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Phytotoxic Effects Of SO_2 On Crop Plants Total Chlorophyll Content

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ABSTRACT

Effect of different SO_2 concentrations on the total chlorophyll content in the leaves of three economically important plant species, viz., *Vigna radiata* (Mung bean), *Solanum esculentum* (=*Lycopersicon esculentum*)[Tomato] and *Zea mays* (Maize) was studied. Controlled fumigation experiments were carried out using three different treatments of SO_2: **T-1**=0.05 ppm (134.0µg m^{-3} SO_2) [x 4h], **T-2**=0.1 ppm (268.0 µg m^{-3} SO_2) [x 2h] and **T-3=**0.2 ppm (536.0 µg m^{-3} SO_2) [x 1h] for 60 days. In Maize, the exposure period was extended to 75 days. Plants of *V. radiata* and *S.esculentum* revealed a common pattern in their response to SO_2 exposure. Reduction in chlorophyll content was T-1>T-2>T-3. Interestingly, SO_2-treatment alone did not exercise a significant effect on the total chlorophyll content. It was the interactive effect of the two variables (SO_2 treatment and fumigation period) which was important. In *Z. mays* the fumigation period was significant only after 15 days of fumigation. The combined effect of both variables was also significant at all ages.

Key words: SO_2, Controlled-fumigation, Chlorophyll content, Mung bean, Tomato, Maize, Statistical regression model.

INTRODUCTION

Sulfur dioxide has since long been recognized as one of the most potent phytotoxicants. The global SO_2 emissions may have shown a decline in the past decade (WHO, 2000), but according to the data released by NASA's aura satellite, the mean SO_2 levels over India have increased by nearly 71% from 2005 to 2012. In other

words, SO_2 presents an ever increasing threat to vegetation in heavily industrialized regions of India. Coal-fired thermal power plants are the biggest contributors of SO_2 emissions.

SO_2 effects on vegetation are two-fold. Whereas even a short duration exposure results in foliar necrosis, it is the chronic injury (resulting from long-term exposure to lower SO_2 concentrations, which is detrimental to crop plants, forests and herbaceous vegetation. This may eventually manifest in terms of poor growth and yield of plants.

In this context, dose-response relationships based on controlled fumigations under quasi-field conditions or well-defined environmental conditions, help in a better understanding of physiological and biochemical events occurring in the affected plants prior to appearance of visible injury symptoms.

Physiological and biochemical effects on SO_2 exposed plants have been documented by many investigators (see Chauhan, 1989a; Lendzian & Unsworth, 1983; Malhotra & Khan, 1984; Chauhan, 1990; Darall, 1989; Rai et al., 2011; Singh et al., 2012; Chauhan, 2015). Decrease in total chlorophyll content is perhaps one of the first biochemical parameters measurable in SO_2-exposed plants. SO_2-induced degradation has been attributed to various factors like phaeophytinization (Rao & LeBlanc, 1965), increased acidity (Arndt, 1970) and free radicals (Peiser & Yang, 1979; Shimazaki et al., 1980; Merzlyak & Kovrizhnikh, 1986; Merzlyak et al., 1991).

Present investigations on three economically important plants were made to study the levels of total chlorophyll content vis-à-vis the mechanisms of plant tolerance to SO_2 -stress. Significance of individual and interactive effects of SO_2 concentration and exposure time upon the green pigment contents has been analyzed by statistical regression model.

MATERIAL AND METHODS

Three economically important cultivated plant species viz., *Vigna radiata* (L.) Wilczek [Mung bean], *Solanum esculentum* [Tomato], and *Zea mays* L. [Maize] were grown from seeds in the nursery. Fifteen-day-old seedlings of these plants were subjected to different SO_2 treatments through an artificial fumigation system. Sulfur dioxide was generated by bubbling $Na_2S_2O_5$ in water and circulated in closed-top fumigation chambers (1 x 1 x1m=1m3) at temperatures ranging between 25-29^{0}C ± 1^{0}C and at a RH of 60 ± 5%. Two 200W metal halide lamps were used for illumination with a light/dark cycle of 12/12 hours.

Treatment protocols of SO_2:

T-1=0.05 ppm (134.0µg m^{-3} SO_2) [x 4h], **T-2**=0.1 ppm (268.0 µg m^{-3} SO_2) [x 2h] and **T-3=**0.2 ppm (536.0 µg m^{-3} SO_2) [x 1h] for 60 days, thus keeping the SO_2 dose constant. *V. radiata* was fumigated for only 45 days. Controls (C) were maintained simultaneously by exposing the plants to air alone.

Estimation of Total Chlorophyll Content:
Fresh leaf tissue (0.2g) was homogenized with 80% aqueous acetone in dark or green light. The homogenate was centrifuged at 1500 x g for 15 min in a K-24 refrigerated centrifuge.The final volume of the supernatant was made up to 10.0 ml with acetone. The extinction E was measured at 645 and 663nm with a Spectronic 20 spectrophotometer. The amount of chlorophyll a and chlorophyll b was determined using the formula given by Maclachlan and Zalik (1963).The values of chlorophyll a and chlorophyll b were added for determining the total chlorophyll content.

Statistical Analysis
Anaysis of variance (ANOVA) and multiple regression analysis were employed to test the significance of individual as well as interactive effects of SO_2 concentration (ppm) and the exposure time (h) upon total chlorophyll content. The relationship between these variables was calculated with the help of an empirical (statistical regression) model and correlation coefficient (R).

RESULTS AND DISCUSSION

Control plants of *Zea mays* were found to have maximum total chlorophyll content, followed by those of *Solanum esculentum* and *Vigna radiata*. A decline in total chlorophyll content was recorded in all the plant species following SO_2 fumigation. *Vigna* recorded maximum loss in chlorophyll content, followed by that in *S. esculentum* and *Z.mays* respectively.

In *V.radiata*, reduction in chlorophylls was observed as T-3>T-2>T-1. Chlorophyll content in 45-day plants of C-1 exposed to SO_2 recorded maximum loss (19.65%) after 30 days of fumigation. Sixty-day old plants with C-2 treatment recorded highest loss in green pigment content (21.30%) after 45 days of SO_2 fumigation. In treatment T-3, highest percent reduction in chlorophyll content (25.73%) was observed in plants after 45 days of SO_2 exposure (Table 1).The fumigation period had an influence on the chlorophyll content initially, i.e., up to 30 days of fumigation (P=0.25-0.001).The interactive effect of the two variables (SO_2 treatment x fumigation period) on total chlorophylls was also significant (P=0.25-0.001). However, SO_2 treatment alone did not exercise a significant effect on the total chlorophyll content (Table 1, Fig. 1).

A similar pattern of chlorophyll loss was observed for *S. esculentum* with age and when subjected to SO_2 exposure. The control tomato plants exhibited increasing chlorophyll loss with age. After SO_2 fumigation for 60 days plants of T-1 recorded a loss of 7.07% whereas the loss of chlorophyll content in plants of T-2 and T-3 sets exposed for the same time period was 14.20 and 16.05% respectively. Here both factors, viz., SO_2 treatment and fumigation period exerted significant effect (P=0.001) on chlorophyll content independently, following 30 days of fumigation. In addition, the combined effect of both these independent variables was also significant (P=0.001) only after 30 days of SO_2-exposure and this effect remained as such up to 60 days of exposure (Table 2, Fig.2).

Total chlorophyll content of *Z.mays* plants was recorded upto75 days. Although there was a gradual decline in the pigment content with age in all the three control sets, it was lesser than that observed in both *V.radiata* and *S. esculentum*. The pattern of reduction in the pigment content was common to all the three treatments T-1, T-2 and T-3. In all of these treatments, the 45-days of fumigation resulted in maximum loss of pigment i.e., 9.10%, 11.09% and 16.32% respectively. The magnitude of loss of chlorophyll was however maximum in the T-3 set. From the statistical viewpoint, the total chlorophyll content in the 30 and 75-day old plants of *Z. mays* was significantly affected (P=0.25-0.001). On the other hand, fumigation period was of significance (P=0.001) only after 15 days of SO_2 fumigation. The combined effect of both these variables was also significant (P=0.25-0.001) at all the ages (Table 3, Fig. 3).

Present study shows that chlorophyll content decreased significantly in all the three plants following SO_2 fumigation. Reduction in total chlorophyll content was maximum in *V.radiata*, followed by that in *S. esculentum* and *Z. mays*. Similar trend for plants exposed to SO_2 has been reported by Gilbert (1968), Steubing et al. (1974), LeBlanc and Rao (1975), Malhotra (1977), and Rabe and Kreeb (1979).

SO_2-induced chlorophyll degradation has been attributed to different factors. Increase in acidity of cell sap has been recognized as one of the major factors for chlorophyll degradation (Rao&LeBlanc,1965). SO_2-induced acidity is thought to convert chlorophyll into phaeophytin where the Mg^{++} of chlorophyll gets replaced by $2H^+$. According to another viewpoint, chlorophyll destruction may not be due to acidity alone, and that SO_2, by virtue of its redox properties, destroys chlorophylls by oxidation (Malhotra, 1977).The present study also did not record any significant change in pH of the leaf cell sap, thereby suggesting that low levels of SO_2 did not bring about sufficient change in the cell pH, necessary for the conversion of chlorophylls into phaeophytin (even in *V.radiata*, which otherwise exhibited maximum reduction in chlorophyll content in response to SO_2-fumigation). This also indicates a possibility of an alternate mechanism in operation.

In vitro experiments by Peiser and Yang (1977,1978) suggest degradation of chlorophylls by free radicals, viz., alkoxy radicals produced by bisulphate-induced linoleic acid hydroperoxide (LOOH) cleavage.This reaction is catalyzed by the enzyme lipoxygenase. The reaction occurs either by a homolytic or heterolytic mechanism (Davies, 1961), and is pH dependant.

Chlorophylls are also reported to be co-oxidized, coupled to a lipoxygenase-linoleate system (Holden, 1965) wherein chlorophyll is destroyed either by co-oxidation during the formation of the fatty acid hydroperoxides (Imamura & Shimizu, 1974) or during the subsequent enzymatic decomposition of the hydroperoxides (Holden, 1965). On the other hand, role of SO_2-induced superoxide radical ($\cdot O_2^-$)in chlorophyll destruction has been clearly demonstrated in spinach leaves (Shimazaki et al., 1980).

Further, scavengers of superoxide radical like tiron (1,2-dihydroxybenzene-3,5-disulphonate), hydroxyquinone, ascorbate and the enzyme superoxide dismutase (SOD) inhibit chlorophyll degradation. A free-radical chain mechanism is thought to be operative where the superoxide radical plays a pivotal role (Shimazaki et al.,

1980). In addition,the superoxide free radical also produces singlet oxygen ($^1O_2$) which causes lipid peroxidation. Free oxygen radicals also participate in chlorophyll allomerization i.e.,formation of degradation products with altered cyclopentanone ring V (Merzylak & Kovrizhnikh,1986; Merzylak et al., 1991).Present investigations tend to support this view of the participation of superoxide radical in chlorophyll degradation. Plants of *Zea mays*, which exhibited a high endogenous level of SOD activity (scavenger of superoxide radical),showed much less effect on their chlorophyll content after SO_2 fumigation, when compared to the other two species investigated (Chauhan 1989a; 1989b). Various mechanisms involved in chlorophyll degradation by SO_2 have been summarized graphically in Fig 4.

The process of plant senescence also involves degradation of chlorophyll. Peroxidases and H_2O_2 have been implicated in chlorophyll bleaching (Kato& Shimizu, 1985).Since peroxidase activity is known to increase with the age of the plant, and is maximum at senescence, any stress which accelerates senescence will also result in an increased peroxidase activity. Companion studies on these three plant species have show an increase in the peroxidase activity and the number of peroxidase isoenzymes with corresponding decrease in chlorophyll content (Chauhan, 1989a).

Table 1. EFFECT OF SO_2 TREATMENTS ON TOTAL CHLOROPHYLL CONTENT IN *V.radiata*

Period of Fumigation (Days)	**15**		**30**		**45**	
TREATMENT Conc. (ppm) Time (h)	**Total Chlorophyll Content (mg/g f wt.)**	**Percent Reduction**	**Total Chlorophyll Content (mg/g f wt.)**	**Percent Reduction**	**Total Chlorophyll Content (mg/g f wt.)**	**Percent Reduction**
C–1 (0×4)	1.0804±0.037		1.159±0.045		0.8694±0.065	
T–1 (0.05×4)	1.0310±0.813	4.50	0.9315±0.14	19.65	0.6891±0.163	19.60
C–2 (0×2)	0.9162±0.045	13.40	0.9585±0.09		0.8924±0.085	
T–2 (0.10×2)	0.7928±0.046	13.40	0.7651±0.01	20.17	0.7026±0.182	21.30
C–3 (0×1)	0.8643±0.025		1.0156±0.05		0.8802±0.034	
T–3 (0.20×1)	0.7659±0.003	13.60	0.7752±0.06	23.60	0.6537±0.127	25.73

Mean (± SD) of 5 replicates C–1, C–2, C–3: Controls [air × time (h)];
T–1, T–2, T–3: Treatments [Conc. of SO_2 (ppm) × Exposure time (h)]

SIGNIFICANCE OF FACTORIAL EFFECTS:

Period of fumigation (Days)	15				30				45			
Source of Variation	**df**	**Sum of Squares**	**Mean Source**	**F**	**df**	**Sum of Squares**	**Mean Sources**	**F**	**df**	**Sum of Squares**	**Mean Sources**	**F**
SO_2 Conc. (ppm)	9	22.74	0.057	0.23	9	26.37	0.1909	1.00*	9	19.14	0.0459	0.12
Exposure Time (h)	14	22.93	0.2468	2.47**	14	26.54	0.3646	1.90**	14	19.34	0.2012	0.54
SO_2 Conc. Exposure Time	29	23.09	0.4080	4.05**	29	26.92	0.7466	3.90**	29	19.71	0.5752	1.55
Error	6		0.0997		6		0.1910		6		0.3693	

*Levels of significance: ** P<0.1; * P<0.25*

Table 2. EFFECT OF SO_2 TREATMENTS ON TOTAL CHLOROPHYLL CONTENT IN *S. esculentum*

Period of Fumigation (Days)	15		30		45		60	
Treatment Conc. (ppm) Time (h)	**Total Chlorophyll Content (mg/g f wt.)**	**Percent Reduction**	**Total Chlorophyll Content (mg/g f wt.)**	**Percent Reduction**	**Total Chlorophyll Content (mg/g f wt.)**	**Percent Reduction**	**Total Chlorophyll Content (mg/g f wt.)**	**Percent Reduction**
C–1 (0×4)	0.8953±0.05		1.445±0.045		1.2507±0.211		1.0704±0.032	
T–1 (0.05×4)	0.8688±0.0245	2.95	1.3908±0.007	3.40	1.2126±0.16	3.30	0.9943±0.056	7.07
C–2 (0×2)	0.8953±0.05		1.436±0.007		1.2507±0.211		1.0704±0.032	
T–2 (0.10×2)	0.8316±0.0464	7.10	1.259±0.070	12.58	1.0864±0.078	14.10	0.9180±0.056	14.20
C–3 (0×1)	1.1349±0.28		1.396±0.34		1.6944±0.0901		1.339±0.20	
T–3 (0.20×1)	1.0408±110	11.50	1.196±0.11	14.30	1.4208±0.10	16.40	1.124±0.099	16.05

Mean (± SD) of 5 replicates C–1, C–2, C–3: Controls [air × time (h)];
T–1, T–2, T–3: Treatments [Conc. of SO_2 (ppm) × Exposure time (h)]

SIGNIFICANCE OF FACTORIAL EFFECTS:

Period of fumigation (Days)	15				30				45				60			
Source of Variation	df	Sum of Squares	Mean Source	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F
SO_2 Conc. (ppm)	9	27.03	0.3112	0.60	9	55.076	0.0744	0.11	9	53.237	0.9372	1.40*	9	35.716	0.3260	0.98*
Exposure Time (h)	14	26.766	0.0435	0.08	14	55.158	0.1557	0.25	14	52.579	0.2790	0.41	14	35.555	0.1650	0.49
SO_2 Conc. Exposure Time	29	27.595	0.8724	1.70	29	55.859	0.8567	1.36	29	54.184	1.8840	2.80	29	36.214	0.8240	2.47
Error	6		0.5177		6		0.6266		6		0.6678		6			

*Levels of significance: * P<0.25*

Table 3. EFFECT OF SO_2 TREATMENTS ON TOTAL CHLOROPHYLL CONTENT IN *Z. mays*

Period of Fumigation (Days)	15		30		45		60	
TREATMENT Conc. (ppm) Time (h)	Total Chlorophyll Content (mg/g f wt.)	Percent Reduction	Total Chlorophyll Content (mg/g f wt.)	Percent Reduction	Total Chlorophyll Content (mg/g f wt.)	Percent Reduction	Total Chlorophyll Content (mg/g f wt.)	Percent Reduction
C–1 (0×4)	1.398±0.52		1.557±0.04		2.199±0.19		1.937±0.046	
T–1 (0.05×4)	1.3212±0.0326	5.50	1.533±0.08	7.20	1.992±0.18	9.10	1.864±0.048	3.90
C–2 (0×2)	1.310±0.067		1.921±0.06		2.284±0.26		1.699±0.066	
T–2 (0.10×2)	1.16±0.059	10.76	1.721±0.13	10.40	2.0314±0.44	11.09	1.570±0.836	7.65
C–3 (0×1)	1.518±0.045		1.526±0.111		1.703±0.25		1.319±0.130	
T–3 (0.20×1)	1.299±0.029	14.56	1.286±0.1415	15.59	1.425±0.178	16.32	1.175±0.026	10.68

*Mean (± **SD**) of 5 replicates C–1, C–2, C–3: Controls [air × time (h)]'*
T–1, T–2, T–3: Treatments [Conc. of SO_2 (ppm) × Exposure time (h)]

SIGNIFICANCE OF FACTORIAL EFFECTS:

Period of fumigation (Days)	15				30				45				60			
Source of Variation	df	Sum of Squares	Mean Source	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F	df	Sum of Squares	Mean Sources	F
SO_2 Conc. (ppm)	9	53.603	0.1602	1.66*	9	78.375	0.8675	1.42*	9	114.875	2.146	1.0*	9	78.389	2.1580	12.93**
Exposure Time (h)	14	53.609	0.1668	1.73**	14	77.776	0.2676	0.489*	14	113.189	0.460	0.21	14	76.336	0.1002	0.60
SO_2 Conc. Exposure Time	29	53.866	0.4233	4.41**	29	78.942	1.4344	2.89**	29	117.481	4.752	2.21**	29	78.656	2.4250	14.53
Error	6		0.0961		6		0.2929		6		2.146		6		0.1668	

*Levels of significance: ** P<0.001; * P<0.25 – 0.1*

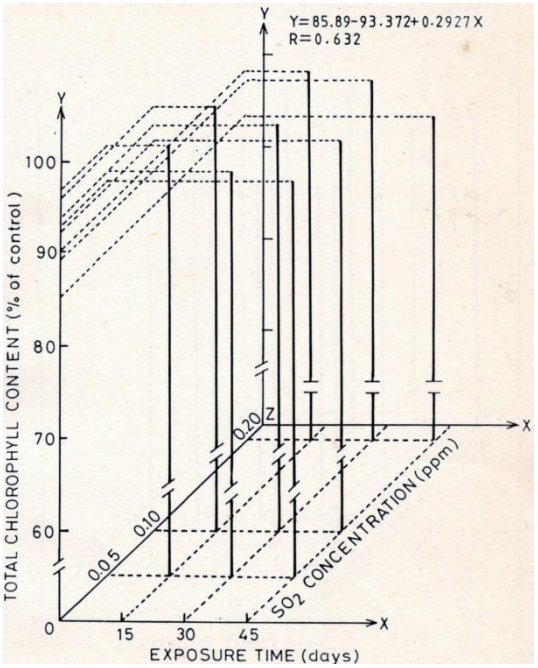

Fig. 1 V.radiata

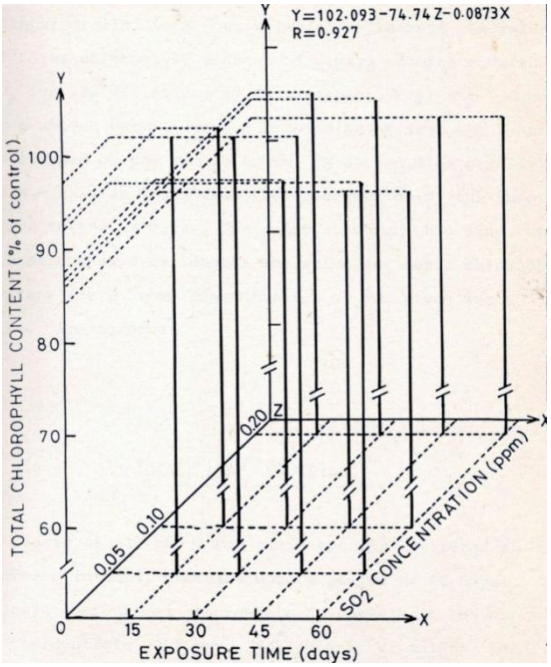

Fig. 2 S.esculentum

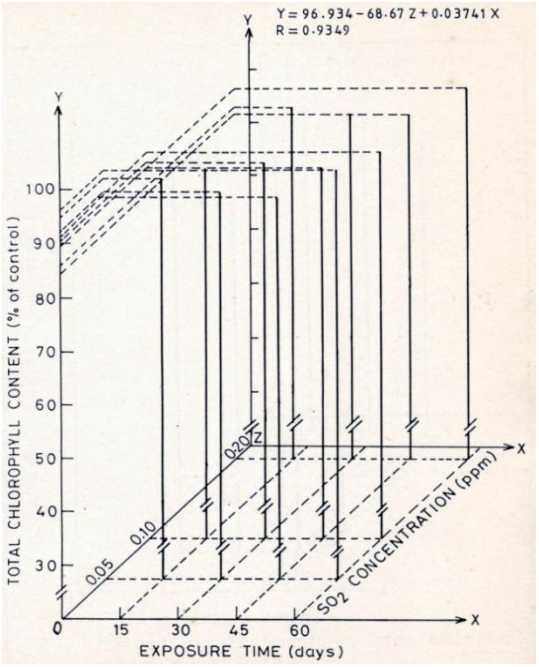

Fig. 3 Zea mays

Figs. 1-3 SIGNIFICANCE OF FACTORIAL EFFECTS

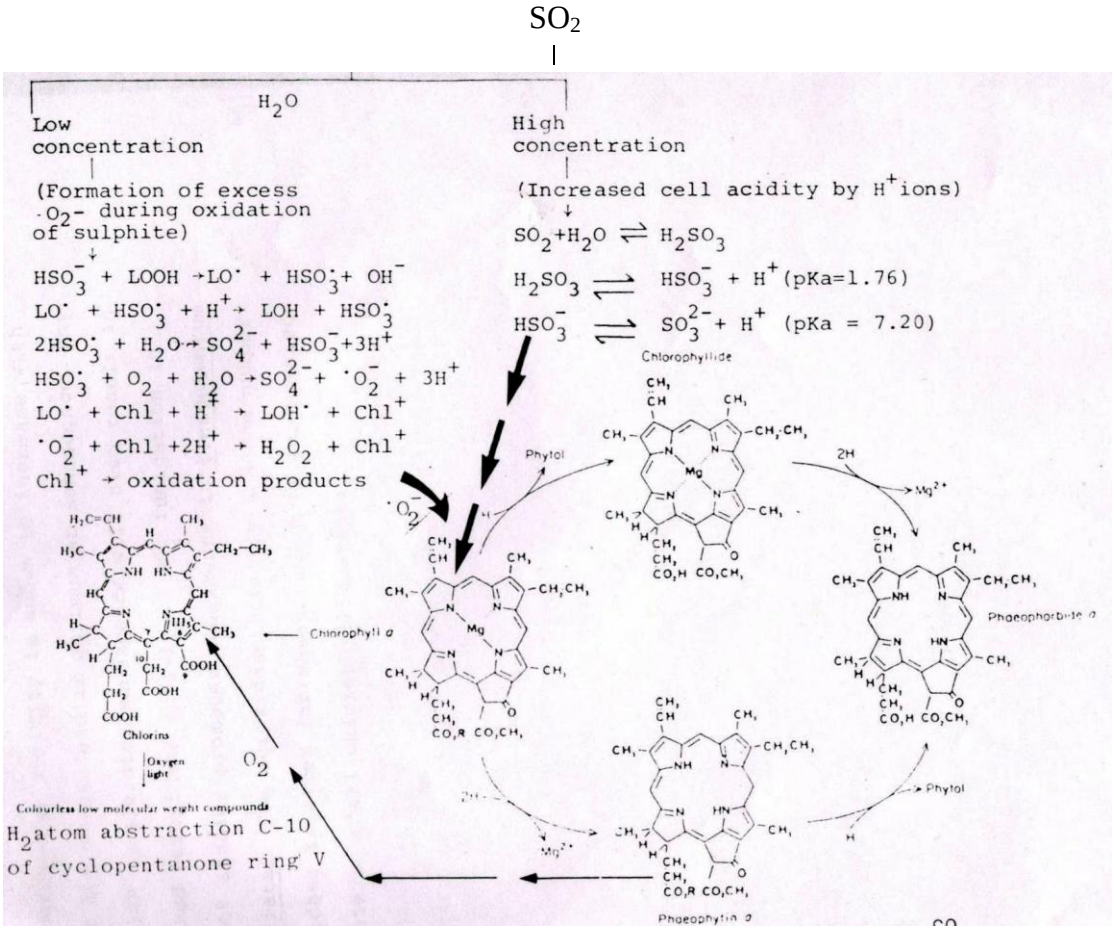

Fig. 4 Summary of Different Mechanisms involved in SO_2-induced Chlorophyll Degradation

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