International Research Journal of Natural and Applied Sciences
Vol. 3, Issue 2, Feb 2016 IF- 2.818 ISSN: (2349-4077)

Website: www.aarf.asia Email : editor@aarf.asia , editoraarf@gmail.com

RESPONSE OF TRICHOME AND STOMATAL FREQUENCY OF LEAVES TO EXPOSURE OF AQUEOUS SULPHUR DIOXIDE BY SCANNING ELECTRON MICROSCOPY IN *CAJANUS CAJAN* AND *AMARANTHUS PANICULATUS*

B. Sujatha, B. Priyadarshini, Ch. Umamahesh, M.V.V.P. Kumar and J. Saraswathi

Department of Botany,
Andhra University,
Visakhapatnam-530003,
A.P., India.

ABSTRACT

The effect of aqueous SO_2 (0, 10, 20, 30, 40, 50, 100 and 250 ppm) on trichome and stomatal frequency of first and fourth week old plants of pigeonpea (Cajanus cajan (L.) Millsp. cv. PDM1) and amaranth (Amaranthus paniculatus L.) were studied. The stomatal frequency of leaves increased both in pigeonpea and amaranth in response to aqueous SO_2 application. Increased trichome frequency can also be seen on both the upper and lower surfaces with the help of scanning electron microscopic photographs in pigeonpea. The trichome frequency however, increased in pigeonpea and decreased in amaranth in response to aqueous SO_2 exposure.

Keywords: Amaranth, aqueous SO_2, pigeonpea, stomatal frequency, trichome frequency.

INTRODUCTION

Air pollution receives one of the prime concerns in India, primarily due to rapid economic growth, industrialization and urbanization with associated increase in energy demands. Lacks of implementation of environmental regulations are contributing to the bad air quality of most of the Indian cities. Air pollutants produced in any air shed are not completely confined, but at time trespassing all the geographical boundaries, hence do not remain only a problem of urban centre's, but spread and affect remote rural areas supporting large productive agricultural land (Richa Rai, 2011). Air pollutants pose risks on yield of crops depending on the emission pattern, atmospheric transport and leaf uptake and on the plant's biochemical defense capacity.

Plant distribution, all over the globe, is dependent on the mode of interaction of plants with their surrounding environment, which in turn depends on the type of environment and the degree of sensitivity or resistance of plants to the environmental stress (Dwivedi and Tripathi, 2007; Tripathi and Gautam, 2007). Sulphur, an essential element for all living plants, is taken up by plants in the form of sulphate from the soil through roots. Additional sulphur, if required, can be obtained by plants from the atmosphere, mostly in the form of sulphur dioxide (SO_2), through leaf stomata (Khan *et al*., 2006). In the urban areas, atmospheric SO_2 level normally varies from 0.05 to 0.5 ppm but in the vicinity of SO_2 sources such as thermal power plants, it goes high and may exceed 2 ppm (Wali *et al*., 2007). High SO_2 concentrations are phytotoxic and disturb stomatal behavior, photosynthesis, transpiration, and formation of secondary metabolites (Agrawal, 2003; Wali *et al*., 2004). In SO_2-exposed plants, sulphur accumulation occurs mainly in the aerial parts through open stomata on leaves (Iqbal *et al*., 2005; Mandal, 2006). In the mesophyll, SO_2 readily dissolves in aqueous phases thereby forming sulphurous acid with dissociation products as sulphite, bisulphite and protons (Rennenberg and Polle, 1994; Rennenberg and Herschbach, 1996). The sulphite and bisulphite anions are phytotoxic.

Cuticular waxes regulate the diffusion of water and gases and serve as a barrier to air pollutants (Schonherr, 1982). A thick cuticle provides a much higher tolerance based on the diffusion resistance to the pollutant. Sulphur dioxide exposure would alter the amount and distribution of epicuticular wax of leaves in plants (Grill, 1973; Paul and Huyah-Long, 1975; Sharma, 1975; Sharma and Butler, 1975; Godzik and Sassen, 1978; Fowler, 1980; Koziol and Cowling, 1981; Shelvey and Koziol, 1986; Pande and Oates, 1986). Plants depending upon the sulphur dioxide tolerance produce waxy depositions to cope up with the unfavourable conditions (Grill, 1973). Leaves of *Loliumperenne* exposed to sulphur dioxide had more epicuticular wax than the leaves of control plants. (Koziol and Cowling, 1981). It is suggested that the deposition of wax in response to sulphur dioxide may indicate a stress avoidance mechanism in plants. Trichomes play a protective role against sulphur dioxide exposure. Tolerant plants to sulphur dioxide exhibits greater trichome density than sensitive plants (Sharma, 1975; Elkiey and Ormrod, 1980; Krizek *et al*., 1982; Khan and Khan, 1993).

Leaves of a plant are the foremost to be affected by environmental changes including air pollution. Gaseous pollutants compete with CO_2 for uptake through stomata. A clear picture of plant-pollutant interaction with reference to crop plants is not yet available. The present study investigates the response of trichome and stomatal frequency of leaves to exposure of aqueous sulphur dioxide by scanning electron microscopy in *Cajanus cajan* (L.) Millsp. cv. PDM1), an important pulse crop and *Amaranthus paniculatus* L. (local cultivar), a popular green leafy vegetable consumed all over India.

MATERIALS AND METHODS

Preparation of aqueous sulphur dioxide

Sulphur dioxide was prepared in the laboratory by reacting sodium metabisulphite with concentrated H_2SO_4 and the generated gas was collected into distilled water. Aqueous SO_2 concentration was determined titrimetrically according to the method of Vogel (1961). Fresh stock solution of 1000 ppm concentration was prepared and from it the various concentrations of SO_2 were prepared by diluting with distilled water. The pH was adjusted to 6.9 by adding dilute NaOH. It was reported that 1 ppm SO_2 in air gives 1000 ppm in aqueous solution (Puckett *et al.*, 1973; Saunders and Wood, 1973; Malhotra, 1977).

Plant material

Seeds of pigeonpea and amaranth were washed with distilled water and surface sterilized with 0.01 M mercuric chloride and were raised in earthen pots filled with soil containing farm yard manure and soil in the ratio of 1:3. The plants were watered on alternate days. The plants were grown under a natural photoperiod of approximately 12 h and average day temperatures of 31 ± 2^{o}C and 21 ± 1^{o}C at night at Andhra university experimental farm. The aqueous SO_2 at concentrations of 0, 10, 20, 30, 40, 50, 100 and 250 ppm was supplied as foliar spray at 8.00 a.m on every third day starting from five days after germination and continued up to one month. The zero SO_2 concentration treatment was called as control. The data were collected at weekly intervals starting from the day of foliar spray. The plants were separated into leaves, stems and roots prior to each analysis.

Number of stomata and trichomes

The stomatal and trichome numbers were determined by taking leaf surface impressions of the leaves both on the upper and lower epidermis by using 'quick fix' (Wembley Laboratories, Bombay) as adhesive. The negative imprint was mounted on a clean slide using a clean coverslip. The counts were made on three randomly chosen spots on each piece and the number of stomata and trichomes in the microscopic field area were counted.

Scanning electron microscopic studies of leaf surface

The selected third leaf from the top of the 3-week old treated plants were fixed in 2.5% glutaraldehyde in 0.025 M phosphate buffer, dehydrated with alcohol series and then subjected to critical point drying in solid carbon dioxide. Ten mm of the dried specimens were coated with gold-palladium and examined in scanning electron microscope (JEOL-JSM-T330A).

RESULTS AND DISCUSSION

The stomatal studies were confined to first and fourth week old plants only. The SO_2 treated plants in the form of foliar spray registered higher stomatal frequency than the control plants in both pigeonpea and amaranth. The stomatal frequency increased with increasing SO_2 concentration. The higher stomatal frequency was recorded in 250 ppm SO_2 treated plants in both the plant species. The stomatal frequency always recorded higher values on the lower surface than on the upper surface. The stomatal frequency increased with increasing SO_2 concentration in both upper and lower surfaces of both pigeonpea and amaranth (Tables-1 and 2). The higher frequency of stomata may be presumably due to the restricted leaf area expansion.

Table 1: The effect of aqueous SO_2 on stomatal frequency of pigeonpea leaves (Number of stomata/mm^2) (Mean of 15 replications with SE)

SO_2 conc. (ppm)	Stomatal frequency			
	Primary leaf (1-week)		Trifoliar leaf (4- week)	
	U.E.	L.E.	U.E.	L.E.
0	160.43 ±1.95	519.61 ±2.95	206.01 ±2.49	662.19 ±6.48
10	163.36 ±1.18	544.65 ±3.22	212.11 ±1.72	682.10 ±6.96
20	166.77 ±1.48	555.59 ±2.66	215.11 ±1.55	704.98 ±2.77
30	173.29 ±1.39	588.39 ±3.44	219.12 ±1.89	710.19 ±3.70
40	175.29 ±1.17	597.30 ±2.66	221.70 ±1.33	720.03 ±2.58
50	178.63 ±1.35	616.44 ±2.84	227.46 ±1.89	729.88 ±4.33
100	186.56 ±1.68	628.79 ±2.74	232.47 ±2.39	745.66 ±5.31
250	193.24 ±2.43	648.69 ±2.69	239.33 ±2.56	765.56 ±5.31

U.E. = Upper epidermis; L.E.=Lower epidermis.

Table 2: Effect of aqueous SO_2 on stomatal frequency of amaranth leaves (Number of stomata/mm^2) (Mean of 15 replications with SE)

Stomatal frequency				
SO_2 conc. (ppm)	Primary leaf (1-week)		Trifoliar leaf (4- week)	
	U.E.	L.E.	U.E.	L.E.
0	81.552 ±1.50	121.869 ±1.90	96.410 ±1.40	153.255 ±3.08
10	85.55 ±1.15	126.544 ±1.74	102.003 ±1.66	156.678 ±1.74
20	93.656 ±0.82	133.739 ±1.88	106.928 ±1.24	163.105 ±1.25
30	101.085 ±1.53	143.839 ±1.67	113.105 ±1.15	169.324 ±1.35
40	106.427 ±1.66	150.434 ±1.82	116.694 ±1.52	175.626 ±1.47
50	107.68 ±1.54	155.018 ±1.65	118.697 ±1.77	178.046 ±1.11
100	110.023 ±1.67	159.699 ±1.88	122.370 ±2.60	189.148 ±1.88
250	114.697 ±1.70	172.215 ±2.98	138.310 ±4.07	195.826 ±2.54

U.E. = Upper epidermis; L.E. =Lower epidermis.

In pigeonpea the control plants registered lowest trichomes frequency both on the upper and lower surface, when compared to the plants treated with foliar application of aqueous SO_2 both in the primary and trifoliate leaves (Table-3). The increase in the trichome frequency becomes more conspicuous with increasing SO_2 concentration. Trichome frequency was greatest in 250 ppm SO_2 treated plants. The trichome frequency was always higher on the lower surface than on the upper surface of pigeonpea leaves.

Table-3: The effect of aqueous SO_2 on trichome frequency of 4-week old pigeonpea leaves (number of trichomes/mm^2) (Mean of 15 replications with SE)

SO_2 Conc. (ppm)	Trichome frequency			
	Primary leaf (1-week)		Trifoliate leaf (4-week)	
	U.E.	L.E.	U.E.	L.E.
0	18.364 ± 1.40	35.225 ±1.29	38.898 ±2.19	55.759 ±2.60
10	21.911 ±1.47	43.823 ±2.32	49.415 ±1.54	66.110 ±1.75
20	34.574 ±2.60	53.773 ±1.34	54.674 ±2.22	76.377 ±1.54
30	38.205 ±1.75	63.689 ± 2.13	58.848 ±2.36	82.470 ±1.93
40	43.289 ±3.20	71.452 ±1.84	64.190 ±1.56	85.559 ±1.15
50	48.889 ±1.55	77.128 ±2.66	67.779 ±1.32	88.063 ±1.60
100	58.070 ±1.40	84.808 ±1.93	70.185 ±1.44	93.072 ±1.93
250	72.280 ±5.60	94.186 ±1.70	78.130 ±1.61	102.921 ±1.95

U.E. = Upper epidermis; L.E. =Lower epidermis

Amaranth showed relatively low trichome frequency when compared to pigeonpea leaves. Trichomes on amaranth leaves are uniseriate with ellipsoidal heads. Their distribution is mainly located on coastal regions (Metcalfe and Chalk, 1950). Somehow, the trichome frequency of amaranth leaves reduced on both the surfaces with increasing concentration of foliar application of aqueous SO_2. Further contrary to pigeonpea, trichome frequency was higher on the upper surface than on the lower surface (Table-4). Interestingly the druse number increased both on the upper and lower sides of amaranth leaves in response to foliar application of aqueous SO_2 (Table-5).

Table-4: The effect of aqueous SO_2 on trichome frequency of 4-week old amaranth leaves (number of trichomes/mm^2) (mean of 15 replications with SE)

SO_2 Conc. (ppm)	Trichome frequency	
	U.E.	L.E.
0	129.79 ±3.25	88.06 ±4.22
10	119.03 ±3.06	80.55 ±2.73
20	103.00 ±3.64	71.36 ±5.48
30	89.73 ±4.73	58.84 ±3.45
40	73.03 ±5.48	47.16 ±4.44
50	58.34 ±4.32	36.31 ±3.35
100	43.82 ±7.36	29.63 ±2.32
250	29.63 ±7.94	20.86 ±4.85

U.E. = Upper epidermis; L.E. =Lower epidermis

Table-5: The effect of aqueous SO_2 on druse frequency of 4-week old amaranth leaves (number of druses/mm^2) (mean of 15 replications with SE)

SO_2 Conc. (ppm)	Druse frequency (average of upper and lower epidermis)
0	430.30 ±7.89
10	440.15 ±3.82
20	458.09 ±2.55
30	471.11 ±4.33
40	497.99 ±3.75
50	521.36 ±5.33
100	557.02 ±4.66
250	598.16 ±3.78

In pigeonpea the increase in trichome frequency can also be seen on both the upper and lower surfaces with the help of scanning electron microscopic photographs (Plate-1,2 a,b,c,d). Somehow, the trichome preparations of amaranth could not be obtained properly since, during the processing of the material they might have lost.

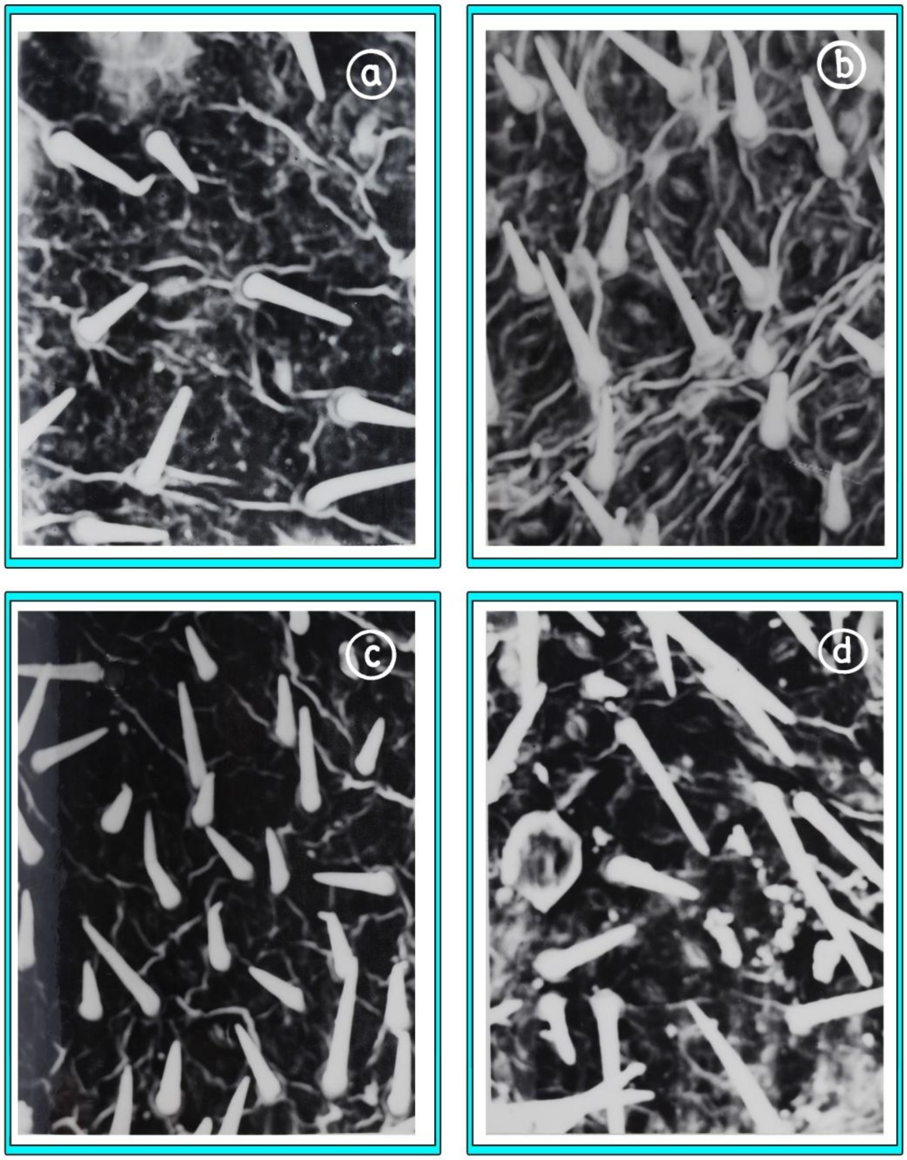

Plate-1: Scanning electron micrographs of pigeonpea, showing trichomes of adaxial leaf surface of 3-week old plant, in response to the foliar application of aqueous SO_2.

a - 0 ppm (x300); b - 30 ppm (x300); c - 100 ppm (x300); d - 250 ppm (x300)

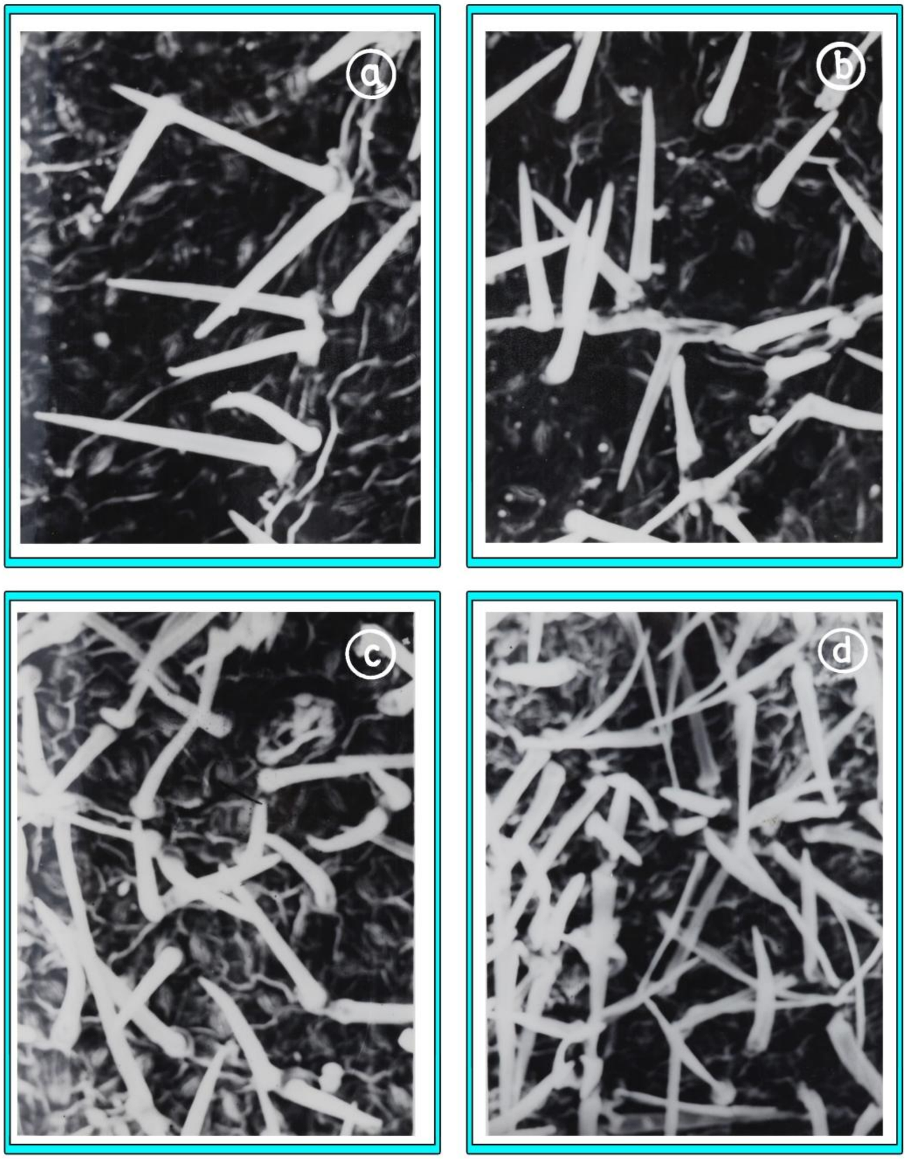

Plate-2: Scanning electron micrographs of pigeonpea, showing trichomes of abaxial leaf surface of 3-week old plant, in response to the foliar application of aqueous SO_2.

a - 0 ppm (x300); b - 30 ppm (x300); c - 100 ppm (x300); d - 250 ppm (x300)

Trichomes play an important role in the protection of plant leaves (Thruston *et al*., 1966). Aqueous SO_2 enhanced the trichome frequency in pigeonpea with increasing SO_2 concentration (Plate-1 and 2; Table-3). On the other hand, the trichome frequency in amaranth declined in response to increasing SO_2 concentration (Table-4). Sharma and Tyree (1973), Sharma and Butler (1975) suggested a positive correlation between the levels of air pollution and trichome density of plant species. The more the number of trichomes the more the tolerance potential of the plant species to SO_2 (Krizek *et al*., 1984; Muthuchelian, 1993). The increase in trichome length has also been taken as a tolerance trait to SO_2 in *Lycopersicum esculentum* cultivars (Khan and Khan, 1993). Sulphur dioxide damage of trichomes and stomata were also noted in *Phaseolus vulgaris* and *Helianthus* (Franke, 1971; Evans *et al*., 1977; Evans and Curry, 1979). One of the reasons for the relative tolerance of pigeonpea to SO_2 exposure may be presumed due to the presence of relatively more pubescent leaf surface than amaranth. Increased trichomes may also act as an extra sink to reduce SO_2 impact on leaf metabolism. The reduction of trichome number in amaranth may be due to the effect of SO_2 on the development of trichomes possibly affecting their differentiation.

CONCLUSIONS

The stomatal frequency increased with increasing SO_2 concentration in both upper and lower surfaces of both pigeonpea and amaranth. Trichome frequency of pigeonpea leaves increased in response to SO_2 exposure. On the other hand interestingly the trichome frequency of amaranth leaves decreased with increasing SO_2 concentration. Increase in trichomes may be considered as an avoidance mechanism in pigeonpea to SO_2 by providing an extra sink to reduce the SO_2 impact on leaf metabolism. The number of druses in the leaves of amaranth decreased with increasing SO_2 concentration and duration of exposure.

REFERENCES

Agrawal M., 2003. Plant responses to atmospheric sulphur. In: Sulphur in plants (Eds. Y.P. Abrol and A. Ahmad). Kluwer Academic Publishers, The Netherlands. pp. 279-294.

Dwivedi AK and Tripathi BD., 2007. Pollution tolerance and distribution pattern of plants in surrounding area of coal-fired industries. *J. Environ. Biol*., **28**(2):257-263.

Elkiey T and Ormrod DP., 1980. Sorption of ozone and sulfur dioxide by *Petunia* leaves. *J. Environ. Qual*., **9**:93-95.

Evans LS and Curry TM., 1979. Differential responses of plant foliage to simulated acid rain. *Ame. J. Bot*., **66**(8): 953-962.

Evans LS., Gmur NF and Da Costa F., 1977. Leaf surface and histological perturbations of leaves of *Phaseolus vulgaris* and *Helianthus annus* after exposure to simulated acid rain. *Ame. J. Bot*., **64**:903-913.

Fowler D., 1980. Removal of sulfur and nitrogen compounds from the atmosphere in rain and by dry deposition. In Drablos and Tollan. pp.22-32.

Franke W., 1971. The entry of residues into plants via ectodesmata (ectocythodes). *Resi. Rev*., **38**:81-115.

Godzik S and Sassen MA., 1978. A scanning electron microscope examination of *Aesculus hippocastanum* L. leaves from control and air -polluted areas. *Environ. pollut*., **17**:13-18.

Grill D., 1973. Rasterelek tromenmikorskopische utersuchungen an SO_2-belastetenfichtenna detn**.** *Phytopath. Z*., **78**:75-80.

Iqbal M, Bano R and Wali, B., 2005. Plant growth responses to air pollution. In: Chaturvedi, S.N., Singh, K.P. (Eds.), Plant Biodiversity, Microbial Interaction and Environmental Biology. Aavishkar Publishers, Jaipur, pp. 166-188.

Khan I, Ahmad A and Iqbal M., 2006. Sulphur in the environment. In: Tandon, P., Khatri, S., Abrol, Y.P. (Eds.), Biodiversity and its Significance. IK International, New Delhi, pp. 90-99.

Khan MR and Khan MW., 1993. The interaction of SO_2 and root-knot nematode on tomato. *Environ. Pollut*., **81:**91-102.

Koziol MJ and Cowling DW., 1981. Effect of exposure to SO_2 on the production of epicutical wax in *Loliumperenne* L. *Environ. Pollut*. (series A), **26:**183-186.

Krizek DT., Wergin WP and Shemeniuk P., 1982. Physiological and morphological properties of leaves and bracts of *Poinsettia* in relation to sulfur dioxide tolerance**.** *Hort.Science*, **17:**519.

Krizek DT., Wergin WP and Shemeniuk P., 1984. Morphological and physiological properties of *Poinsettia* leaves and bracts in relation to sulfur dioxide sensitivity. *Environ. Expt. Bot*., 165-173.

Malhotra SS., 1977. Effects of aqueous sulphur dioxide on chlorophyll destruction in *Pinus contorta*. *New Phytol*., **78**:101-109.

Mandal, Madhumanjari, 2006. Physiological changes in certain test plants under automobile exhaust pollution. *J. Environ. Biol*.., **27**:43-47.

Metcalfe CR and Chalk L., 1950. 212. Amaranthaceae. In: Anatomy of the dicotyledons. Vol.II. Clarendon press Oxford, UK. pp. 1067-1069.

Muthuchelian K, Nedunchezlian N and Kulandaivelu G., 1993. Effect of simulated acid rain on $^{14}CO_2$ fixation, ribulose-1,5-bisphosphate carboxylase and nitrate and nitrite reductases in *Vigna sinensis* and *Phaseolus mungo*. *Photosynthetica*, **28**:361-367.

Pande PC and Oates K., 1986. SEM analysis of *Commelina communis* L**.** leaves after exposure to SO_2 and NO_2 pollution. *Environ. pollut*. (series A) **42**:353-360.

Paul R and Huynh-Long V., 1975. Premieres observations, realisees au microscope electronique a balayage, sur les effects du dioxyde de sufre an du dioxyde de soufre an niveau de l'epidorme de fevilles de *Phaseolus vulgaris* L**.** *Parasitica*, **31**:30-39.

Puckett KJ, Niebor E, Flora WP and Richardson DHS., 1973. Sulphur-dioxide: Its effect on photosynthetic ^{14}C fixation in lichens and suggested mechanisms of phototoxicity. *New Phytol*., **72**:141-154.

Rennenberg H and Herschbach C., 1996. Responses of plants to atmospheric sulphur. In: Yunus, M., Iqbal, M. (Eds.), Plant Response to Air Pollution. Wiley, Chichester, pp. 285-293.

Rennenberg H and Polle A., 1994. Metabolic consequences of atmospheric sulphur influx into plants. In: Alscher, R.G., Wellburn, A.R. (Eds.), Plant Responses to the Gaseous Environment. Chapman & Hall, London, pp. 165-180.

Richa Rai, Madhu Rajput, Madhoolika Agrawal and S.B. Agrawal 2011. Gaseous air pollutants : a review on current and future trends of emissions and impact on agriculture. *J. Sci. Res*., **55**:77-102.

Saunders PJW and Wood CM., I973. SO_2 in the environment, its production, dispersal, and fate. In: Air Pollution and Lichens (Ed. by B. W. Ferry, M. S. Baddeley, and D.L. Hawksworth), pp. 6. Athlone Press, London.

Schonherr J., 1982. Resistance of plant surfaces to water loss: Transport properties of cutin, suberin and associated lipids. In:Lange *et al*., vol.**12**B:153-180.

Sharma GK and Tyree J., 1973. Geographic cuticular and gross morphological variations in *Liquidambar styraciflua* and their possible relationship to environmental pollution. *Bot. Gaz*., **134**:179-184.

Sharma GK and Butler J., 1975. Environmental pollution: Leaf cuticular patterns in *Trifolium pratense* L. *Ann. Bot*., **39**:1087-1090.

Sharma GK., 1975. Leaf surface effects of environmental pollution on sugar maple (*Acersaccharum*) in Montreal. *Can. J. Bot*., **53**:2312-2314.

Shelvey JD and Koziol MJ., 1986. Seasonal and SO_2 induced changes in epicuticular wax of rye grass. *Phytochemistry*, **25**:415-420.

Thruston R, Smith WT and Cooper BP., 1966. Alkaloid secretion by trichomes of *Nicotiana* species and resistance to aphids. *Entomol. Exp. App*., **9**:428-432.

Tripathi AK and Mukesh Gautam, 2007. Biochemical parameters of plants as indicators of air pollution. *J. Environ. Biol*., **28**:127-132.

Vogel AI., 1961. A text book of quantitative inorganic analysis including elementarary instrumental analysis. The English language book society and longman. pp.370.

Wali B, Mahmooduzzafar and Iqbal M., 2004. Plant growth, stomatal response, pigments and photosynthesis of *Althea officinalis* as affected by SO_2 stress. *Ind. J. Plant Physiol.*, **9**:224-233.

Wali B, Iqbal M and Mahmooduzzafar, 2007. Anatomical and functional responses of *Calendula officinalis* L. to SO_2 stress as observed at different stages of plant development. *Flora*, **202**:268-280.