International Journal of Engineering Research & Management Technology
ISSN: 2348-4039
January-2019 Volume 6, Issue-1
Email: editor@ijermt.org
www.ijermt.org

EFFECT OF SULPHUR DIOXIDE ON PLANT CHLOROPHYLL ON THE FAMILY OF BRASSICACEAE

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ABSTRACT

The effect of SO_2 on chlorophyll in (*Brassica juncea [L.] Czern.and Coss.cv.Pusa Bold; Raphanus sativus L.cv. Mino Early Long White*).In exposed seedlings SO_2 concentration ranging (653, 1306, 2612&3918 µg m^{-3} SO_2), resulted in a sharp decrease in total chlorophyll content. Both chlorophyll a and chlorophyll b got reduced and declined following the exposure of SO_2. This decrease could be due to disturbance in chloroplast ultra structure and because of conversion of chlorophyll into phaeophytin upon exposure to SO_2. After spraying of calcium hydroxide the yield in C+ SO_2 exposed set was higher than the set of plant exposed to SO_2 alone. Maximum reductions were noticed at 3918 µg m^{-3} of SO_2. The breakdown of chlorophyll molecules by SO_2 as measured by loss of Mg^{++} and total chlorophyll.

INTRODUCTION

Sulphur dioxide is one of the major air pollutants in industrialized areas that can damage vegetation. The process of photosynthesis appears to be mainly affected. A current view is that plant must exhibit visible symptoms for injury to exist. Many studies have tried to establish certain relationship between visible symptoms caused by exposure to SO_2 and injury. However several glasshouse exposure studies (Bogorad L 1966; Reinert RA and JS Sanders, 1982) have shown a net reduction of growth and yield without the development of visual symptoms. The present study was initiated to determine if low concentration of SO_2 have any effect on the process of photosynthesis without producing visual symptoms.

The effect of SO_2 was studied on the following aspects of pigment metabolism : (a) Effect on chlorophyll conversion into phaeophytin,(b)Effect on the activity of chlorophyllase and (c) Effect of low cytoplasmic pH caused by SO_2 on (a) and (b)

Growth Conditions

Plants from the family Brassicaceae (*Brassica juncea (L.) Czern.and Coss.cv.Pusa Bold; Raphanus sativus L.cv.Mino Early Long White*) seeds were washed with sterile distilled water and then treated with 0.1% mercuric chloride for 5 minutes and finally washed with sterile distilled water for 15 minutes. Surface sterilized seeds were allowed to imbibe water for 6 hrs.and thereafter sown on petriplates lined with cotton over which Whatman no.40 filter paper was placed. Seeds were placed on filter paper. For each variety, five sets each

having 150 seeds were maintained. Then seeds were sown in polythene bags/earthen ware containing garden soil. The soil was sandy loam and homogenously mixed with farmyard manure. All experiment will carried out in a fumigation chamber (1m*1m*1m) made of iron rods. Each chamber was portable and covered with transparent polythene sheets. Then Plants were exposed to SO_2 from 5th day onwards. Plants of each species were divided into five sets. Out of five four sets were exposed to four different concentration of Sulphur dioxide (653, 1306, 2612, 3918 µg m^{-3}) while the fifth one was used as control.

Sulphur dioxide Treatment

Sulphur dioxide was prepared by allowing a reaction of dilute sulphuric acid (10% H_2SO_4) and sodium sulphite (NA_2SO_3) under controlled condition of temperature and humidity. Complete reaction of 1M NA_2SO_3 with 10% H_2SO_4 produces 1M SO_2 or 126 mg of NA_2SO_3 yields 64 mg SO_2 .

The chemical reaction for SO_2 preparation is as follows-

$$NA_2SO_3 + H_2SO_4 \longrightarrow SO_2 \uparrow + NA_2SO_4 + H_2O$$

(126 mg) (64 mg)

Hence, 1.968 of NA_2SO_3 is required to produce 1 mg (1000 µg m^{-3}) of sulphur dioxide. Therefore, on the basis of this equation 1.285, 2.571, 5.142, 7.713 mg of NA_2SO_3 were used to obtain 653, 1306, 2612, 3918 µg m^{-3} of sulphur dioxide,respectively,inside the exposure system. The plants were given the treatment of SO_2 on alternate day for 2 hrs.

Calcium hydroxide treatment

Calcium hydroxide (0.5% aqueous solution) was used for amelioration studies. Results of germination studies showed that lower concentration of sulphur dioxide (653 and 1306 µg m^{-3}) did not cause any appreciable reduction. On the contrary, higher concentration (2612 and 3918 µg m^{-3}) of sulphur dioxide proved to be highly toxic. Plants treated with 2612 and 3918 µg m^{-3} of SO_2 were selected for amelioration studies and calcium hydroxide was used as an ameliorating agent.

For ameliorating study set treated with 0.5% $Ca(OH)_2$, 0.5% $Ca(OH)_2$+2612 µg m^{-3} SO_2 , 2612 µg m^{-3} SO_2 , 0.5% $Ca(OH)_2$+3918 µg m^{-3} SO_2 and 3918 µg m^{-3} SO_2 were designated as C,C+T_1, T_1,C+ T_2 and T_2 sets, respectively. The calcium hydroxide solution was sprayed on sulphur dioxide (2612 µg m^{-3} and 3918 µg m^{-3} SO_2) treated plants once in a week with the help of a sprayer.

The study on both plants was carried out till maturity (90 d in *Brassica juncea* and 120 d in *Raphanus sativus*).the observations for various attributes were recorded in 15 d, 30 d, 45 d, 60 d, 75 d, 90 d old plants of *Brassica juncea* and *Raphanus sativus* respectively.

Chlorophyll Extraction &Determination

Chlorophyll content was estimated according to Arnon's (1949) method. Fresh leaves (100 mg) were homogenized with acetone (80%) and a pinch of sodium bicarbonate was added. The homogenate was

centrifuged for 5 minutes and final volume of supernatant was made to 1o ml. by adding 80% acetone. The optical density (OD) of the extract was recorded at 645 nm and 663 nm on a spectrophotometer against a blank (80 % acetone).

The amount of chlorophyll a, chlorophyll b and total chlorophyll was calculated using the following formulae-

Chlorophyll a= (12.7 A_{663}-2.69 A_{645}) * V/W*1000(mg g^{-1} fresh weight)

Chlorophyll b= (22.9 A_{645}-4.68 A_{663}) * V/W*1000(mg g^{-1} fresh weight)

Total chlorophyll = (8.02 A_{663}-20.2 A_{645}) * V/W*1000(mg g^{-1} fresh weight)

RESULT & DISSCUSION

Reduction in chlorophyll a and chlorophyll b content of treated seedlings in comparison to control ones was observed at higher SO_2 level.SO_2 also reduced the stability index of chlorophyll. Chlorophyll content was estimated to determine the effect of SO2 on photosynthetic machinery of plants. Data analysis reveals that so2 exposures cause significant loss of chlorophyll in both experimental crops. Amount of chlorophyll a, chlorophyll b &total chlorophyll increased rapidly first 30 days and then remained almost changed but thereafter a sharp decline was observed in all sets. Max reduction in chlorophyll a content was recorded at 3918 µg m^{-3} of SO_2 in 60 d old leaves. Maximum %decrease in chlorophyll a content was 33.67 in *Brassica juncea* and 58.33 in *Raphanus sativus*. On other hand maximum decline in chlorophyll b content was 28.24 and 39.73 %in *Brassica juncea* and *Raphanus sativus* respectively at 3918 µg m^{-3} of sulphur dioxide. Influence of SO_2 was also observed on stability index of chlorophyll. Stability index of chlorophyll in treated plant was lower in comparison to control one. Maximum reduction noticed at 3918 µg m^{-3} of SO_2.

Table 3a: Estimation of some biochemical components in leaves of *Brassica juncea* fumigated with different concentration of sulphur dioxide at 45 d and 60 d plant age.

Plant age(d)														
	45 d							60 d						
Attribute	concentration of sulphur dioxide(µg m^{-3})					CD		concentration of sulphur dioxide(µg m^{-3})					CD	
	0	653	1306	2612	3918	5%	1%	0	653	1306	2612	3918	5%	1%
Chlorophyll a (mg g^{-1} f.wt.)	4.861 ±0.358	4.973 ±0.281	3.230** ±0.272	3.440** ±0.541	2.697** ±0.1754	1.193	1.275	4.086 ±0.283	4.166 ±0.283	2.877** ±0.302	2.876** ±0.102	2.669** ±0.046	0.890	0.952
Chlorophyll b (mg g^{-1} f.wt.)	1.406 ±0.056	1.403 ±0.231	1.321 ±0.125	1.123 ±.025	1.204 ±0.102	0.824	0.882	1.813 ±0.099	1.904 ±0.023	1.373** ±0.178	1.323** ±0.178	1.301** ±0.043	0.382	0.407
Stability index of chlorophyll	100.00	101.739	72.618	72.809	62.246	--	--	100.00	102.895	72.029	78.669	73.495	--	--

Table 3b: Estimation of some biochemical components in leaves of *Raphanus sativus* fumigated with different concentration of sulphur dioxide at 45 d and 60 d plant age.

Plant age(d)														
	45 d							60 d						
Attribute	concentration of sulphur dioxide(µg m^{-3})					CD		concentration of sulphur dioxide(µg m^{-3})					CD	
	0	653	1306	2612	3918	5%	1%	0	653	1306	2612	3918	5%	1%
Chlorophyll a (mg g^{-1} f.wt.)	1.121 ±0.022	0.983** ±0.008	0.780** ±0.037	0.681** ±0.041	0.587** ±0.013	0.098	0.120	0.948 ±0.035	0.864** ±0.032	0.735** ±0.011	0.526** ±0.038	0.395** ±0.020	0.028	0.083
Chlorophyll b (mg g^{-1} f.wt.)	0.354 ±0.005	0.353 ±0.002	0.390 ±0.029	0.238** ±0.051	0.263** ±0.005	0.090	0.108	0.307 ±0.021	0.286* ±0.014	0.255** ±0.014	0.186** ±0.011	0.186** ±0.009	0.010	0.052
Stability index of chlorophyll	100.00	89.843	82.882	62.058	57.653	--	--	100.00	91.669	79.384	57.044	46.582	--	--

Tables show that Chlorophyll content of C+T1&C+T2 sets are higher than the T1&T2 sets respectively. On 60th day % increase in chlorophyll a content of C+T1 set was 36.50 in *Brassica juncea* and 32.09 in *Raphanus sativus*. Over their T1sets.the corresponding value for C+T2 sets were 27.32 &22.58 in *Brassica juncea* and *Raphanus sativus* respectively. Level of chlorophyll b also increased in C+ SO_2 set than set treated with SO_2 alone. The total chlorophyll contents of C+ SO_2 set were higher than those of SO_2 treated plants. The application of calcium hydroxide reduced the loss in chlorophyll stability.

Table 4a: Estimation of some biochemical components in leaves of *Brassica juncea* treated with SO_2 and calcium hydroxide at 15 d and 30 d plant age

Plant age(d)															
	15 d								30 d						
Attribute	Treatment						CD		Treatment						CD
	Control	C	C+T_1	C+T_2	T_1	T_2	5%	1%	Control	C	C+T_1	C+T_2	T_1	T_2	5%	1%
Chlorophyll a (mg g^{-1} f.wt.)	1.336 ±0.294	1.453 ±0.236	1.460 ±0.225	1.266 ±0.108	1.054 ±0.124	1.073 ±0.036	0.595	0.837	3.055 ±0.315	2.562 ±0.430	2.755 ±0.101	2.349 ±0.484	2.468 ±0.467	1.738 ±0.274	1.333	1.882
Chlorophyll b (mg g^{-1} f.wt.)	0.766 ±0.252	0.783 ±0.222	0.766 ±0.252	0.573 ±0.093	0.650 ±0.096	0.415 ±0.087	0.564	0.796	1.225 ±0.237	1.282 ±0.3189	1.189 ±0.087	1.366 ±0.225	1.059 ±0.077	1.068 ±0.205	0.734	1.036

Stability index of chlorophyll	100.00	100.17	99.726	82.388	81.060	70.789	--	--	100.00	89.813	92.140	86.790	82.406	65.560	--	--

Table 4b: Estimation of some biochemical components in leaves of *Raphanus sativus* treated with SO_2 and calcium hydroxide at 15 d and 30 d plant age

Plant age(d)																
	15 d								**30 d**							
Attribute	**Treatment**						**CD**		**Treatment**						**CD**	
	Control	**C**	**C+T_1**	**C+T_2**	**T_1**	**T_2**	**5%**	**1%**	**Control**	**C**	**C+T_1**	**C+T_2**	**T_1**	**T_2**	**5%**	**1%**
Chlorophyll a (mg g^{-1} f.wt.)	0.567	0.557	0.499	0.499	0.436**	0.404**	0.092	0.129	0.814	0.793	0.693**	0.642**	0.641**	0.562**	0.082	0.116
	±0.005	±0.024	±0.049	±0.048	±0.007	±0.011			±0.003	±0.015	±0.035	±0.056	±0.008	±0.015		
Chlorophyll b (mg g^{-1} f.wt.)	0.134	0.125	0.105*	0.107*	0.108*	0.085**	0.026	0.036	0.201	0.233	0.221	0.189	0.157	0.139	0.094	0.133
	±0.001	±0.014	±0.004	±0.061	±0.002	±0.005			±0.003	±0.032	±0.012	±0.045	±0.017	±0.013		
Stability index of chlorophyll	100.00	97.517	86.729	86.329	76.953	71.291	--	--	100.00	99.737	88.910	81.844	78.693	69.060	--	--

Malhotra (1977) & Shimazaki *et.al.* (1980) reported that stress treatment either inhibits or increase its destruction. After treatment with SO_2, chlorophyll content of plants was positively affected at low conc.; same was found to be decreased at higher conc.

Chlorophyll Breakdown

In order to understand the mechanism of chlorophyll destruction, the effect of SO_2 on Mg^{++} loss from chlorophyll (conversion of chlorophyll into pheophytin) was determined. Having entered inside the leaves, SO_2 with the help of water forms sulphurous acid, which then dissociates into H^+ and HSO_3^- ions and causes degradation of chlorophyll. chlorophyll a is degraded to phaeophytin through replacement of Mg^{+2} ions present in chlorophyll molecules by free H^+ ions, while chlorophyll b forms chlorophyllide through the removal of phytol group of the molecule (Rao and LeBlanc,1966).however the phaeophytinization of chlorophyll caused by SO_2 occurs when the pH of outer solution is lower(Malhotra,1977). Acids such as hydrofluoric and hydrochloric acids have been shown to convert chlorophyll into pheophytin with the release of Mg^{++} (Rao and LeBlanc,1966). Mg^{++} is replaced by two molecules of hydrogen with a resulting change in the light absorption spectral properties of chlorophyll molecule.

Sulphur dioxide and Chlorophyll destruction

SO_2 exposure resulted in simultaneous loss of both chlorophyll a &chlorophyll b. This implies that the loss of chlorophyll b was resultant upon inhibited biosynthesis of chlorophyll a (Bogorad, 1966).The decrease in chlorophyll content at higher concentration of SO_2 could be due to very low pH which seems to be very fatal as described by Gilbert (1968) and Grunwald (1981). Some other investigator reported that SO_2 is oxidized to SO_4^{-2} through intermediary sulphite ions. Excess of Sulphite leads to break down of chlorophyll (Ricks&William, 1975). SO_2 may react with chlorophyll to form superoxide radicals (Shimazaki et al. 1980; William and Banerjee, 1995). These radicals cause wide spread damage to membrane and associated molecules including chlorophyll pigment (Peiser and Yang, 1978).

CONCLUSION

Both crops (*Brassica juncea* & *Raphanus sativus*) showed negative response to elevated SO_2 levels, the extend of response was different between both crops. Both plants adopted some protective strategies to fight against SO_2 pollution. Study reports that initial concentration of SO_2 (653 µg m^{-3}) caused slight stimulation in growth of *Brassica juncea* thus *Brassica juncea* can tolerate or grow better up to a certain critical level of SO_2, the level which is higher than other crops. it was found that photosynthetic pigments were positively affected at low concentration in *Brassica juncea*, the same found to be decreased at higher concentration reduction in chlorophyll led to impairment of photosynthetic vis-a –vis reduction in growth and yield of plants. Chlorophyll degradation was found more in *Raphanus sativus* than in *Brassica juncea*. The present investigation led us to believe that calcium hydroxide acts as an antidote against SO_2 stress in two test crops *Brassica juncea* and *Raphanus sativus*. The yield improvement was better in *Brassica juncea* than that in *Raphanus sativus*, the reason behind this may be that *Raphanus sativus* is very sensitive to SO_2 pollution & is not able to recover from SO_2 stress. On other hand *Brassica juncea* being relatively resistant to SO_2 responded potentially to calcium hydroxide treatment and showed better growth than non sprayed plants.

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