	8.27
Leaf number	45.24	55.74	36.84
Root biomass (g)	49.43	21.15	1.68
Shoot biomass (g)	6.82	3.69	4.03
Leaf area (cm^2)	12.07	9.23	8.39

Table 3 represent the data of *Vicia faba* exposed to 1306 μgm^{-3} sulphur dioxide, a higher value of the pollutant gas. The results show the positive values of RL, SL and LN signifying the growth even on the final fortnight time scale. The values of RL, SL and LN were found (16.45, 24.22, 16.97); (28.03, 22.19, 8.52) and (41.67, 66.67, 41.18), respectively showing a pattern of stunted growth. The negative value of rest of the parameters e.g., RB, SB and LA on 60th day of harvesting signifies reduced growth response.

Table 3 : Comparative data on morphological parameters obtained from the samples of *Vicia faba* L. exposed to 1306 μgm^{-3} SO_2

Parameters	Per cent variation		
	Exposed to SO_2 (1306 μgm^{-3})		
	15-30d	30-45d	45-60d
Root length (cm)	16.45	24.22	16.97
Shoot length (cm)	28.03	22.19	8.52
Leaf number	41.67	66.67	41.18
Root biomass (g)	46.71	25.75	-3.25
Shoot biomass (g)	7.04	3.47	-4.92
Leaf area (cm^2)	12.15	9.27	-1.42

Table 4 represent the data of *Vicia faba* exposed to 2612 μgm^{-3} sulphur dioxide. The results show the stunted growth with almost all the negative values of % variation exhibiting the reduced pattern of growth.

Table 4 : Comparative data on morphological parameters obtained from the samples of *Vicia faba* L. exposed to 2612 μgm^{-3} SO_2

Parameters	Per cent variation		
	Exposed to SO_2 (2612 μgm^{-3})		
	15-30d	30-45d	45-60d
Root length (cm)	16.03	25.06	-1.49
Shoot length (cm)	29.20	22.15	-3.58
Leaf number	48.28	27.15	-45.46
Root biomass (g)	-13.38	-5.96	-14.86
Shoot biomass (g)	-0.471	-1.69	-15.49
Leaf area (cm^2)	-1.04	-1.72	-38.42

From the results given, it was observed in closely controlled experimental conditions that a range of atmospheric pollutant such as SO_2 affect the metabolism of plant cells and appreciably decrease macroscopic growth. Usually plant performance is decreased on the increase of sulphur dioxide concentrations. The adverse effects of SO_2 on plant growth parameters were earlier reviewed by Thomas (1951), and were re established by the researchers all over the world (Yunus and Iqbal, 1996; Cui *et al.*, 2006; Saquib *et al.*, 2010; Akabueze *et al.*, 2012).

Bio-molecular response of plants exposed to sulphur dioxide:

Biomolecular study has been suggested to ascertain the ecophyaiological impact of a pollutant gas (Yunus and Iqbal, 1996; Iqbal *et al.*, 2010). The content of foliar chemical and biochemical materials (chlorophyll, malondialdehyde, protein

etc.) changes to differing extents when subjected to the environmental pollutants (Wannaz *et al*., 2003). In the present study, the primary productivity in terms of chlorophyll content has been considered as a one of the molecular growth and Carotenoids as indicator of stress.

From the Table 5, it is quite indicative that on increase of concentration of sulphur dioxide causes damage to chlorophyll-a in *Vicia faba* with a value ranging from 1.418 ± 0.58 mgg^{-1}FW (milligram per gram fresh weight of leaves) in control to 0.019 ± 0.01 on a subjection of 15 days to the pollutant gas. However, further exposition was observed to be reductive as far as the chlorophyll contents are concerned. The value dropped to a minimum level of 0.0440 ± 0.91 on 60th day being exposed to 2612 μgm^{-3} SO_2.

A similar pattern was also found in chlorophyll –b content, the value of which found to be quite normal, varying only in time scales with a value 0.498 ± 0.82 on 15th day raised upto 0.921 ± 0.05 on 60th day along with almost consistent and gradual increments. On the exposure of SO_2 gas sharp and sudden drop in the content was observed (Table 6) whereby Chl-b content in *Vicia faba* under exposed condition were found to range from 0.198 ± 0.01 to 0.070 ± 0.10 mgg^{-1} FW on the 60th day. 0.070 ± 0.10 is the minimum value recorded in the leaves of *Vicia faba* exposed to 2612 μgm^{-3} SO_2. Total chlorophyll content was also found to drop in each exposition *viz.*, 653, 1306, 2612 and no values could be obtained in samples of plant exposed to 3918 μgm^{-3} SO_2 (table 7). carotenoid content express the strategy of plant to with stand stressful conditions, Table 8 represent the carotenoid content in *Vicia faba* on the same expositions as mentioned above. There is a sharp increase of carotenoids in all the concentrations of SO_2 at every fortnight ranging the values between 0.518 ± 0.11 in the samples exposed to 653 μgm^{-3} on the 15th day to a value 0.390 ± 0.15 with same exposition attributes. The higher content were observed in the single set of samples of 15th day old samples exposed to 3918 μgm^{-3} with a value 0.712 ± 0.15.

Table 9 represent the data of leaf extract pH and total sulphate content accumulated in the leaves. Results are in agreement with these findings. SO_4^{-2} content was found range between 0.62 ± 0.0 to 0.63 ± 0.9 in control, 0.67 ± 0.4 to 0.47 ± 0.91 at 653 μgm^{-3} SO_2, 0.69 ± 0.23 at 1306 μgm^{-3} SO_2, 0.61 ± 0.22 to BDL (below detection limit) at 2612 μgm^{-3} and 0.49 ± 0.20 to BDL at 3918 μgm^{-3} in *Vicia faba*. The pH extract values were also found to reduce to acidic may be because of sulphurous acid.

Sij (1971) suggested the SO_2 inhibition of apparent photosynthesis may result from this uncoupling effect of the

Table 5 : Effect of various concentrations of SO_2 on chlorophyll content in the leaves of *Vicia faba* L.

Chlorophyll content	Days of exposition	Control	653 μgm^{-3}	1306 μgm^{-3}	2612 μgm^{-3}	3918 μgm^{-3}
Chll-a (mgg^{-1}FW)	15d	1.418 ± 0.58	0.942 ± 0.83	0.901 ± 1.0	0.519 ± 0.39	0.019 ± 0.01
	30d	1.849 ± 1.20	0.846 ± 0.30	0.788 ± 0.50	0.672± 0.51	--
	45d	2.019 ± 0.72	0.833 ± 0.12	0.631 ± 0.34	0.541± 0.52	--
	60d	2.321 ± 0.95	0.783 ± 0.91	0.610 ± 0.77	0.440± 0.91	--

Table 6 : Effect of various concentrations of SO_2 on chlorophyll content in the leaves of *Vicia faba* L.

Chlorophyll content	Days of exposition	Control	653 μgm^{-3}	1306 μgm^{-3}	2612 μgm^{-3}	3918 μgm^{-3}
Chll-b	15d	0.498 ± 0.82	0.320 ± 0.35	0291 ± 0.02	0.116 ± 1.03	--
(mgg^{-1}FW)	30d	0.849 ± 0.98	0.288 ± 0.06	0.273 ± 0.01	0.092 ± 0.01	--
	45d	0.893 ± 1.02	0.208 ± 0.04	0.193 ± 0.38	0.083 ± 0.09	--
	60d	0.921 ± 0.05	0.198 ± 0.01	0.161 ± 0.90	0.070 ± 0.10	--

Table 7 : Effect of various concentrations of SO_2 on the total chlorophyll contents in the leaves of *Vicia faba* L.

Chlorophyll content	Days of exposition	Control	653 μgm^{-3}	1306 μgm^{-3}	2612 μgm^{-3}	3918 μgm^{-3}
T. Chll (mgg^{-1}FW)	15d	1.789 ± 1.80	1.097 ± 0.91	0.906 ± 0.08	0.765 ± 0.31	0.092 ± 0.03
	30d	1.983 ± 0.86	1.091 ± 0.83	0.898 ± 0.01	0.664 ± 0.09	--
	45d	2.018 ± 0.77	1.076 ± 0.45	0.882 ± 0.35	0.561 ± 0.51	--
	60d	2.172 ± 1.05	1.071 ± 0.30	0.799 ± 0.04	0.411 ± 0.02	--

Table 8 : Effect of various concentrations of SO_2 on carotenoids of the leaves of *Vicia faba* L

Parameter	Days of exposition	Control	653 μgm^{-3}	1306 μgm^{-3}	2612 μgm^{-3}	3918 μgm^{-3}
Carotenoids	15d	0.479 ± 0.15	0.518 ± 0.11	0.661 ± 0.15	0.669 ± 0.14	0.712 ± 0.15
(mgg^{-1}FW)	30d	0.361 ± 0.05	0.403 ± 0.15	0.596 ± 0.13	0.602 ± 0.10	--
	45d	0.353 ± 0.15	0.377 ± 0.13	0.481 ± 0.05	0.580 ± 0.10	--
	60d	0.311 ± 0.15	0.390 ± 0.15	0.462 ± 0.05	0.492 ± 0.11	--

Table 9 : Leaf extract pH and total sulphate accumulation in the leaves of *Vicia faba* exposed to various concentration of SO_2

Parameter	Attributes		Control	653 μgm^{-3}	1306 μgm^{-3}	2612 μgm^{-3}	3918 μgm^{-3}
Vicia faba	15d	pH	5.03 ± 1.01	5.0 ± 0.1	4.91 ±0.3	4.63 ± 0.71	4.02 ± 1.4
		SO_4^{-2}	0.62 ± 0.0	0.67 ± 0.4	0.69 ± 0.23	0.61 ± 0.22	0.49 ± 0.20
	30d	pH	5.03 ± 2.01	3.93 ± 0.90	4.01 ± 0.89	3.78 ± 0.8	3.03 ± 0.1
		SO_4^{-2}	0.79 ± 0.31	0.79 ± 0.5	0.62 ± 0.5	0.58 ± 023	0.52 ± 0.9
	45d	pH	5.09 ± 0.3	4.0 ± 0.8	3.83 ± 0.3	3.90 ± 0.1	3.39 ± 0.3
		SO_4^{-2}	0.85 ± 0.4	0.81 ± 0.2	0.70 ± 0.09	0.69 ± 0.1	0.23 ± 0.5
	60d	pH	5.90 ± 0.1	4.01 ± 0.5	4.09 ± 0.8	4.11 ± 0.45	3.09 ± 0.1
		SO_4^{-2}	0.63 ± 0.9	0.47 ± 0.91	0.38 ± 0.7	BDL	BDL

Table 10 : Effect of various concentrations of SO_2 on total protein content in *Vicia faba* L

Parameter	Days of exposition	Control	653 μgm^{-3}	1306 μgm^{-3}	2612 μgm^{-3}	3918 μgm^{-3}
Protein	15d	8.03 ± 1.09	6.87 ± 4.02	3.46 ± 0.09	3.01 ± 0.76	--
($mgg^{-1}FW$)	30d	8.19 ± 0.03	6.13 ± 2.09	3.24 ± 0.62	2.86 ± 0.12	--
	45d	9.01 ± 1.01	6.25 ± 1.50	3.31 ± 1.21	2.05 ± 1.90	--
	60d	9.15 ± 0.82	6.41 ± 0.90	2.97 ± 0.23	1.91 ± 4.01	--

Table 11 : Production of malondialdehyde (MDA) in *Vicia faba* on treatment with various concentrations of SO_2 in various time periods

LPO (MDA produced)	Days of exposition	Control	653 μgm^{-3}	1306 μgm^{-3}	2612 μgm^{-3}	3918 μgm^{-3}
	15d	0.21* ± 0.01	0.490 ± 0.90	0.650 ± 0.32	0.509 ± 0.84	0.099 ± 0.23
Vicia faba	30d	0.24 ± 0.01	0.931 ± 0.01	1.75 ± 0.95	0.400 ± 0.40	0.101 ± 0.87
	45d	0.20 ± 0.03	0.960 ± 0.51	1.70 ± 0.01	0.793 ± 0.63	--
	60d	0.19 ± 0.11	1.020 ± 0.91	1.81 ± 0.89	0.803 ± 0.11	--

sulphur anions. In the cells, SO_2 dissolve to give bisulfite and sulfite ion, sulfite is toxic and at low concentrations it is metabolized by the chloroplasts to sulphate a nontoxic mineral ion for cells (Zeiger, 2006). Thus the results (Table 9) show the least accumulation in higher concentrations of SO_2 while those survive accumulates higher contents of sulphate ions. Chlorophyll-a was found to be more severely affected than chlorophyll-b. Sulphite may react with chlorophyll to produce superoxide radicals (William and Banerjee, 1995). Chlorophyll-a is degraded to pheophytin through replacement of Mg^{+2} ions from chlorophyll molecules, but degradation of chlorophyll-b by SO_2 leads the formation of chlorophyllide-b due to removal of phytol group of the chlorophyll-b molecules (Rao and Le Blank, 1966). The sensitivity of chlorophyll a to air pollution is four and half times higher than carotenoides (Saquib *et al.*, 2010).

Table 10 represent the impact of SO_2 concentrations on protein contents of broad bean. Result show the continuous decrease in protein content ranging within a value 6.87 ± 4.02 to 3.01 ± 0.76 at 2612 μgm^{-3} SO_2 in 15 days and 6.41 ±0.90 at 653 μgm^{-3} to 1.91 ± 4.01 at 2612 μgm^{-3} in 60 days of exposure in *Vicia faba* (broad bean). There were not enough plant samples available in the sets treated with 3918 μgm^{-3} SO_2 for the protein assay. The present study show the impact of sulphur dioxide on the leaf protein in broad bean having a trend of declining protein content which is in agreement of Agarwal and Deepak (2003) who determined that sulphur dioxide enrichment results in protein reduction in the leaves by 13%. Zeiger (1975) suggested that such decrease could be attributed to break down of existing protein and reduction in synthesis.

Table 11 present the result of lipid peroxidation (LPO) further caused as a result of oxidative stress caused by sulphur dioxide. The control sets of broad bean were having the least values of malondialdehyde produced with a range 0.21 ± 0.01 to 0.19 ± 0.1 $nmolMDAg^{-1}$. The exposition of SO_2 at various concentrations for various days exhibited an increase in the MDA production with no plant sample available at the highest concentrations of SO_2. The values ranges between 0.49 ± 0.9 to 1.02 ± 0.9 at 653 μgm^{-3}, 0.65 ± 0.3 to 1.81 ± 0.9 at 1306 μgm^{-3}, 0.51 ± 0.8 to 0.80 ± 0.1 at 2612 μgm^{-3} and 0.09 ± 0.2 to BDL at extreme concentration 3918 μgm^{-3}. The LPO results of *Vicia faba* show the oxidative stress caused by the SO_2 as described by Nandi *et al.* (1990).

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