Indian J. Plant Physiol., Vol. 9, No. 3, (N.S.) pp. 224-233 (July-Sept., 2004)

PLANT GROWTH, STOMATAL RESPONSE, PIGMENTS AND PHOTOSYNTHESIS OF *ALTHEA OFFICINALIS* AS AFFECTED BY SO_2 STRESS

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Received on 12 Sept., 2003, Revised on 6 Aug., 2004

SUMMARY

Growth responses of marshmallow (*Althea officinalis* L.) to SO_2 (0.5, 1.0 and 2.0 ppm) stress were studied at pre-flowering, flowering and post-flowering stages. Compared to controls, dry masses of treated leaves, stems and roots were higher at 0.5 ppm SO_2 concentration and significantly decreased with the higher doses at each stage. Number of leaves, leaf area, length of stems and roots and number of flowers and fruits were also greater at 0.5 ppm concentration, but showed a decrease with higher concentrations. Roots showed greater reductions than stem. Size of stomatal aperture, stomatal density and stomatal index declined under higher SO_2 stress, though 0.5 ppm concentration hardly caused any noticeable difference over the control. The net photosynthetic rate of the leaves significantly increased, and the stomatal conductance and intercellular CO_2 concentration decreased at 0.5 ppm SO_2; the reverse was true for 1 ppm and 2 ppm concentrations. The photosynthetic pigments significantly declined under higher SO_2 stress at each stage of plant development, but the lowest SO_2 concentration exhibited stimulatory effect.

***Key words: Althea officinalis*, chlorophyll, leaf morphology, net photosynthetic rate, SO_2, stomata**

INTRODUCTION

SO_2, a major atmospheric pollutant, damages plants by altering photosynthesis, respiration and other metabolic processes and causes chlorotic and necrotic foliar injuries (Agrawal 2003). Increased tissue acidity due to SO_2 absorption and the subsequent breakdown of photosynthetic pigments and oxidation of chlorophyll effectively reduce CO_2 fixation rate and hence the growth and yield (Chaudhary and Rao 1977). Manifestation of toxicity symptoms depends largely on concentration of the pollutant, duration of exposure and the genetic constitution of plants. SO_2 may have both negative and positive effects on plant productivity depending on sulphur status of the plant and the soil (Agrawal 2003). Sulphur is involved in the formation of disulphide bonds in proteins and is a major component of a variety of secondary metabolites. However, it disturbs copper metabolism in plants even in the absence of visible symptoms (Bergmann 1992). Studies on responses of medicinal plants to SO_2 pollution are only few. Marshmallow (*Althea officinalis* L., Malvaceae), an annual herb, common in the Himalayan region has considerable medicinal properties (Weiner 1990, Kirtikar and Basu 1993). The present study elucidates growth responses of SO_2-treated marshmallow at different stages of plant development.

MATERIALS AND METHODS

The study was conducted at Jamia Hamdard Campus, New Delhi, situated at 28.38' N latitude and 77.11' E longitude, about 228 m above the mean sea level. The soil

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was a coarse-textured sandy loam with pH around 8.0, E.C. 0 – 198 dSm^{-1} and the nitrogen and sulphur contents around 10.4 ppm and 2.8 ppm, respectively. Experiments were laid out in plots (12.5 x 9 square feet), each having three rows of plants, maintaining a row to row distance of 30 cm and a plant to plant distance of 15 cm. Seeds were sown in October when monthly means of minimum and maximum temperatures lay around 19°C and 32°C while those of RH around 49% and 80% respectively. Thirty-days-old seedlings of *A. officinalis* were fumigated for a week, for one hour daily in the morning, in a specially fabricated fumigation chamber (12.5 x 9.0 x 3.5 ft), with 0.5, 1.0 and 2.0 ppm SO_2 concentrations released from a gas cylinder and controlled by a regulator. The untreated plants were maintained as control. Sampling (10 plants for each treatment) was done at pre-flowering (30 days after fumigation, DAF), flowering (60 DAF) and post-flowering stages (120 DAF).

For growth and biomass, plants containing intact roots were carefully dug at random from control as well as treated plots. These were thoroughly washed to remove soil. Morphological characteristics, viz. length of root and stem, number of leaves per plant, and leaf area were recorded. Leaf area was measured by a LI-3000A leaf area meter (Li-cor, Lincoln, USA). For dry mass, component plant parts were separated and oven dried at 65°C until the weight became constant. In the last harvesting (post-flowering), treatment effect on yield components (*i.e.* flower and fruits per plant and seeds per fruit) was also assessed.

Stomatal pore, stomatal density and stomatal index were determined in epidermal peels obtained by the method of Ghouse and Yunus (1972) using hot nitric acid. The peels, processed in the customary ethanol series for dehydration and stained with safranin, were mounted in Canada balsam. Stomata were measured on a compound light microscope, and stomatal index (SI) was calculated as

$$\text{S.I.} = \frac{S}{S+E} \times 100$$

Where, S and E represent number of stomata and epidermal cells per mm^2, respectively (Salisbury 1927).

The chlorophyll and carotenoid contents of fresh leaves were estimated by the method of Hiscox and Israelstam (1979) using dimethylsulphoxide (DMSO) and by applying the formulae of MacLachlan and Zalik (1963), and Duxbury and Yentsch (1956). Stomatal conductance, intercellular CO_2 concentration and net photosynthetic rate were measured by a portable LI-6200 Photosynthesis System (Li-cor, Lincoln, USA). Leaf gas exchange measurements were made on cloud-free days between 8.00-9.00 a.m.The level of significance of the variations observed was determined by the Student's 't' test.

RESULTS

The overall growth at different stages of plant development declined under the influence of high concentrations (1-2 ppm) of SO_2, as observed for both vegetative and reproductive plant parts. However, with low SO_2 concentration (0.5 ppm), the effect was rather positive (Table 1). For instance, the roots and stem of plants exposed to 0.5 ppm SO_2 were longer than those of controls, but were significantly reduced with the higher SO_2 concentrations, showing the maximal decrease at pre-flowering stage. The root-stem length ratio was significantly higher than control at 0.5 ppm concentration and did not show any marked variation with higher doses. Dry mass of roots and stems increased with age of the plant and was relatively greater in plants exposed to 0.5 ppm SO_2. A significant reduction, however, was observed at higher concentrations, the maximal difference figuring at the flowering stage in both roots and stems. The root-stem dry mass ratio was maximum at pre-flowering stage in control plants. In the treated plant, it was always lower at pre-flowering stage, and higher at post-flowering stage, compared to control. Flowers and fruits per plant were significantly more in number at 0.5 ppm SO_2 but lesser in number at higher concentrations. However, number of seeds per fruit was unaffected at lowest concentration but significantly decreased at higher concentrations used (Table 1).

Leaf number and area

The number of leaves per plant increased with plant age. It decreased under a heavy SO_2 stress even though exposure to 0.5 ppm SO_2 had a positive effect (Table 2). The maximal decrease (45%) figured in the post-flowering

Table 1. Growth and yield of *Althea officinalis* as affected by SO_2 treatments.

Parameters	Control	0.5 ppm SO_2	1 ppm SO_2	2 ppm SO_2
Root length (cm)				
Pre-flowering	21.50 ± 2.29	22.00 ± 2.22 ($+2.32^{NS}$)	15.50 ± 2.29 (-27.90^{**})	11.16 ± 2.56 (-48.09^{**})
Flowering	25.66 ± 2.25	31.50 ± 2.85 ($+22.76^{**}$)	21.40 ± 1.79 (-16.60^{**})	16.43 ± 2.25 (-35.97^{**})
Post-flowering	33.50 ± 4.09	37.50± 2.50 ($+11.94^{*}$)	27.43 ± 2.56 (-18.11^{**})	23.50 ± 2.50 (-29.85^{**})
Stem length (cm)				
Pre-flowering	68.50 ± 3.27	70.50 ± 2.99 ($+2.91^{NS}$)	46.50 ± 3.77 (-32.11^{**})	35.00 ± 4.44 (-48.90^{**})
Flowering	147.83 ± 13.40	162.00 ± 8.11 ($+9.58^{*}$)	126.83 ± 9.38 (-14.20^{**})	99.00 ± 3.27 (-33.03^{**})
Post-flowering	167.33 ± 11.67	178 ± 7.77 ($+6.67^{*}$)	138.50 ± 6.50 (-17.22^{**})	115.16 ± 12.29 (-31.17^{**})
Root-stem length ratio				
Pre-flowering	0.31 ± 0.02	0.34 ± 0.03 ($+9.67^{**}$)	0.33 ± 0.02 ($+6.07^{NS}$)	0.32 ± 0.03 ($+0.94^{NS}$)
Flowering	0.17 ± 0.01	0.22 ± 0.03 ($+29.14^{**}$)	0.16 ± 0.005 (-5.88^{NS})	0.17 ± 0.02 (0.00^{NS})
Post-flowering	0.20 ± 0.02	0.25 ± 0.02 ($+25.00^{**}$)	0.20 ± 0.01 (-0.00^{NS})	0.20 ± 0.006 (0.00^{NS})
Root dry mass (g)				
Pre-flowering	4.75 ± 0.25	4.80 ± 0.53 ($+1.05^{NS}$)	3.55 ± 0.18 (-25.26^{**})	2.84 ± 0.13 (-40.12^{**})
Flowering	10.50 ± 0.67	12.22 ± 1.00 ($+16.38^{**}$)	6.81 ± 0.42 (-35.14^{**})	5.54 ± 0.40 (-47.23^{**})
Post-flowering	12.45 ± 0.96	15.05 ± 1.32 ($+20.88^{**}$)	10.05 ± 0.96 (-19.34^{**})	8.10 ± 0.38 (-34.99^{**})
Stem dry mass (g)				
Pre-flowering	10.91 ± 0.99	11.66 ± 0.86 ($+6.87^{NS}$)	7.98 ± 0.28 (-26.85^{**})	6.46 ± 1.19 (-40.78^{**})
Flowering	45.94 ± 3.44	49.49 ± 3.33 ($+7.72^{*}$)	32.07 ± 2.94 (-30.19^{**})	26.61 ± 1.90 (-42.07^{**})
Post-flowering	52.33 ± 2.97	53.17 ± 3.91 ($+1.60^{NS}$)	40.81 ± 4.39 (-22.01^{**})	36.26 ± 1.68 (-30.70^{**})
Root-stem dry mass ratio				
Pre-flowering	0.46 ± 0.03	0.38 ± 0.02 (-17.39^{**})	0.44 ± 0.02 (-3.47^{NS})	0.45 ± 0.06 (-2.17^{NS})
Flowering	0.23 ± 0.02	0.26 ± 0.02 ($+13.04^{**}$)	0.22 ± 0.009 (-2.63^{NS})	0.20 ± 0.002 (-12.28^{**})
Post-flowering	0.23 ± 0.01	0.29 ± 0.03 ($+26.08^{**}$)	0.24 ± 0.004 ($+4.34^{NS}$)	0.27 ± 0.004 ($+17.39^{**}$)
Flowers per plant	112.00 ± 10.53	115.00 ± 7.33 ($+2.67^{NS}$)	82.33 ± 11.71 (-26.49^{**})	55.66 ± 10.69 (-50.30^{**})
Fruits per plant	101.00 ± 15.13	105.00 ± 9.66 ($+3.96^{NS}$)	66.66 ± 11.59 (-34.00^{**})	48.66 ± 16.77 (-51.82^{**})
Seeds per fruit	32.66 ± 1.52	32.00 ± 2.25 (-2.02^{NS})	29.66 ± 2.88 (-9.18^{**})	25.66 ± 2.88 (-21.43^{**})

** = Significant at 1% level, * = Significant at 5% level, NS = Non-significant
Values are the mean ± S.D. of observations obtained from 10 plants for each treatment. Figures in parentheses indicate per cent variation from the control.

stage with 2 ppm concentration of SO_2. The total leaf area also increased with application of low dose of SO_2 but it was markedly reduced under heavier stress, the difference from the control being the maximum at the post-flowering stage (Table 2). Total leaf dry mass estimations also exhibited similar trends.

Size of stomata

The length and width of stomatal apertures on both adaxial and abaxial surfaces of leaf increased consistently with plant age. The apertures were relatively small in the treated plants, more significantly with 2 ppm concentration (Fig. 1). Stomatal density (SD), and stomatal index (SI) increased slightly with age of the plant, being relatively low in the corresponding stages of treated plants. SD varied maximally in flowering stage (adaxial epidermis) and post-flowering stage (abaxial epidermis), whereas SI did so in the pre-flowering and post-flowering stages, respectively. However, application of 0.5 ppm concentration hardly caused any noticeable difference over the control (Table 3).

Photosynthetic pigments

Photosynthetic pigments were less abundant in the treated plants than in controls (Table 4). The lowest SO_2 concentration used showed some stimulatory effect on pigment concentration, but higher concentrations were clearly depressive. Chlorophyll *a* content was more significantly affected than chlorophyll *b*, with the maximum decline occurring at the flowering stage. The total chlorophyll content declined significantly at 1 ppm and 2 ppm doses, the maximal impact occurring during pre-

Table 2. Foliar growth of *Althea officinalis* plants as affected by SO_2 stress.

Parameters	Control	0.5 ppm SO_2	1 ppm SO_2	2 ppm SO_2
Leaves per plant				
Pre-flowering	28.33 ± 4.50	40.00 ± 3.88 (+41.19**)	20.33 ± 1.52 (-28.33**)	17.00 ± 2.00 (-39.99**)
Flowering	53.66 ± 5.85	61.66 ± 4.54 (+14.91**)	40.66 ± 0.82 (-24.22**)	31.33 ± 3.51 (-41.61**)
Post-flowering	65.66 ± 4.04	65.00 ± 3.22 (-1.00^{NS})	49.33 ± 7.02 (-24.48**)	36.00 ± 5.56 (-45.17**)
Single leaf area (cm)^2				
Pre-flowering	33.02 ± 5.79	42.57 ± 4.22 (+28.92**)	36.43 ± 4.16 (+8.86^{NS})	33.50 ± 8.93 (+1.66^{NS})
Flowering	45.59 ± 3.72	47.82 ± 4.47 (+4.89^{NS})	44.99 ± 8.46 (-1.25^{NS})	46.48 ± 4.06 (+1.71^{NS})
Post-flowering	43.38 ± 3.52	46.52 ± 3.33 (+7.23^{NS})	42.91 ± 6.31 (-1.34^{NS})	44.08 ± 12.05 (+2.45^{NS})
Total leaf dry mass (g)				
Pre-flowering	6.31 ± 0.41	7.55 ± 0.88 (+19.65**)	5.23 ± 0.39 (-17.11**)	4.48 ± 0.65 (-29.00**)
Flowering	14.23 ± 0.37	16.08 ± 1.44 (+13.00**)	12.66 ± 0.54 (-11.03**)	7.89 ± 0.27 (-44.55**)
Post-flowering	15.49 ± 1.06	17.33 ± 1.31 (+11.87**)	13.36 ± 0.69 (-13.75**)	10.71 ± 1.42 (-30.85**)

** = Significant at 1% level, * = Significant at 5% level, NS = Non-significant

Values are the mean ± S.D. of observations obtained from 10 plants for each treatment. Figures in parentheses indicate per cent variation from the control.

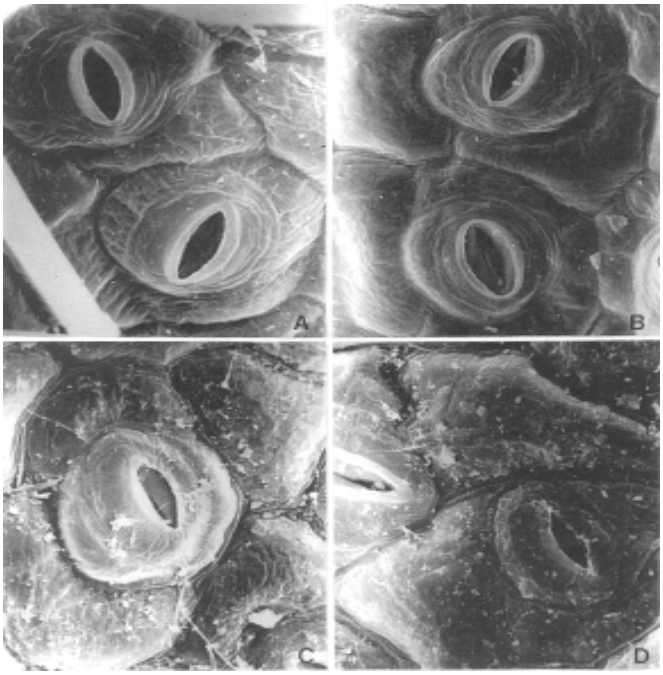

Fig.1. Electron micrographs of the adaxial epidermis of *A. officinalis* leaves at post- flowering stage. The stomata in control material (A) are bigger than in SO_2-treated plants particularly in those exposed to 1 ppm (C) and 2 ppm (D) concentrations. All at x 1800.

flowering and flowering stages (Fig. 2). Carotenoid content was maximum at the flowering stage and its decline due to heavy SO_2 stress was also maximum at this stage.

Physiological responses

Foliar stomatal conductance was invariably minimum at pre-flowering stage. SO_2 stress did not cause much variation up to 1 ppm dose but the conductance increased significantly at 2 ppm concentration at each stage of growth, showing the maximum effect during flowering. The intercellular CO_2 content was maximum at post-flowering stage; it declined with the initial treatment, was little altered with 1 ppm and increased markedly with 2 ppm SO_2 concentration at each stage (Table 4). The net photosynthetic rate, almost stable with plant age, was relatively higher at initial SO_2 treatment but declined at higher doses of SO_2 particularly at 2 ppm concentration (Fig. 3).

DISCUSSION

Leaves of the treated plants developed chlorosis within a week of SO_2 fumigation. Chlorotic patches enlarged and

Table 3. Stomatal morphology of *Althea officinalis* plants as affected by SO_2 stress.

Parameters	Control	0.5 ppm SO_2	1 ppm SO_2	2 ppm SO_2
Length of stomatal pore: adaxial (µm)				
Pre-flowering	30.50 ± 0.39	30.62 ± 0.33 ($+0.39^{NS}$)	29.50 ± 0.32 (-3.27^{**})	22.50 ± 0.23 (-26.22^{**})
Flowering	34.25 ± 0.48	34.31 ± 0.52 ($+0.18^{NS}$)	34.20 ± 0.41 (-0.14^{NS})	31.00 ± 0.38 (-9.48^{**})
Post-flowering	40.25 ± 0.89	39.10 ± 2.75 (-2.85^{NS})	36.75 ± 0.75 (-8.69^{**})	34.50 ± 0.56 (-14.28^{**})
Length of stomatal pore: abaxial (µm)				
Pre-flowering	27.25 ± 0.39	26.95 ± 0.75 (-1.10^{NS})	27.20 ± 0.16 (-0.18^{NS})	24.25 ± 0.19 (-11.00^{**})
Flowering	32.50 ± 0.12	31.88 ± 1.35 (-1.90^{NS})	31.25 ± 0.16 (-3.84^{**})	29.25 ± 0.16 (-10.00^{**})
Post-flowering	37.00 ± 0.15	37.05 ± 0.23 ($+0.135^{NS}$)	34.00 ± 0.18 (-8.10^{**})	33.25 ± 0.18 (-10.13^{**})
Width of stomatal pore: adaxial (µm)				
Pre-flowering	11.25 ± 0.29	11.16 ± 0.24 (-0.80^{NS})	10.75 ± 0.09 (-4.44^{**})	10.62 ± 0.06 (-5.60^{**})

Parameters	Control	0.5 ppm SO_2	1 ppm SO_2	2 ppm SO_2
Flowering	12.87 ± 0.11	12.45 ± 1.29 (-3.26^{NS})	12.52 ± 0.12 (-2.71**)	11.75 ± 0.03 (-8.70**)
Post-flowering	13.75 ± 0.32	14.00 ± 0.65 (+1.82^{NS})	13.37 ± 0.08 (-2.76**)	13.12 ± 0.09 (-4.58**)
Width of stomatal pore: abaxial (µm)				
Pre-flowering	11.37 ± 0.03	11.40 ± 0.09 (+0.26^{NS})	11.30 ± 0.03 (-0.61**)	11.07 ± 0.02 (-2.63**)
Flowering	12.50 ± 0.03	12.45 ± 0.15 (-0.40^{NS})	12.12 ± 0.03 (-3.04**)	11.62 ± 0.03 (-7.04**)
Post-flowering	14.74 ± 0.04	14.50 ± 0.65 (-1.63^{NS})	13.50 ± 0.04 (-8.41**)	12.75 ± 0.04 (-13.50**)
Stomatal density: adaxial (mm^{-2})				
Pre-flowering	8.05 ± 0.06	8.00 ± 0.15 (-0.62^{NS})	8.03 ± 0.03 (-0.25^{NS})	7.80 ± 0.06 (-3.10^{NS})
Flowering	8.80 ± 0.08	8.95 ± 0.45 (+1.70^{NS})	8.45 ± 0.07 (-3.97**)	8.35 ± 0.04 (-5.11**)
Post-flowering	9.25 ± 0.04	9.28 ± 0.12 (+0.32^{NS})	9.15 ± 0.07 (-1.08**)	9.05 ± 0.02 (-2.16**)
Stomatal density: abaxial (mm^{-2})				
Pre-flowering	8.35 ± 0.03	8.25 ± 0.35 (-1.19^{NS})	8.05 ± 0.05 (-3.59**)	8.05 ± 0.07 (-3.59**)
Flowering	8.80 ± 0.03	8.60 ± 0.52 (-2.27^{NS})	8.50 ± 0.06 (-3.40**)	8.45 ± 0.04 (-3.97**)
Post-flowering	9.75 ± 0.07	9.82 ± 0.25 (+0.72^{NS})	8.95 ± 0.02 (-8.20**)	8.65 ± 0.06 (-11.28**)
Stomatal index: adaxial (%)				
Pre-flowering	16.02 ± 0.19	16.00 ± 0.18 (-0.12^{NS})	15.65 ± 0.19 (-2.30**)	14.78 ± 0.20 (-7.74**)
Flowering	16.28 ± 0.11	16.25 ± 0.12 (-0.18^{NS})	16.22 ± 0.11 (-0.36^{NS})	16.10 ± 0.17 (-1.10**)
Post-flowering	18.05 ± 0.12	17.90 ± 0.65 (-0.83^{NS})	17.72 ± 1.11 (-1.82**)	16.80 ± 0.12 (-6.92**)
Stomatal index: abaxial (%)				
Pre-flowering	14.51 ± 0.13	14.35 ± 0.09 (-1.10^{NS})	13.57 ± 0.13 (-6.47**)	12.52 ± 0.19 (-13.71**)
Flowering	15.68 ± 0.15	15.70 ± 0.13 (+0.13^{NS})	15.29 ± 0.13 (-2.48**)	15.09 ± 0.10 (-3.76**)
Post-flowering	18.94 ± 0.17	18.85 ± 0.35 (-0.48^{NS})	18.18 ± 0.13 (-4.01**)	15.94 ± 0.12 (-15.83**)

** = Significant at 1% level, NS = Non-significant

Values are the mean ± S.D. of 10 independent reading. Figures in parentheses indicate per cent variation from the control.

Table 4. Pigment content of leaves, stomatal conductance and internal CO_2 concentration in *Althea officinalis* plants as affected by SO_2 stress.

Parameters	Control	0.5 ppm SO_2	1 ppm SO_2	2 ppm SO_2
Chlorophyll a (µg g^{-1} dry wt.)				
Pre-flowering	4.52 ± 0.12	5.51 ± 0.28 (+21.90**)	4.20 ± 0.46 (-7.07**)	3.88 ± 0.24 (-14.15**)
Flowering	10.36 ± 0.24	10.57 ± 0.60 (+2.03^{NS})	9.32 ± 0.68 (-10.03**)	6.80 ± 0.16 (-34.36**)
Post-flowering	7.28 ± 0.24	8.10 ± 0.33 (+11.26**)	6.80 ± 0.12 (-6.59**)	6.48 ± 0.16 (-10.98**)
Chlorophyll b (µg g^{-1} dry wt.)				
Pre-flowering	2.88 ± 0.36	2.98 ± 0.66 (+3.47^{NS})	2.72 ± 0.36 (-5.55^{NS})	2.01 ± 0.16 (-30.20**)
Flowering	2.24 ± 0.32	2.33 ± 0.54 (+4.01^{NS})	2.04 ± 0.28 (-8.92^{NS})	1.08 ± 0.28 (-51.78**)
Post-flowering	1.76 ± 0.12	1.80 ± 0.80 (+2.27^{NS})	1.56 ± 0.16 (-11.36**)	1.12 ± 0.08 (-36.36**)
Total chlorophyll (µg g^{-1} dry wt.)				
Pre-flowering	7.56 ± 0.40	8.71 ± 0.25 (+15.21**)	6.52 ± 0.36 (-13.75**)	5.96 ± 0.44 (-21.16**)
Flowering	12.52 ± 0.16	13.32 ± 0.88 (+6.38*)	11.16 ± 0.64 (-10.86**)	7.76 ± 0.48 (-38.01**)
Post-flowering	8.92 ± 0.87	9.11 ± 0.88 (+2.13^{NS})	8.20 ± 0.08 (-8.07**)	7.52 ± 0.09 (-15.69**)
Carotenoid (µg g^{-1} dry wt.)				
Pre-flowering	3.72 ± 0.12	3.80 ± 0.86 (+2.15^{NS})	3.36 ± 0.20 (-9.67**)	3.12 ± 0.20 (-16.12**)
Flowering	5.24 ± 0.28	5.27 ± 0.15 (+0.57^{NS})	4.32 ± 0.36 (-17.55**)	3.12 ± 0.36 (-40.45**)
Post-flowering	3.36 ± 0.16	3.45 ± 0.31 (+2.67^{NS})	3.32 ± 0.16 (-1.90^{NS})	3.16 ± 0.12 (-5.95**)
Stomatal conductance (mol m^{-2} s^{-1})				
Pre-flowering	0.24 ± 0.02	0.20 ± 0.01 (-16.66**)	0.27 ± 0.09 (+12.50^{NS})	0.28 ± 0.07 (+16.66^{NS})
Flowering	0.46 ± 0.08	0.41 ± 0.07 (-10.86^{NS})	0.52 ± 0.09 (+13.04^{NS})	0.64 ± 0.08 (+39.13**)
Post-flowering	0.45 ± 0.09	0.43 ± 0.07 (-4.44^{NS})	0.51 ± 0.08 (+13.33^{NS})	0.61 ± 0.07 (+35.55**)
Internal CO_2 concentration (ppm)				
Pre-flowering	229.60 ± 32.40	194.36 ± 17.46 (-15.35**)	243.12 ± 48.30 (+5.88^{NS})	278.07 ± 35.57 (+21.11**)
Flowering	219.21 ± 28.70	187.72 ± 14.54 (-14.36**)	244.64 ± 30.48 (+11.60^{NS})	268.03 ± 29.78 (+22.27**)
Post-flowering	300.40 ± 30.08	255.98 ± 26.33 (-14.78**)	304.23 ± 36.82 (+1.27^{NS})	316.61 ± 40.65 (+5.39^{NS})

** = Significant at 1% level, * = Significant at 5% level, NS = Non-significant

Values are the mean ± S.D. of 30 independent readings. Figures in parentheses indicate per cent variation from the control.

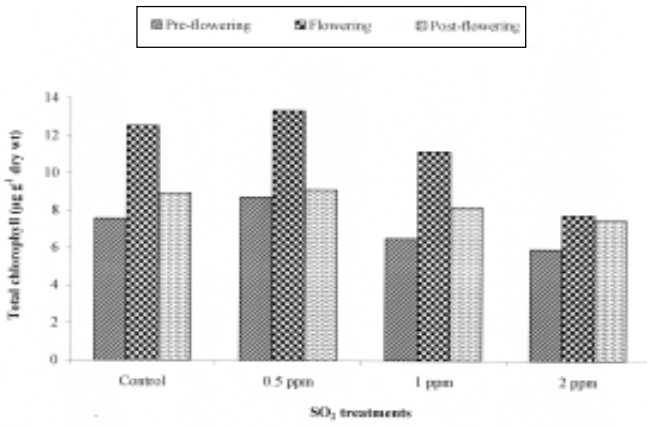

Fig.2 Concentration of total chlorophyll in the leaves of *A. officinalis* at pre-flowering, flowering and post-flowering stages of control and SO_2-treated plants.

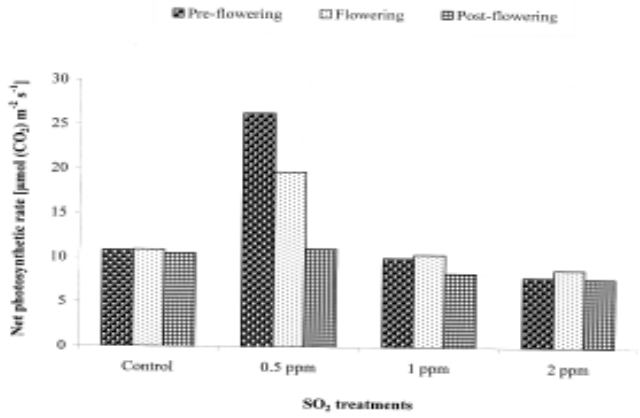

Fig.3 Net photosynthetic rate in the leaves of *A. officinalis* at pre-flowering, flowering and post-flowering stages of control and SO_2-treated plants.

turned into gray or brown necrotic spots. SO_2 dissolved in water in the leaf tissues, seemingly caused local injury by generating toxic ions (Malhotra and Hocking 1976, Manninen *et al.* 1996). These injuries retard the net assimilation rate of the plant (Weinstein and McCune 1970).

A. officinalis exhibited poor growth which could be due to translocation of SO_2 derivatives to meristematic regions (Crittenden and Read 1978). SO_2 may possibly slow down the rate of division and expansion of cells (Chang and Thompson 1966). Stem length was more significantly affected than root length, the root-stem length ratio was markedly reduced during late stages of plant growth. In *Anagallis arvensis*, root length and root biomass have shown positive correlation with increasing distance of plants from the point source of pollution (Khan and Ghouse 1988). The reduction in stem mass was significant throughout the course of plant development of *A. officinalis*. SO_2 may affect root growth either through the soil or by altering the production of photosynthates and their availability to the root (Khan and Khan 1993). This conforms to the report on rye grass exposed to 70 µg m^{-3} SO_2 for 56 days (Crittenden and Read 1978). There may also be a damage of chlorenchyma of the leaf by SO_2 exposure. The decline in fruit/seed yield, as observed in the present study, might involve failure of pollination or fertilization due to SO_2 stress. Various air pollutants are known to inhibit the mechanism of flowering by injuring leaves, which may decrease the amount of available carbohydrates, hormonal growth regulators and the physiological efficiency of the foliage (Kozlowski and Constantinidou 1986). Number of seeds per fruit also declined in the treated *A. officinalis* plants, conforming to observations on *Triticum aestivum* (Deepak and Agrawal 1999). SO_2 enters the leaf through stomata and reaches the intercellular spaces of mesophyll cells where it combines with water to form sulphite (SO^{2-3}) and bisulphite (HSO_3) and other phytotoxic ionic derivatives (Malhotra and Hocking 1976). Decrease in total leaf area was highly significant throughout the course of plant development with a maximum during post-flowering stage. This could also be a morphological adaptation, as opined by Murray and Wilson (1991).

Absorption of air pollutants depends on their concentration gradient from leaf exterior to leaf interior and on the dimensions of stomata. In the present study, stomatal pore size got reduced under SO_2 stress, more so at higher concentrations. Narrowing down of stomatal aperture or its closure may be a protective measure (Gupta and Ghouse 1987) associated with the accumulation of CO_2 in sub-stomatal cavities following SO_2 caused inhibition of photosynthesis or with the changes in permeability of guard cell membrane (Sij and Swanson 1973). Moreover, reduced leaf area, as in *A. officinalis*, must accommodate lesser number of stomata, thereby, reducing the volume of SO_2 entering into the leaves. Stomatal indices increased with the age of the plant, being relatively low in the treated plants. The photosynthetic

productivity, based on the quantity of chloroplast pigment, is directly proportional to the photosynthetic area of leaves. The amount of total chlorophyll in the fumigated plants was significantly low, chlorophyll *a* being more severely affected than chlorophyll *b*. Sulphite may react with chlorophyll to produce superoxide radicals (Shimazaki *et al.* 1980, Williams and Banerjee 1995). Chlorophyll *a* is degraded to phaeophytin through replacement of mg^{+2} ions in chlorophyll molecules, while chlorophyll *b* forms chlorophyllide *b* through the removal of phytol group of the molecule (Rao and Le Blanc 1966). Reduction in carotenoid level increased with increasing SO_2 concentration.

Stomatal conductance and internal CO_2 concentration were higher in plants treated with 2 ppm SO_2, but photosynthetic rate was lower as compared to control. This shows that at this level of SO_2, plants are able to take up CO_2 from the atmosphere effectively, but utilization of the same is low. This low utilization of CO_2 might be due to inactivation of Rubisco, the main enzyme responsible for photosynthesis. The reduced utilization of CO_2 in photosynthesis will result in further accumulation of the internal CO_2.

Excessive sulphur levels in plants can affect plant growth and development by disturbing copper metabolism which may cause Cu-deficiency in plants, hamper photosynthesis and respiration, etc and impair the reproductive phase by (a) reducing the number of flowers and (b) causing production of sterile pollen (Bergmann 1992).

It emanates from our observations that *A. officinalis* gains in growth when exposed to 0.5 ppm SO_2 concentration whereas higher concentrations prove injurious. The apparently positive effect of mild SO_2 treatment could be because the soil at the experimental site was S-deficient, having an average of 2.8 ppm sulphur content and therefore, SO_2 application did possibly compensate the sulphur deficiency.

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