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ASSESSMENT OF GROWTH, YIELD AND BIOCHEMICAL COMPONENTS IN *ABELMOSCHUS ESCULENTUS* MOENCH CV. PUSA MAKHMALI FUMIGATED WITH SULPHUR DIOXIDE.

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Abelmoschus esculentus cv. (Okra) Pusa Makhmali was subjected to 320, 667 and 1334 $\mu g\ m^{-3}$ of SO_2. Slight reductions in most of the growth and biochemical attributes were observed at 320 $\mu g\ m^{-3}$ SO_2 concentration in earlier stages of plant growth, but these reductions became significant with increase in concentrations of the pollutant and plant age. In the leaves of mature plants of okra, potassium, phosphorus and carbohydrate contents were found to be reduced significantly. Foliar pH and ascorbic acid contents were also decreased. In fruits, the amounts of K, P, N, protein and carbohydrate were decreased appreciably, while sulphur content was considerably increased both in leaves and fruits. Yield, in terms of number of fruits $plant^{-1}$ and their fresh and dry weights, was also adversely affected.

Key Words: SO_2, growth, yield, biochemical components, reduction.

Sulphur dioxide, a noxious air pollutant, is a byproduct of fuel combustion. The fumigant may enter the plants through roots as sulphite ions, through stomata in gaseous form or after having dissolved in water as sulphite ions. At higher concentrations of the pollutant the plants may show visible injury symptoms and at lower concentrations, these may show many physiological and biochemical alterations (Ziegler, 1972; Jager and klein, 1980; Pierre and Queiroz, 1981; Wellburn, 1982 and Koziol and Whatley, 1984). Recent evidences indicate that these negative impacts are mainly caused by free oxygen radicals (Jager, 1982; Dubey, 1992 and Singh and Rao, 1992). The present study reveals changes in growth, biochemicals and yield of *Abelmoschus esculentus* Moench exposed to various levels of SO_2.

MATERIALS AND METHODS

The seeds of *Abelmoschus esculentus* cv. Pusa Makhmali procured from I.A.R.I., New Delhi, were grown in four plots. The size of each experimental plot was 10 x 4 m^2. One of these plots was used for control and the remaining plots were used for 320, 667 and 1334 $\mu g\ m^{-3}$ SO_2 treatment, respectively. The seed spacing was kept 25 cm from plant to plant and 35 cm from line to line. Watering was done in the form of free surface flow once a week. Fifteen day old plants were treated with SO_2 concentration of 320, 667 and 1334 $\mu g\ m^{-3}$. A control with identical conditions of growth, but without SO_2 exposure, was used. The treatment was given in 1 m^3 ployethylene chambers supported on aluminium frames. Sulphur dioxide was produced by passing a continuous air current using suction pump through aqueous solution of sodium metabisulphite which was ionized under pressure to generate SO_2 (Agrawal *et al.*, 1982). The gas thus generated, was passed gradually into the chambers through a teflon tubing and mixed with the ambient air of the chamber. The gas in the chamber was monitored at the interval of two hours following Dennis (1913).

Three samplings of five plants each for each treatment, were made after 15, 30 and 45 days of exposure. The intact plants, excavated from each plot at every sampling time, were washed with smooth run tap water and then analysed. The plants were analysed with regard to leaf injury percentage, growth parameters (Plant length, root and shoot length, number of leaves $plant^{-1}$ and leaf area), fresh and dry matter accumulations in shoot and root and net primary productivity. Development of foliar injury, if any, in the treated plants was observed periodically. The plant parts were dried in an oven at 80°C for 24 hours to find out phytomass accumulation.

Chlorophyll *a* and *b* and total chlorophyll contents were determined as given by Arnon (1949).

Leaf extract pH was determined with a digital pH meter by homogenizing 5 g fresh leaves with 25 ml double distilled water. Ascorbic acid content was

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Table 1: Growth response of *Abelmoschus esculentus* cv. Pusa Makhmali exposed to various concentrations of sulphur dioxide.

Parameter	Days	SO_2 (μ gm^{-3})				C.D.	
		0	320	667	1334	5%	1%
		a	ab	ab	b		
Shoot length	30	19.30	18.08	17.68	16.50	2.66	–
(cm)		a	ab	bc	c		
	45	32.80	29.78	28.41	26.11	3.44	5.11
		a'	a'b'	a'b'	b'		
		a	ab	bc	c		
	60	64.40	55.45	50.11	45.41	9.47	14.17
		a'	a'b'	b'	b'		
		a	ab	ab	b		
Root length	30	8.20	7.66	7.45	6.98	0.78	–
(cm)		a	ab	b	b		
	45	11.20	10.09	9.68	8.85	1.51	–
		a	b	bc	c		
	60	16.70	14.11	12.30	11.43	2.39	3.43
		a'	a'b	b'	b'		
		a	ab	ab	b		
Plant height	30	27.50	25.74	25.13	23.48	4.16	–
(cm)			a	ab	bc	c	
	45	44.00	39.87	38.09	34.96	4.69	7.03
		a'	a'b'	a'b'	b'		
		a	b	bc	c		
	60	81.10	69.56	62.41	56.84	9.84	14.64
		a'	a'b'	b'	b'		
		a	ab	ab	b		
Number of leaves	30	6.30	5.95	5.75	5.41	0.65	–
plant^{-1}		a	ab	b	b		
	45	12.20	11.00	10.40	9.60	1.73	2.58
		a'	a'b'	a'b'	b'		
		a	b	bc	c		
	60	17.70	14.90	13.48	12.14	2.32	3.23
		a'	a'b'	b'	b'		
		a	ab	ab	b		
Leaf area	30	33.40	31.52	30.29	28.12	4.38	--
(cm)		a	ab	bc	c		
	45	96.40	87.20	81.32	74.42	12.47	18.66
		a'	a'b'	a'b'	b'		
		a	b	bc	c		
	60	122.50	102.53	93.22	82.44	14.33	21.64
		a'	a'b'	b'	b'		

Similar superscripts and underscripts in a parameter represent insignificant difference at 5% and 1% level respectively.

calculated by the method given by Keller and Schwager (1977).

At the final harvest after 60 days, phosphorus, potassium, sulphur and carbohydrate contents were determined in leaves and fruits while soluble and protein nitrogens and total protein contents were determined only in fruits. Phosphorus content was determined by using stannous chloride method following Allen (1940), postassium by volumetric method (Ward and Johnston, 1962), carbohydrate by acetone colorimetric method (Yemm and Willis, 1954) and sulphur by following the method of Patterson (1958).

The protein content was determined by the method suggested by Layne (1957) and that of nitrogen by micro-Kjeldahl method of Jackson (1958). Number, length, fresh weights and dry weights of fruits were also recorded in the mature crop of okra.

The recorded data were analysed statistically using Analysis of Variance (ANOVA) and then by determining the critical differences (C.D.) at 5% and 1% levels of significance.

Table 2: Fresh weight, dry weight fractions and net primary productivity (N.P.P.) of *Abelmoschus esculentus* cv. Pusa Makhmali exposed to 320, 667 and 1334 $\mu g\ m^{-3}$ sulphur dioxide.

Parameter	Days	SO_2 ($\mu g\ m^{-3}$)				C.D.	
		0	320	667	1334	5%	1%
Fresh weight of shoot (g)	30	19.302^{a}	17.550^{ab}	17.030^{ab}	16.081^{b}	3.038	--
	45	$85.320^{a}_{a'}$	$72.875^{b}_{a'b'}$	$70.143^{b}_{a'b'}$	$65.871^{b}_{b'}$	10.787	15.969
	60	$115.504^{a}_{a'}$	$93.562^{b}_{b'}$	$88.593^{b}_{b'}$	$80.555^{b}_{b'}$	14.243	21.189
Fresh weight of root (g)	30	$1.303^{a}_{a'}$	$1.162^{ab}_{a'b'}$	$1.130^{b}_{a'b'}$	$1.051^{b}_{b'}$	0.163	0.239
	45	$2.602^{a}_{a'}$	$2.203^{b}_{a'b'}$	$2.103^{b}_{a'b'}$	$1.970^{b}_{b'}$	0.351	0.520
	60	$5.505^{a}_{a'}$	$4.366^{b}_{b'}$	$4.134^{b}_{b'}$	$3.733^{b}_{b'}$	0.670	0.990
Total fresh weight $plant^{-1}$ (g)	30	20.605^{a}	18.712^{ab}	18.160^{ab}	17.132^{b}	2.615	--
	45	$87.922^{a}_{a'}$	$75.078^{b}_{a'b'}$	$72.246^{b}_{a'b'}$	$67.841^{b}_{b'}$	12.00	17.956
	60	$121.009^{a}_{a'}$	$97.928^{b}_{a'b'}$	$92.724^{b}_{b'}$	$84.588^{b}_{b'}$	15.875	23.640
Dry weight of shoot (g)	30	5.324^{a}	4.860^{ab}	4.711^{ab}	4.466^{b}	0.811	--
	45	$23.671^{a}_{a'}$	$20.404^{b}_{a'b'}$	$19.623^{b}_{a'b'}$	$18.416^{b}_{b'}$	3.163	4.740
	60	$31.792^{a}_{a'}$	$25.624^{b}_{b'}$	$24.635^{b}_{b'}$	$22.159^{b}_{b'}$	4.082	5.938
Dry weight of root (g)	30	$0.330^{a}_{a'}$	$0.297^{ab}_{a'b'}$	$0.284^{b}_{a'b'}$	$0.263^{b}_{b'}$	0.044	0.065
	45	$0.674^{a}_{a'}$	$0.572^{b}_{a'b'}$	$0.535^{b}_{b'}$	$0.490^{b}_{b'}$	0.094	0.138
	60	$1.464^{a}_{a'}$	$1.166^{b}_{b'}$	$1.105^{bc}_{b'}$	$0.970^{c}_{b'}$	0.193	0.288
Phytomass (g)	30	5.654^{a}	5.157^{ab}	4.995^{ab}	4.729^{b}	0.689	--
	45	$24.345^{a}_{a'}$	$20.975^{b}_{a'b'}$	$20.158^{b}_{a'b'}$	$18.906^{b}_{b'}$	3.365	5.045
	60	$33.256^{a}_{a'}$	$26.790^{b}_{b'}$	$25.743^{b}_{b'}$	$25.129^{b}_{b'}$	4.430	6.445
N.P.P. (g $plant^{-1}$ day^{-1})	30	0.188^{a}	0.172^{ab}	0.167^{ab}	0.158^{b}	0.025	–
	45	$0.541^{a}_{a'}$	$0.466^{b}_{a'b'}$	$0.448^{b}_{a'b'}$	$0.420^{b}_{b'}$	0.070	0.103
	60	$0.554^{a}_{a'}$	$0.447^{b}_{b'}$	$0.429^{b}_{b'}$	$0.385^{b}_{b'}$	0.072	0.105

Similar superscripts and underscripts in a parameter represent insignificant difference at 5% and 1% level respectively.

Table 3: Effects of different concentrations of SO_2 on biochemical parameters in the leaves of *Abelmoschus esculentus* cv. Pusa Makhmali.

Parameter	Days	SO_2 ($\mu g\ m^{-3}$) 0	320	667	1334	C.D. 5%	1%
Chlorophyll *a* ($mg\ gfw^{-1}$)	30	$0.953^{a}_{a'}$	$0.855^{ab}_{a'b'}$	$0.808^{b}_{a'b'}$	$0.731^{b}_{b'}$	0.127	0.179
	45	$1.780^{a}_{a'}$	$1.484^{b}_{a'b'}$	$1.339^{b}_{b'}$	$1.263^{b}_{b'}$	0.254	0.373
	60	$2.135^{a}_{a'}$	$1.640^{b}_{b'}$	$1.520^{b}_{b'}$	$1.362^{b}_{b'}$	0.322	0.467
Chlorophyll *b* ($mg\ gfw^{-1}$)	30	0.640^{a}	0.604^{ab}	0.587^{ab}	0.586^{b}	0.053	--
	45	1.150^{a}	1.050^{ab}	1.010^{b}	0.942^{b}	0.140	--
	60	$1.350^{a}_{a'}$	$1.119^{b}_{a'b'}$	$1.060^{b}_{b'}$	$0.990^{b}_{b'}$	0.193	0.281
Total chlorophyll ($mg\ gfw^{-1}$)	30	$1.593^{a}_{a'}$	$1.459^{ab}_{a'b'}$	$1.395^{b}_{a'b'}$	$1.287^{b}_{b'}$	0.189	0.270
	45	$2.930^{a}_{a'}$	$2.534^{b}_{a'b'}$	$2.409^{b}_{a'b'}$	$2.205^{b}_{b'}$	0.383	0.560
	60	$3.485^{a}_{a'}$	$2.759^{b}_{b'}$	$2.580^{b}_{b'}$	$2.352^{b}_{b'}$	0.487	0.718
Leaf extract pH	30	6.74^{a}	6.37^{ab}	6.22^{ab}	6.04^{ab}	0.58	--
	45	6.78^{a}	6.21^{ab}	6.04^{ab}	5.83^{b}	0.87	--
	60	6.87^{a}	6.04^{b}	5.89^{b}	5.61^{b}	0.78	1.14
Ascorbic acid ($mg\ g\ dw^{-1}$)	30	$0.040^{a'}$	$0.037^{a'b'}$	$0.036^{a'b'}$	$0.034^{b'}$	--	--
	45	$0.080^{a}_{a'}$	$0.072^{ab}_{a'b'}$	$0.069^{b}_{a'b'}$	$0.063^{b}_{b'}$	0.010	0.014
	60	$0.100^{a}_{a'}$	$0.085^{b}_{a'b'}$	$0.079^{bc}_{b'}$	$0.014^{c}_{b'}$	0.014	0.020

Similar superscripts and underscripts in a parameter represent insignificant differene at 5% and 1% level respectively.

OBSERVATIONS

Plants exposed to 1334 $\mu g\ m^{-3}$ of SO_2 showed foliar lesions much earlier than those of plants fumigated to the other two concentrations. Plants exposed to 320, 667 and 1334 $\mu g\ m^{-3}$ SO_2 showed first visible injury symptoms at 37, 26 and 23 days of plant age, respectively, and the injury initially being confined to the mature leaves of treated plants. The injury after earlier exposures was in the form of pale yellowish/chlorotic patches mostly confined to the margins and tips of leaves. However, after prolonged exposures the injury was in the form of necrosis and the symptoms extended to interveinal areas also. At this stage younger leaves were also affected.

Shoot length, root length and hence the total plant length was adversely affected by SO_2 exposure. Such effects became more pronounced with the increase of SO_2 level and number of days of exposures. Initially i.e. after 15 days of exposures the reductions in shoot and root lengths, number of leaves plant $^{-1}$ and leaf area could attain only at 5% level of significance. Further, with an increase in the plant age highly considerable reductions in these parameters were also observed i.e. at 1% level of significance (Table 1).

Table 4: Phosphorus, Potassium, Sulphur, Carbohydrate, Nitrogen and Protein Contents in mature crop of *Abelmoschus esculentus* cv. Pusa Makhmali on exposure to a series of concentrations of Sulphur dioxide.

Parameter	SO_2 ($\mu g\ m^{-3}$)				C.D.	
	0	320	667	1334	5%	1%
LEAVES						
Phosphorus ($mg\ gdw^{-1}$)	$0.420^{a}_{a'}$	$0.345^{b}_{a'b'}$	$0.323^{b}_{b'}$	$0.299^{b}_{b'}$	0.058	0.085
Potassium ($mg\ gdw^{-1}$)	$0.750^{a}_{a'}$	$0.600^{b}_{b'}$	$0.571^{b}_{b'}$	$0.528^{b}_{b'}$	0.089	0.129
Sulphur ($mg\ gdw^{-1}$)	$0.350^{a}_{a'}$	$0.401^{b}_{a'b'}$	$0.419^{bc}_{b'}$	$0.450^{c}_{b'}$	0.035	0.052
Carbohydrate ($mg\ gdw^{-1}$)	$73.702^{a}_{a'}$	$61.836^{b}_{a'b'}$	$58.814^{b}_{b'}$	$55.125^{b}_{b'}$	9.310	13.905
FRUITS						
Phosphorus ($mg\ gdw^{-1}$)	$0.530^{a}_{a'}$	$0.443^{b}_{a'b'}$	$0.424^{b}_{a'b'}$	$0.399^{b}_{b'}$	0.074	0.107
Potassium ($mg\ gdw^{-1}$)	$0.940^{a}_{a'}$	$0.783^{b}_{b'}$	$0.751^{b}_{b'}$	$0.708^{b}_{b'}$	0.106	0.155
Sulphur ($mg\ gdw^{-1}$)	$0.275^{a}_{a'}$	$0.306^{b}_{a'b'}$	$0.318^{bc}_{a'b'}$	$0.338^{c}_{b'}$	0.031	0.045
Carbohydrate ($mg\ gdw^{-1}$)	$58.201^{a}_{a'}$	$51.974^{ab}_{a'b'}$	$49.932^{b}_{a'b'}$	$47.273^{b}_{b'}$	7.028	10.416
Soluble Nitrogen ($mg\ gdw^{-1}$)	$0.547^{a}_{a'}$	$0.425^{b}_{b'}$	$0.393^{b}_{b'}$	$0.350^{b}_{b'}$	0.077	0.112
Protein Nitrogen ($mg\ gdw^{-1}$)	$2.848^{a}_{a'}$	$2.249^{b}_{b'}$	$2.078^{b}_{b'}$	$1.915^{b}_{b'}$	0.393	0.580
Total Nitrogen	$3.395^{a}_{a'}$	$2.674^{b}_{b'}$	$2.471^{b}_{b'}$	$2.267^{b}_{b'}$	0.481	0.716
Protein content P.N. x 6.25 ($mg\ gdw^{-1}$)	$17.802^{a}_{a'}$	$14.063^{b}_{b'}$	$12.994^{b}_{b'}$	$11.975^{b}_{b'}$	2.432	3.634

Similar superscripts and underscripts in a paramenter represent insignificant difference at 5% and 1% level respectively.

Reductions in plant length, leaf area and leaf number/plant led to reduction in the fresh and dry weights of the plants and therefore, the net primary productivity was decreased (Fig. 1). After 15 days of exposures highly significant reductions i.e. at 1% level were observed only in the fresh and dry weights of root, while reductions in fresh and dry weights of shoot and whole plant remained significant only at 5% level. After 30 and 45 days of exposures all these attributes showed highly significant reductions (Table 2).

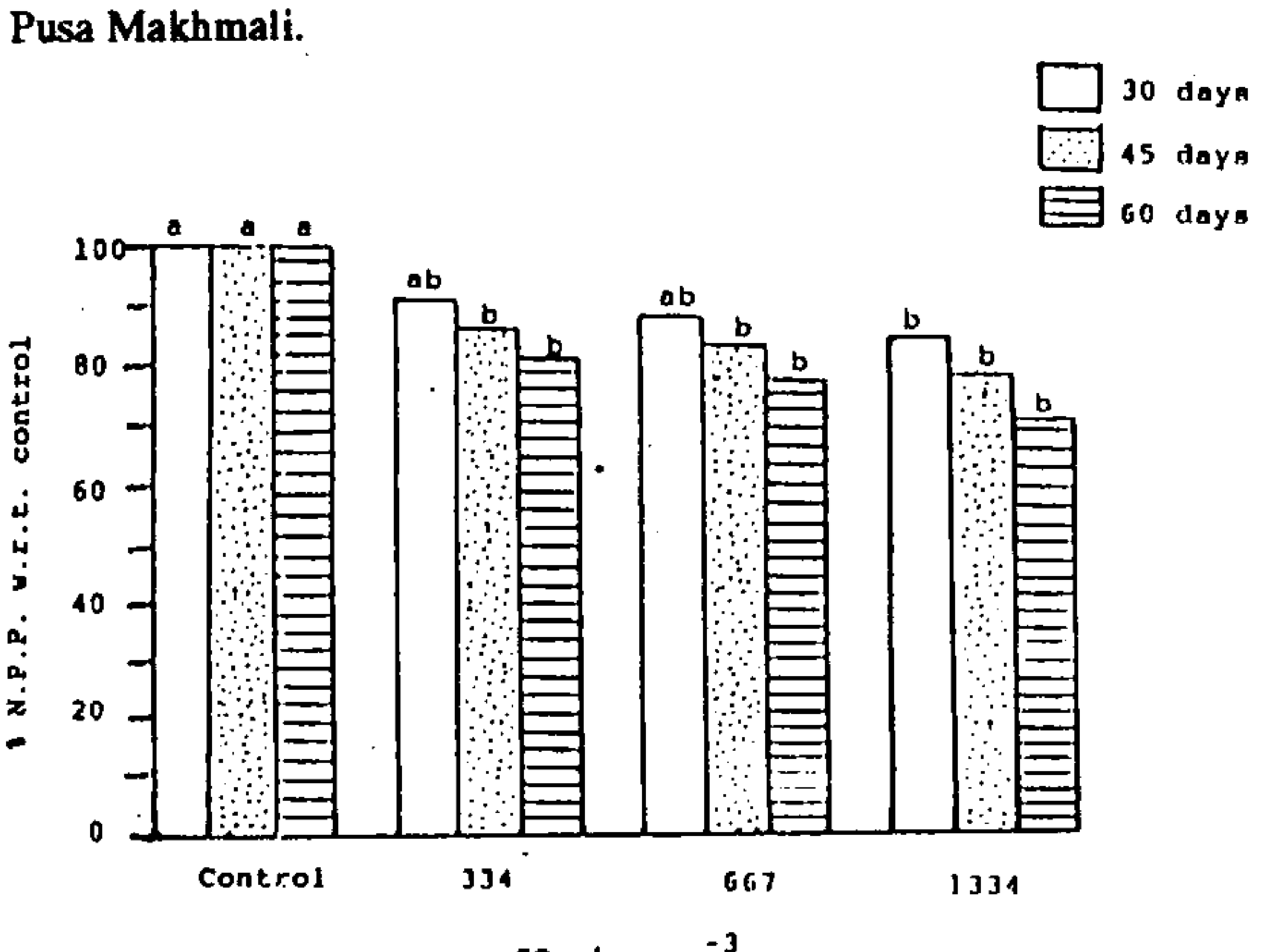
Fumigations with sulphur dioxide also rendered reductions in chlorophyll contents. Reduction in chlorophyll *a* was found to be higher than noted in chlorophyll *b*. Amounts of chlorophyll *a* and total chlorophyll were reduced more appreciably at all the stages of plant growth, while chlorophyll *b* showed such reductions only at the crop maturity. Foliar pH and ascorbic acid contents were also adversely affected in plants exposed to all the three concentrations of SO_2 used. Leaf extract pH showed appreciable reductions at 30 and 45 days of plant

Table 5: Yield response of *Abelmoschus esculentus* cv. Pusa Makhmali exposed to Sulphur dioxide.

Parameter	SO_2 (μgm^{-3})				C.D.	
	0	320	667	1334	5%	1%
Number of fruits plant^{-1}	$12.63^{a}_{a'}$	$9.52^{b}_{b'}$	$8.90^{b}_{b'}$	$8.10^{b}_{b'}$	2.09	3.05
Length of fruit (cm)	12.50^{a}	10.80^{b}	10.69^{b}	10.60^{b}	1.68	—
Fresh weight of fruits plant^{-1} (g)	$115.565^{a}_{a'}$	$76.757^{b}_{b'}$	$70.004^{bc}_{b'c'}$	$62.058^{c}_{c'}$	9.129	13.283
Dry weight of fruits plant^{-1} (g)	$30.694^{a}_{a'}$	$19.875^{b}_{b'}$	$17.956^{bc}_{b'}$	$15.717^{c}_{b'}$	4.133	6.014

Similar superscripts and underscripts in a parameter represent insignificant different at 5% and 1% level respectively.

Fig. 1: Effect of different concentrations of SO_2 at different days on N.P.P. (Net Primary Productivity) in *Abelmoschus esculentus* cv. Pusa Makhmali.



Different superscripts in respective days represent significant differences at 5% level of significance employing Critical Difference (C.D.).

Values in Control :
30 days : 0.180 g plant^{-1} day^{-1}
45 days : 0.541 g plant^{-1} day^{-1}
60 days : 0.544 g plant^{-1} day^{-1}

growth and more appreciable later on. Ascorbic acid content exhibited non-significant reduction at 30 days of plant growth but later on it got reduced significantly (Table 3).

Analysis of K, P and carbohydrate contents in the treated plants revealed reductions even at higher level of sighificance. Nitrogen, phosphorus, potassium, protein and carbohydrate levels in fruits of fumigated plants were decreased significantly at both (5 and 1%) levels of significance. On the contrary, an appreciable increase in sulphur level of leaves and fruits was also observed. Ultimately, the pollutant incurred a great reduction in the economic yield of okra (Table 4).

Yield of fruits : number of fruits plant^{-1}, length and their fresh and dry weights was decreased significantly by all the three concentrations of SO_2 applied (Table 5).

DISCUSSION

On the basis of present findings it may be inferred that *Abelmoschus esculentus* cv. Pusa Makhmali is highly susceptible to SO_2 pollution. The damage rendered in plants by the pollutant may be due to the accumulation of sulphur compounds in the mesophyll tissues of the leaves. These compounds may cause destruction of chloroplasts leading to the interveinal chlorosis, necrosis, and reduced leaf area (Wellburn *et al.*, 1972; Farroq *et al.*, 1988). Mature leaves were found to be more susceptible to the gas than the younger leaves as they have more functional stomata and larger intercellular spaces which facilitate rapid gas flow (Evans and Ting, 1974; Davies, 1980).

The decrease in leaf number, area and the photosynthetic activity of plants, produced an unfavourable effect on the overall growth and development of plants. Reduction in length, fresh and dry weights of shoot and root may be ascribed to the reduced photosynthetic activity and thereby lowering the translocation of photosynthetic products in shoot and root (Noyes, 1980; Saxe, 1983).

Further, the reduced photosynthetic ability of chlorophyll molecules is associated with the formation of toxic ions (H^+, HSO_3^{1-}, SO_3^{2-} and SO_4^{2-}) on the dissoultion of SO_2 in water and displacing Mg^{2+} ions from the chlorophyll molecule and converting it to a photosynthetically inactive brown pigment phaeophytin (Rao and Le Blanc, 1966; Malhotra and Hocking, 1976; Saxe, 1983). The chief cause for reduced photosynthetic activity is the competition between SO_2 and CO_2 for reaction site on RuBP-Carboxylase enzyme (Ziegler 1972; Mansfield and Jones, 1984).

Sulphurous and sulphuric acids formed by SO_2 and H_2O inside the leaf tissues seem to lower the foliar pH level (McClean *et al.*, 1968; Prasad and Rao, 1982). This alteration in the pH may influence the pH dependent enzymatic activities in plant cells which in turn would adversely affect the metabolic phenomena of the treated plants.

Mapson (1958) opined that lowering of ascorbic acid level in the SO_2 treated plants is owing to the sulphonation of S-H groups, while Hogler and Herman (1973) and Young and Loewus (1975) suggested that the formation of sulphite is accompanied with generation of free oxygen radicals which convert ascorbic acid into DHA or oxalic acid.

A gradual decline in total phosphorus and potassium contents of leaves and fruits was also noted in the experimental taxon. This lowering of potassium and phosphorus is found to be involved in increased susceptibility of the plants.

The reduction in nitrogen, protein and carbohydrate contents might be the result of decreased photosynthesis (Constantinidou and Kozlowski, 1979; Koziol and Jordon, 1978; and Saxe, 1983) or inhibition of protein synthesis or enhanced protein degradation (Rabe and Kreeb, 1979). The increase in the amount of sulphur in SO_2 exposed plants is due to its uptake through stomata, the metabolic utilization and ultimately its deposition into different tissues of the plants (Garsed and Read, 1977; Pandey, 1982).

Advanced flowering in treated plants as compared to untreated ones may be due to the fact that under stress conditions plants would have been in hurry to complete their life cycles. Sulphur dioxide adversely affects flowering and fertilization by reducing the reproductive physiology of plants which finally leads to significant reductions in the quality, quantity and dimensions of fruits (Manning and Wardaro, 1974; Oshima *et al.*, 1977; Varshney and Varshney, 1981).

Overtly, it appears that the presently studied cultivar Pusa Makhmali of ladies finger is highly susceptible to prolonged exposures with 320, 667 and 1334 $\mu g\ m^{-3}$ of SO_2.

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