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RESEARCH ARTICLE

STOMATAL STUDY OF HELIANTHUS ANNUUS L. (SUNFLOWER) IN RESPONSE TO SULPHUR DIOXIDE POLLUTION

Ritu Goswami*

Department of Botany, R.R. Bawa DAV College for Girls, Batala – 143505, Dist. Gurdaspur (Punjab)
Email: dr_ritu22@yahoo.com

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Abstract: One of the major problems now a days is pollution and among various air pollutants, sulphur dioxide (SO_2) is probably the most widespread and intensively studied. Some of the environmental effects of SO_2 include acidification of soils, lakes and rivers on one hand and injuries and devastating damage to vegetation under natural and controlled conditions on the other. The aim of the present study was to find out the impact of sulphur dioxide pollution on the oil yielding cultivar KBSH – 1 of sunflower i.e., *Helianthus annuus* L. (family Asteraceae) on fumigation with four cumulative doses 2612, 3265, 3918 and 4571 µg m^{-3} of SO_2. The findings of the study manifested a decline in the various stomatal parameters (number of stomata and epidermal cells, size of stomatal complex, stomatal index, density and coverage area) on both adaxial (upper) and abaxial (lower) surfaces of leaves. The stomatal attributes were studied at 30th, 50th, 70th and 90th day of the fumigated cultivar along with a control set.

Keywords: Air pollution, Stomata, Sulphur dioxide, *Helianthus*

INTRODUCTION

Various environmental problems like air pollution which has appeared because of industrialization which is no doubt synonymous with progress in the modern world but has added various chemical and noxious gases like CO_2, CO, SO_2, HF, (NO)x, fly ash, and particulate matter released by various industrial units, thermal and nuclear power plants to the atmosphere. Of these, sulphur dioxide (SO_2) is one of the principal contaminants of air. During last few decades, several studies revealed the phytotoxic effects of SO_2 on higher plants (Hogestu and Shishikura, 1994). Sulphur dioxide cause severe damage to vegetation under natural and control conditions (Verma and Agarwal, 1996). If SO_2 concentration increases beyond certain critical level, various fundamental plant processes are affected (Thomas, 1961). However, sensitivity of SO_2 varies within and amongst plant species (Yunus *et al*.,1985) and also depends upon the plant age, its development and various ecological conditions (Heck and Dunning, 1978). The present study deals with the long term effects of different concentrations of SO_2 on various stomatal parameters of the oil-yielding cultivar of sunflower i.e., *Helianthus annuus* L. cv. KBSH – 1 (family Asteraceae).

MATERIALS AND METHODS

Seeds of *Helianthus annuus* L. cv. KBSH – 1 were sown in polythene bags filled with sandy loam soil. The plants were exposed to four cumulative doses i.e., 2612, 3265, 3918 and 4571 µg m^{-3} SO_2 for 2h daily from 11th day to maturity of the crop using 1m^3 polythene chambers. The SO_2 gas was prepared chemically by reacting sodium sulphite with concentrated sulphuric acid. A control set was also run in identical conditions but without exposure to SO_2. The leaves isolated from 30, 50, 70 and 90d old plants were analysed for various stomatal studies (number of stomata and epidermal cells, size of stomatal complex, stomatal index, density and coverage area) on both adaxial and abaxial surfaces of leaves. The stomata were studied by Conservative Facsimile Technique (Prakash and Kumar, 1995) and by fresh peelings also in which the epidermis of leaves were peeled off manually, stained with safranin and mounted in glycerine. The microscopic analysis was carried out using 45x objective and 5x ocular. The variations induced in epidermal pattern were evaluated by using following formulae:

$$\text{Stomatal density} = \frac{\text{no.of stomata}}{\text{unit area}}$$

$$\text{Stomatal coverage area} = \frac{\text{no.of stomata unit area}^{-1} \times \text{size of stomata}}{\text{area studied}}$$

*Corresponding Author

Stomatal index was calculated according to Salisbury (1972).

$$\text{Stomatal index} = \frac{S}{e+S} \times 100$$

where, S = no. of stomata microscopic field area^{-1}
e = no. of epidermal cells microscopic field area^{-1}
The data obtained for various attributes in treated and control both were subjected to statistical analyses.

RESULTS

The continuity of epidermis is interrupted by minute openings known as stomata. They are primary meant for gaseous exchange and transpiration but air pollutants also enter in the plant through them. The leaves of the studied cultivar were amphistomatic having higher number of stomata and epidermal cells per unit area at abaxial surface.

Treatment of plant with different concentrations of sulphur dioxide reduced the number of stomata and epidermal cells per unit area on both the surfaces of leaves but the effect was more pronounced on the adaxial surface (upper surface). Percent reduction in number of stomata recorded were 41.50 and 40.38 on adaxial and abaxial surface of leaves in 90d old plants exposed with 4571mg m^{-3} SO_2 (Table 1a and 1b). With an increase in SO_2 concentration, the number of stomata decreased and the distance between two nearest stomata got increased (Fig 1 & 2). The distance was more on adaxial surface than on abaxial surface because of the occurrence of less stomata. Similarly, number of free cells increased with the increase in concentration of sulphur dioxide. On the other hand, size of guard cells and aperture, density, coverage area of stomata and stomatal index on both the leaf surfaces showed a marked reduction following SO_2 exposure (Table 1a and 1b).

Table 1 a. Effect of different concentrations of SO_2 on the number of stomata and their associated cells per unit area (mm^2), change in average distance (mm) between nearest stomata, size (mm^2) of guard cell and stomatal aperture, density, coverage area (mm^{-2}) and stomatal index in the leaves (adaxial surface) of *Helianthus annuus* L.cv.KBSH-1.

Plant age, d	SO_2 (µg m^{-3})	Attribute								
		Stomata	Arrested Cell	Free Cell	Distance b/w two nearest stomata	Size of guard cell	Size of aperture	Density	Coverage area	Stomatal index
30	0	36.00 ± 5.8309	92.20 ± 6.3686	16.80 ± 3.3105	0.048 ± 0.0025	9.18×10^{-4}	3.62×10^{-4}	211.76	0.5421	24.82
	2612	33.60 ± 5.5353	82.20 ± 4.5343	19.40 ± 4.2708	0.057 ± 0.0019	7.68×10^{-4}	2.88×10^{-4}	197.64	0.4174	24.66
	3265	29.80 ± 5.5641	71.40 ± 4.6733	22.60 ± 3.5552	0.068 ± 0.0029	6.73×10^{-4}	2.22×10^{-4}	175.29	0.3137	24.07
	3918	27.60 ± 5.5353	62.40 ± 4.9638	26.80 ± 3.4871	0.076 ± 0.0029	5.45×10^{-4}	1.79×10^{-4}	162.35	0.2350	23.63
	4571	24.50 ± 5.2153	51.60 ± 5.1224	31.00 ± 5.4037	0.086 ± 0.0032	4.97×10^{-4}	1.58×10^{-4}	144.11	0.1887	22.87
50	0	55.40 ± 6.6513	102.80 ± 7.2498	13.80 ± 3.3105	0.044 ± 0.0025	8.60×10^{-4}	3.52×10^{-4}	325.88	0.7899	32.20
	2612	49.40 ± 4.8414	90.60 ± 6.3749	17.00 ± 3.5440	0.049 ± 0.0019	7.16×10^{-4}	2.20×10^{-4}	290.58	0.5439	31.46
	3265	42.60 ± 5.3141	77.80 ± 6.2737	18.20 ± 3.8678	0.056 ± 0.0025	5.99×10^{-4}	1.88×10^{-4}	250.58	0.3944	30.73
	3918	40.00 ± 5.6568	70.20 ± 5.3065	23.20 ± 4.5782	0.061 ± 0.0019	5.37×10^{-4}	1.38×10^{-4}	235.29	0.2219	29.98
	4571	31.60 ± 5.3141	61.80 ± 6.0464	27.40 ± 5.3141	0.071 ± 0.0029	4.78×10^{-4}	1.19×10^{-4}	185.88	0.2219	26.15
70	0	69.60 ± 5.0039	112.80 ± 5.3814	9.600 ± 1.8547	0.036 ± 0.0029	7.81×10^{-4}	2.86×10^{-4}	409.41	0.8736	36.25
	2612	60.80 ± 7.4135	101.40 ± 6.1514	13.40 ± 3.2000	0.048 ± 0.0032	6.27×10^{-4}	1.91×10^{-4}	357.64	0.5851	34.62

	3265	52.60 ± 6.1514	90.00 ± 4.7328	15.40 ± 3.7202	0.044 ± 0.0029	5.00x10^{-4}	1.30x10^{-4}	309.41	0.3898	33.29
	3918	48.40 ± 4.8414	81.60 ± 5.3141	19.80 ± 5.1923	0.050 ± 0.0019	4.40x10^{-4}	1.02x10^{-4}	284.70	0.3086	32.30
	4571	38.60 ± 5.0039	74.00 ± 6.2289	23.60 ± 4.4090	0.064 ± 0.0029	3.45x10^{-4}	0.66x10^{-4}	227.05	0.1866	28.34
90	0	80.00 ± 5.0990	121.60 ± 5.1224	8.800 ± 1.7204	0.026 ± 0.0040	5.93x10^{-4}	1.83x10^{-4}	470.58	0.7303	38.09
	2612	72.00 ± 5.7619	109.00 ± 5.6568	10.60 ± 2.4166	0.032 ± 0.0039	4.96x10^{-4}	1.19x10^{-4}	423.52	0.5209	37.57
	3265	65.40 ± 4.4090	101.60 ± 5.5713	13.40 ± 3.2619	0.040 ± 0.0043	4.22x10^{-4}	0.86x10^{-4}	384.70	0.3908	36.25
	3918	54.40 ± 4.2708	92.20 ± 4.7497	18.20 ± 3.7629	0.044 ± 0.0016	3.11x10^{-4}	0.56x10^{-4}	320.00	0.2348	33.00
	4571	46.80 ± 3.7629	83.80 ± 5.6356	20.40 ± 5.0039	0.058 ± 0.0032	2.06x10^{-4}	0.35x10^{-4}	275.29	0.1326	30.99

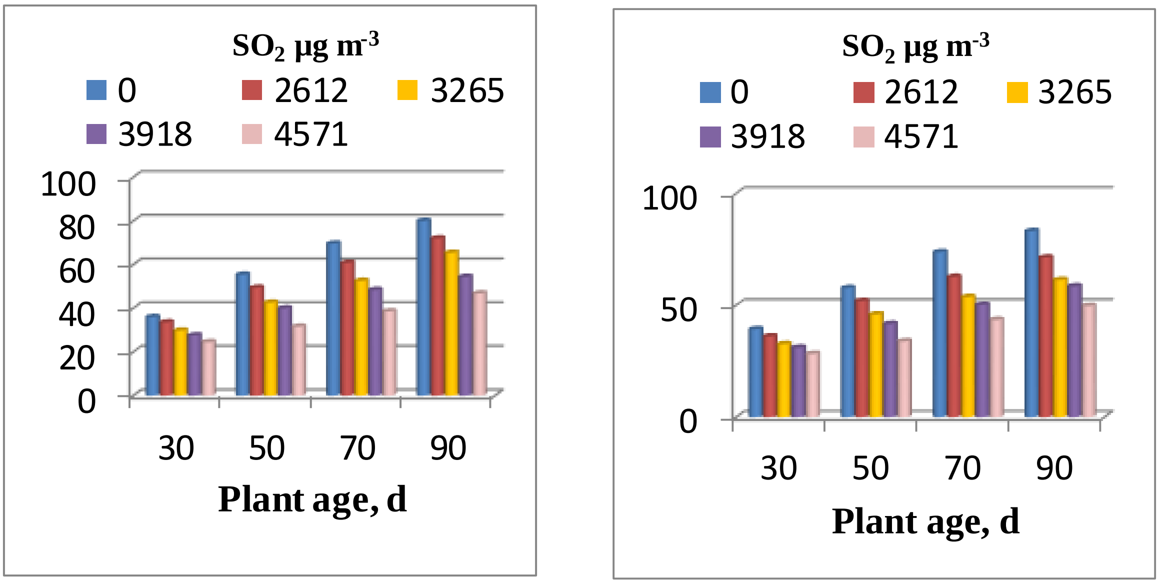
± Standard deviation

Table 1 b. Effect of different concentrations of SO_2 on the number of stomata and their associated cells per unit area (mm^2), change in average distance (mm) between nearest stomata, size (mm^2) of guard cell and stomatal aperture, density, coverage area (mm^{-2}) and stomatal index in the leaves (abaxial surface) of *Helianthus annuus* L.cv.KBSH-1

Plant age,d	**SO_2 (µg m^{-3})**	**Attribute**								
		Stomata	**Arrested Cell**	**Free Cell**	**Distance b/w two nearest stomata**	**Size of guard cell**	**Size of aperture**	**Density**	**Coverage area**	**Stomatal index**
30	0	39.20 ± 4.1665	76.00 ± 4.000	10.20 ± 1.7204	0.038 ± 0.0019	9.67x10^{-4}	3.16x10^{-4}	230.58	0.5916	31.25
	2612	36.00 ± 4.1472	70.60 ± 4.2708	13.40 ± 1.8547	0.042 ± 0.0019	8.60x10^{-4}	2.49x10^{-4}	211.76	0.4696	30.00
	3265	32.60 ± 3.8262	64.60 ± 4.4090	15.40 ± 3.2619	0.052 ± 0.0025	6.32x10^{-4}	1.97x10^{-4}	191.76	0.3179	28.95
	3918	31.00 ± 3.2863	60.60 ± 4.6733	17.60 ± 3.5552	0.060 ± 0.0016	5.06x10^{-4}	1.34x10^{-4}	182.35	0.2334	28.38
	4571	28.20 ± 3.8678	55.80 ± 2.8565	20.20 ± 3.5440	0.068 ± 0.0028	3.84x10^{-4}	0.92x10^{-4}	165.88	0.1579	27.06
50	0	57.80 ± 5.2687	105.80 ± 4.7074	8.600 ± 1.3564	0.029 ± 0.0032	7.93x10^{-4}	2.76x10^{-4}	340.00	0.7255	33.56
	2612	52.00 ± 4.0000	94.80 ± 3.7629	10.40 ± 2.0591	0.036 ± 0.0025	5.29x10^{-4}	2.15x10^{-4}	305.88	0.4551	33.07
	3265	45.80 ± 4.9558	81.40 ± 4.1279	12.60 ± 2.8705	0.041 ± 0.0032	4.64x10^{-4}	1.76x10^{-4}	269.41	0.3448	32.76
	3918	41.60 ± 4.0792	77.20 ± 3.7094	15.00 ± 2.8284	0.056 ± 0.0046	3.59x10^{-4}	1.20x10^{-4}	244.70	0.2344	32.04
	4571	33.80 ± 3.3704	68.00 ± 5.0990	17.00 ± 3.6878	0.062 ± 0.0019	2.66x10^{-4}	0.81x10^{-4}	198.82	0.1379	28.21
70	0	73.80 ± 5.5641	120.40 ± 10.744	6.200 ± 1.7204	0.024 ± 0.0025	5.37x10^{-4}	2.59x10^{-4}	434.11	0.6911	36.82
	2612	62.80 ± 5.1536	104.20 ± 7.3864	7.800 ± 1.8330	0.031 ± 0.0029	4.36x10^{-4}	2.08x10^{-4}	369.41	0.4758	35.92
	3265	53.80 ± 5.6709	91.80 ± 5.2687	9.400 ± 1.3564	0.036 ± 0.0016	3.64x10^{-4}	1.47x10^{-4}	316.47	0.3234	34.70
	3918	50.20 ± 5.5641	85.20 ± 5.1146	13.20 ± 3.0594	0.044 ± 0.0025	2.72x10^{-4}	1.15x10^{-4}	295.29	0.2285	33.78
	4571	43.40 ± 4.5431	76.60 ± 5.2383	14.80 ± 3.7629	0.056 ± 0.0025	1.83x10^{-4}	0.71x10^{-4}	255.29	10.1296	32.19
90	0	83.20 ± 5.9464	128.60 ± 11.825	5.800 ± 1.3266	0.020 ± 0.0025	4.99x10^{-4}	2.11x10^{-4}	489.41	0.6949	38.23
	2612	71.40 ± 7.0028	110.00 ± 11.296	6.200 ± 1.6000	0.026 ± 0.0032	3.76x10^{-4}	1.38x10^{-4}	420.00	0.4317	37.46
	3265	61.40 ± 5.0039	98.00 ± 7.8230	8.600 ± 1.8547	0.034 ± 0.0032	2.35x10^{-4}	0.98x10^{-4}	361.17	0.2405	36.54
	3918	58.60	94.80	11.00	0.040	1.40x10^{-4}	0.51x10^{-4}	344.70	0.1316	35.64

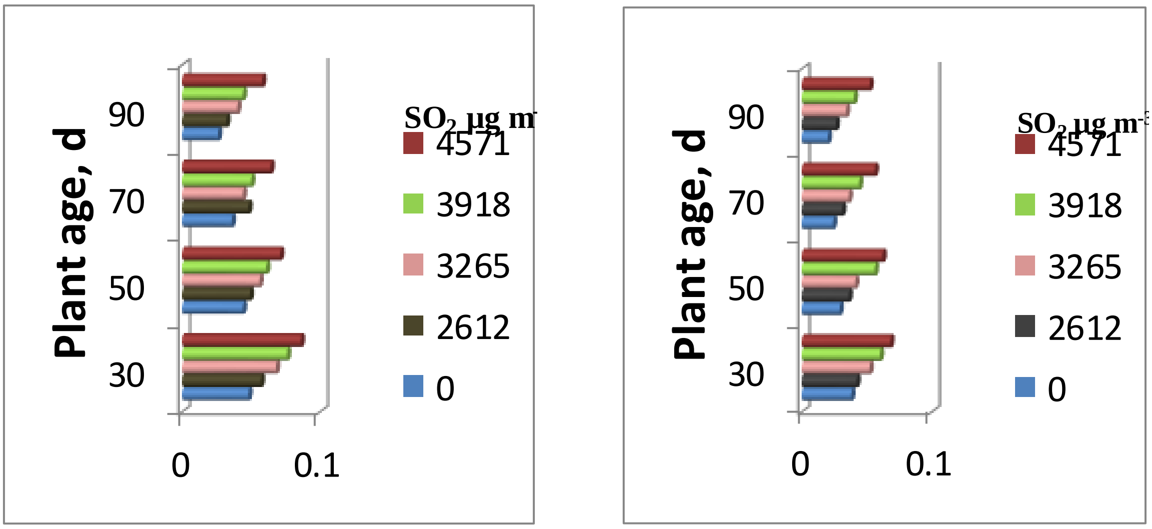
		± 4.7581	± 5.9464	± 1.7888	± 0.0029					
	4571	49.60 ± 4.1279	83.60 ± 5.8172	13.80 ± 2.4819	0.052 ± 0.0025	1.15×10^{-4}	0.30×10^{-4}	291.76	0.0846	33.74

± Standard deviation



Adaxial Surface **Abaxial Surface**

Fig 1: Effect of different concentrations of SO_2 on no. of stomata per unit area (mm^2) on the adaxial and abaxial surface of *Helianthus annuus* L. cv. KBSH – 1.



Adaxial Surface **Abaxial Surface**

Fig 2: Effect of different concentrations of SO_2 on distance (mm) between two nearest stomata on the adaxial and abaxial surface of *Helianthus annuus* L. cv. KBSH – 1.

DISCUSSION

Stomata provide the exchange of gases between the internal air spaces the ambient environment. but due to sulphur dioxide pollution the exchange of gases is adversely affected on account of inhibited number of stomata and epidermal cells on both surfaces of fumigated leaves. The decrease in number of stomata might be a sort of adaptive feature developed by the plants so as to cope up with the effect of SO_2 which may enter the leaf, injure the tissue and cause death (Chattopadhyay, 1996). Along with decrease in number of stomata and epidermal cells, size of stomatal complex, stomatal index, density and coverage area also reduced appreciably an exposure of the studied cultivar with different concentrations of sulphur dioxide. Reductions were observed on both adaxial and abaxial surfaces of leaves, however, it was more noticeable on adaxial surface because of greater exposure to pollutant. Such findings were also given by Gupta and Ghouse (1986). Suppresions in number and size of stomata are also reported by

Khan and Khan (1996) in *Solanum melongena* L. and reductions in stomatal index, density and coverage area are reported by Prakash *et al.* (2001) in *Dahlia* and *Tegetes* following exposure with SO_2. Reduction in number of stomata as well as stomatal closure may be some line of defence of plant against SO_2 stress which in turn protect leaf against further entry of the pollutant but also curtails photosynthesis.

CONCLUSION

After investigating the oil-yielding cultivar of *Helianthus annuus* L. cv. KBSH – 1 following SO_2 exposure, it can be deduced that sulphur dioxide, as a pollutant has exerted a disadvantageous influence on the plant as stress and cause appreciable reductions in stomatal response.

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