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Effect of SO_2 on Growth and Development of *Dahlia rosea* Cav.

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Sulphur dioxide, being a by-product of fossil fuel consumption is one of the major air pollutants prevailing in Indian environment. The pernicious effects of SO_2 on plant growth and yield have been well documented (Mudd and Kozlowski 1975, Linzon 1978). However, it remained debatable for many years whether low concentrations of SO_2 are adequate to induce injury to agricultural and horticultural crops. In recent years, there has been a growing interest in the study of the mechanism of SO_2 phytotoxicity (Malhotra and Hocking 1976; Jager 1977; Schlee 1977). Takemoto and Noble (1982) and Alscher (1984) studied the effect of SO_2 on light and dark reactions of photosynthesis and respiration. The break-down of chlorophylls was reported by Rao and LeBlanc (1966) and Syratt and Wanstall (1969) in lower plants. Godzik and Linskens (1974) noted a remarkable decrease in protein content of SO_2-exposed Phaseolus vulgaris leaves accompanied by an increase in free amino-acids pool. Unsworth et al. (1972) demonstrated that relative humidity modified the response of stomata to SO_2. Bonte (1982) reviewed the effects of air pollutants on flowering and fruiting of plants.

In the light of above reports, the present study was undertaken to investigate the effect of long-term fumigation with SO_2 on plant foliage, photosynthetic pigments, protein content, stomatal response, phytomass and flowering in Dahlia rosea Cav. a popular ornamental annual plant.

MATERIALS AND METHODS

Seedlings of Dahlia rosea Cav. (30-day-old) were transplanted in six 1 m^2 plots at a distance of 20 cm from plant to plant in rows, 25 cm apart. At age 60 days, the plants of two plots were fumigated with 1 ppm SO_2 and that of another two plots with 2 ppm SO_2. The fumigation was carried out inside 1 m^2 polythene chambers for two hours on alternate days between the age of 30 and 75-days as scheduled in Table 1 (Singh et al. 1985). The plants of remaining two plots were not treated with SO_2 and they served as controls. However, control plants were also covered with polythene chambers during fumigation period in order to simulate the conditions of treated

Table 1. Fumigation and sampling schedule of Dahlia plants

Plant age in days	Experimental conditions	Stage of growth	Cumulative dise (c x t) 1 ppm SO_2	Cumulative dise (c x t) 2 ppm SO_2	Sampling order
1-60	Normal	Vegetative	-	-	
75	Fumigation	Pre-flowering	14	22	I
90	Fumigation	Flowering	28	56	II
105	Fumigation	Post-flowering	42	84	III

plants. The plant samples were periodically collected from control and treated plants at pre-flowering (75-day-age), flowering (90-day-age) and post-flowering (105-day-age) stages of growth and analysis with respect to foliar injury, photosynthetic pigments, protein content, stomatal response, phytomass and flower size.

The plants were excavated from each plot at every sampling time and washed carefully under tap water. The intact plants were partitioned into shoot and root and their lengths were measured. The plant parts were oven-dried at 80°C for 24 hours to estimate phytomass accumulation.

The photosynthetic pigments were extracted in 80% ice cold acetone along with a small amount of sodium carbonate. The optical density of clear pigment extract was measured at 480, 510, 645 and 663 nm wavelengths with EC Junior Spectrophotometer (GS 886_1 C). The amount of chlorophyll a and b and carotenoids (mg g^{-1} dry leaf) were calculated using the formulae given by MacLachlam and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Protein estimation in plant leaves was done following the biuret method (Layne 1957).

Scanning electron microscopic (SEM) studies were carried out on control and treated foliar surfaces to observe the stomatal response to SO_2 pollution. For the SEM study, small strips (about 0.5 cm^2) were trimmed from areas between the margin and midrib of leaves fixed in FAA (formaldehyde:Acetic acid:50% alcohol; 9:0.5:0.5 v/v). The material was dehydrated through ethanol series (from 30% to absolute). The foliar strips were then dried in a critical point drier using liquid CO_2. After drying, small pieces of leaf (about 1.00 cm^2) were cut from the strips. Two pieces of material, one with the abaxial surface, and the other with the adaxial surface, on top, were mounted, side by side, on the specimen stubs using double-sided adhesive tape.

The specimens were coated with a thin layer of gold (about 200 Å)

in a sputter coater and then examined under a JEOL-JSM 35C scanning electron microscope at an accelerating voltage of 10 kv and an aperture 100 μm. The image was observed at a magnification range from 200-5000 and photographs were taken on Superpan Agfa 120 roll film.

RESULTS AND DISCUSSION

The development of foliar injury symptoms is the direct manifestation of phytotoxic nature of SO_2 (Hill et al. 1974). In the present study, it was observed that chlorotic symptoms appeared after 20 days of fumigation first on the leaves of 2 ppm SO_2-treated plants followed by 1 ppm SO_2-treated plants. With an increase in pollutant dose, the chlorotic patches on the leaves enlarged in size and gradually turned grey or brown with the death of leaf tissues (Linzon 1967). It was also interesting to note that injury symptoms were localized and developed simultaneously on both the surfaces (adaxial and abaxial) of treated leaves. As shown in Table 2, the areas of foliar injury were 63.36 and 88.10 cm^2 in 1 and 2 ppm SO_2-fumigated plants, respectively at post flowering stage, when pollutant dose augmented to 42 and 84 ppm hr in 1 and 2 ppm SO_2-treated plants, respectively.

Table 2. Areas of foliar injury (cm^2) of Dahlia plants treated with SO_2 at different stages of growth (average of 5 replicates)

Experimental conditions	Stages of growth		
	Pre-flowering	Flowering	Post-flowering
Control	No injury	No injury	No injury
1 ppm SO_2	No injury	30.46±2.15	63.36±5.18
2 ppm SO_2	No injury	40.18±4.28	88.10±5.82

± standard deviation

The appearance of localized injury symptoms clearly indicates that SO_2 on being dissolved in H_2O inside the leaf tissues, is not transported through transpirational pull (MacClean et al. 1968) and so causes local injury by generating toxic ions (Malhotra and Hocking 1976). The plant's ability to detoxify the toxic ions determines the plant's susceptibility to SO_2 (Thomas et al. 1943, Taoda 1973) to a greater extent.

The levels of photosynthetic pigments were significantly lowered by SO_2 in treatd plants with respect to their controls ($P < 0.05$). The concentrations of Chl a, b and carotenoids attained maximum values at the pre-flowering stage in both control and treated plants then declined gradually as the plants matured. It is interesting to note that the extent of reduction in pigment contents kept on increasing with both the pollutant dose and plant age. In 1 ppm SO_2-treated plants, the concentrations of Chl a, b and carotenoids were reduced by 15.68, 8.13 and 3.22%, respectively at the post-flowering stage when the pollutant dose was 42 ppm hr (Table 3). At the same stage

Table 3. Concentrations of Chl a, b, carotenoids and total (mg g^{-1}) dry wt in the leaves of control and treated Dahlia plants (average of three replicates)

Stages of growth	Experimental condition	Concentrations of photosynthetic pigments				
		Chl a	Chl b	Carote-noids	Total	Chl a/ Chl b/ ratio
Pre-flowering	Control	6.13±0.04	4.45±0.02	3.81±0.02	14.39	1.37
	1 ppm	5.73±0.06	4.35±0.04	3.75±0.01	13.83	1.31
	2 ppm	5.62±0.07	4.28±0.02	3.70±0.03	13.61	1.31
Flowering	Control	5.26±0.05	4.08±0.06	2.54±0.05	11.88	1.28
	1 ppm	4.75±0.07	3.91±0.03	2.48±0.02	11.14	1.21
	2 ppm	4.62±0.04	3.80±0.05	2.45±0.02	10.87	1.21
Post-flowering	Control	3.45±0.09	2.48±0.02	1.55±0.03	7.48	1.39
	1 ppm	2.90±0.05	2.27±0.05	1.50±0.01	6.67	1.27
	2 ppm	2.67±0.07	2.17±0.04	1.47±0.04	6.31	1.23

-standard deviation.

of growth, 2 ppm SO_2 exposure lowered Chl a by 22.32%, Chl b by 12.25 and carotenoids by 5.16% when the pollutant dose reached 84 ppm hr. This indicates that Chl a was about 2 times more susceptible to SO_2 than Chl b and 4 times more than carotenoids. Since Chl a is the most important pigment for the photosynthesis in plants, its high sensitivity to SO_2 affects CO_2 fixation adversely and thereby hampers the plant growth and development. The lower levels of these pigments in treated leaves might be attributed to either degradation or decreased synthesis of pigments caused by SO_2. The break down of chlorophyll to pheophytin with the removal of Mg^{++} ions by SO_2 has been reported by several workers (Rao and LeBlanc 1966; Malhotra 1977; Suwannapinut and Kozlowski 1979). However, it is also argued that superoxide radicals formed on the thylakoid membranes when chloroplasts are exposed to SO_2 under illumination, induced chloroplast swelling (Wellburn et al. 1972) or disintegration of membrane (Malhotra 1976; Suwannapinut and Kozlowski 1979).

With the SO_2 treatment, the protein content was also decreased in the leaves of exposed plants at all stages of plant growth and development with respect to their controls ($P<0.01$). It was maximum at pre-flowering stage and progressively declined with the advancement in plant age and pollutant dose. As evident from the Table 4 the protein content was lowered by 20.13 and 39.27% in 1 and 2 ppm SO_2-treated plants, respectively when the respective pollutant doses were 42 and 84 ppm hr. A decrease in the protein content was observed by Godzik and Linskens (1974) in SO_2-exposed leaves of Phaseolus vulgaris. Inhibition of protein synthesis or enhanced

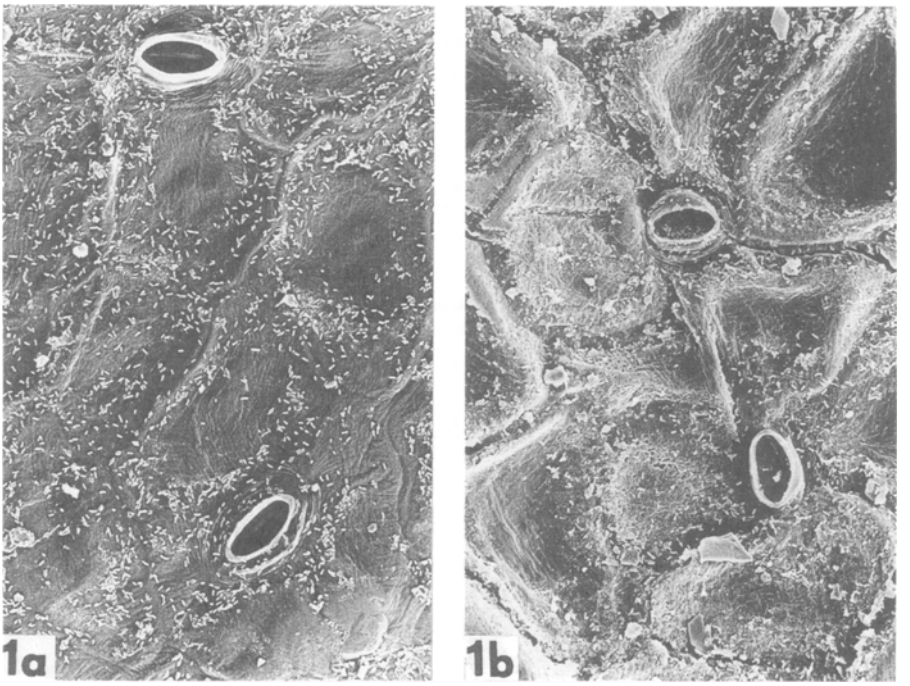

Fig. 1: Scanning electron micrographs of Dahlia rosea Cav. leaf showing SO_2 induced widening and deshaping of stomatal pores, and also a change in surface configuration (x 520): 1a. control, 1b. treated.

Table 4. Protein content (% dry wt) in leaves of control and treated Dahlia plants at different stages of growth (average of three replicates)

Experimental condition	Stages of growth		
	Pre-flowering	Flowering	Post-flowering
Control	7.32±0.05	6.31±0.04	3.03±0.03
1 ppm	6.16±0.03 (15.84)	5.15±0.09 (18.38)	2.42±0.07 (20.13)
2 ppm	5.15±0.07 (29.64)	4.04±0.08 (35.97)	1.84±0.08 (39.27)

protein degradation might account for the decreased contents of protein in treated plants (Robe and Kreeb 1980; Sardi 1981). SEM study revealed that SO_2 induced the widening and deshaping of stomatal pores and also brought about changes in foliar surface configuration (Majernik and Mansfield 1970, 1971; Mansfield and Majernik 1970). These changes were more conspicuous in 2 ppm SO_2-treated leaves than in 1 ppm SO_2-exposed leaves (Fig. 1). Similar observations have been made by Unsworth et al. (1972) and Black and Black (1979).

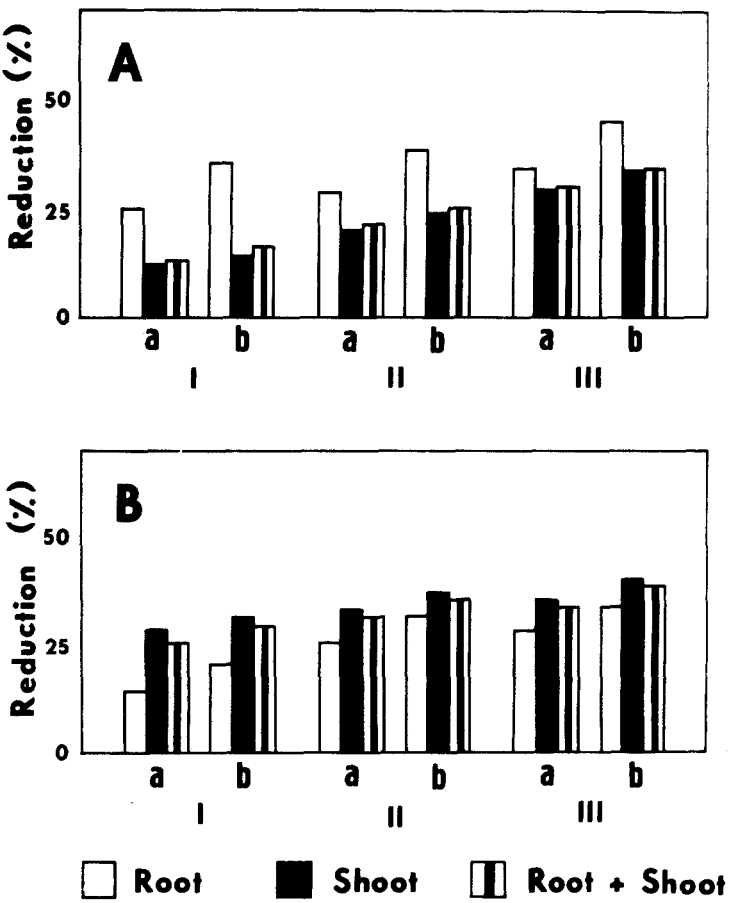

Fig. 2: Per cent reduction in phytomass (A) and length of root and shoot (B) of SO_2 treated Dahlia rosea Cav. plants at pre-flowering (I), flowering (II) and post-flowering (III) stages: a. 1 ppm, b. 2 ppm.

Opening and closure of stomata are the functions of turgidity of subsidiary cells. It is presumed that a rapid and preferential absorption of SO_2 by subsidiary cells, followed by changes in membrane permeability caused a decrease in cell turgor which could have widened the stomatal aperture (Puckett et al. 1977; Squire and Mansfield 1973; Biscoe et al. 1973). Obviously, widening of stomatal aperture facilitates the entry of SO_2 and thereby causes more injury to plants.

Sulphur dioxide treatment retarded the growth and development of plants as evidenced by their decreased root and shoot lengths in comparison to that of control plant (Shimizu et al. 1980). The root and shoot lengths of treated plants continued to increase despite increasing pollutant dose till post-flowering stage as in the case of control plants. However, the root and shoot lengths of treated plants remained invariably smaller than that of control plants at

all stages of growth and development. It is obvious from Fig. 2 that overall plant height of 1 and 2 ppm SO_2-treated plants decreased maximally by 34.39 and 39.33%, respectively when the respective pollutant dose were 42 and 84 ppm hr at post-flowering stage. However, the root and shoot lengths were reduced separately 28.87 and 35.20% in 1 ppm SO_2-treated plants and 34.07 and 40.07 in 2 ppm SO_2-treated plants at the same stage of growth (Fig. 2). The reduction in the root and shoot lengths may be attributed to adverse effects of SO_2 on photosynthesis (Sij and Swanson 1974). As a sequel, the food supply to root development was also affected.

There was a significant decrease in the phytomass accumulation of exposed plants (Shimizu et al. 1980). It is evident from the Fig. 2 that like control plants, phytomass of treated plants also continued to increase despite augmenting pollutant dose, but was always lower than that of control plants at all stages of growth and development. The maximum phytomass values of 53.01, 36.90 and 34.87 g per plant for control and 1 and 2 ppm SO_2-treated plants, respectively were recorded at the stage of post-flowering when the pollutant dose culminated to 42 and 84 ppm hr (Fig. 2). When compared to control plants, the phytomass of treated plants had decreased by 36.90 and 34.21% in 1 and 2 ppm SO_2-exposed plants, respectively. If root and shoot masses were considered separately, they were reduced by 34.60 and 30.10% in 1 ppm SO_2-treated plants and by 45.12 and 33.43% in 2 ppm SO_2-treated plants, respectively at the same stage of growth and pollutant dose. It was also observed that in both the treatments, the root mass was more reduced than shoot mass. Such a reduction may be attributed to several factors including leaf injury, chlorophyll degradation and other disturbances in metabolic processes (Sij and Swanson 1974; Totsuka 1980). Further, greater reduction in the root mass clearly indicates that food material manufactured by treated plants was not adequate to meet the requirements of the growth of above ground parts and consequently its supply to underground parts was adversely affected.

No significant difference was observed in the size of flowers of control and treated plants. And also both the plants aged simultaneously unlike the Calendula plants in which ageing was accelerated by SO_2 (Singh et al. 1985). Besides the number of flowers per plant also remained almost the same in both control and treated plants. This indicates that in Dahlia plants, flowering and ageing was not influenced by their exposure to SO_2.

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