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Total Leaf Protein Content: A Measure to Study the Phytotoxic Effects of Sulfur Dioxide on Crop Plants

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Abstract: ***Effect of different concentrations on total leaf protein content was studied in three economically important plant species, viz., tomato, mung bean and maize. Different treatments of SO_2 exposure were administered in artificial fumigation chambers. Maize showed least reduction in total proteins. Tomato exhibited maximum decline in protein content after SO_2 fumigation. Relationship between individual and interactive effects of SO_2 concentration were analyzed with the help of a statistical regression model.***

Present study helps to establish a correlation between the total leaf protein content, free radicals, activities of antioxidant enzymes like superoxide dismutases and peroxidases and plant sensitivity to SO_2 under ambient conditions as well as in greenhouse environment.

Keywords: controlled SO_2-fumigation, total leaf protein content, tomato, mung bean, maize.

I. INTRODUCTION

Sulfur dioxide is one of the dangerous phytotoxicants in ambient as well as under greenhouse conditions. Exposure to low concentrations of SO_2 is known to induce several physiological and biochemical modifications in plants, as documented and reviewed by many investigators like Chauhan, (1989a), Darall, (1989), Varshney et al., (2009), Rai et al., (2011), Singh et al., (2012), Brahmachari and Kundu, (2017),and Lee et al., (2017) . Total leaf protein content constitutes one of the important parameters for healthy plant metabolism. Investigators like Agrawal et al., (2006), Varshney et al., (2009), Singh et al., (2012), and Lee et al., (2017) have observed a decrease in total proteins in SO_2-fumigated plants in many plants . Alohisen (2014) attributes this decline primarily to the free-radical induced breakdown of existing proteins, resulting in an increase in the total free amino acid content .Reduced *de novo* synthesis of proteins is another reason for the decrease in protein content. The relationship between total protein content and the activity of antioxidant enzymes like peroxidases and superoxide dismutase (SOD) helps to explain the possible mechanisms of SO_2-tolerance by many plants. Present investigation on three economically important crop plants explains the levels of total leaf proteins in relation to SO_2 stress. The combined effects of SO_2 dosage and exposure time have been analysed using a regression model.

II. METERIAL AND METHODS

A. Plant Material and Fumigation System

Three economically important cultivated plant species viz., *Vigna radiata* (L.) Wilczek [Mung bean], *Solanum esculentum*(= *Lycopersicon esculentum* Mill.[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 from an aqueous solution of $Na_2S_2O_5$ and circulated over the plants kept in specially designed closed-top fumigation chambers (1 x 1 x1m= $1m^3$) at temperatures ranging between 25-29^{0}C ± 1^{0}C and at a Relative humidity (RH) of 60 ± 5%. As a source of illumination, two metal halide lamps of 200W each were employed following a light/dark cycle of 12/12 hours..

B. Fumigation Protocol

Artificial fumigations were carried out according to the following protocols detailed by Chauhan, (1989a):

Treatments: 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, the SO_2 dose was kept constant. Among the above, *V. radiata* was fumigated for only 45 days. Controls (C) were maintained simultaneously by exposing the plants to air alone. Fresh leaf tissue (0.2g) was homogenized in 10 ml of 5%TCA and total protein content was estimated following the method of Lowry et al.,(1951).

III. RESULTS AND DISCUSSION

Leaves of *Vigna radiata* showed considerable reduction in total leaf proteins following SO_2 treatment. A progressive reduction in protein content was observed with respect to the dosage, viz., 39.34, 22.10 and 16.57 per cent for treatments T-3, T-2, and T-1 respectively for 45-day fumigation (Table 1, Fig. 1). Whereas the total leaf protein content in control plants of *Solanum esculentum* (=*Lycopersicum esculentum*) was slightly less than that recorded for *Vigna*, there was much pronounced reduction in leaf proteins in all the three treatments. The reduction values were much higher in T-1, T-2 and T-3 over the corresponding values in *Vigna*. For instance, values for total leaf protein content in the treatments T-1, T-2, and T-3 for 60 days were 30.21,57.94, and 62.68% respectively (Table 2 , Fig. 2), which were considerably higher than the other plant species investigated.SO_2-fumigated plants of *Zea mays* exhibited least reduction in total leaf protein content. 60-days fumigation cycle with T-1, T-2, and T-3 treatments resulted in a reduction in protein content of only 14.14, 18.75, and 21.87% respectively (Table 3,Fig. 3).

Investigators like Malhotra and Khan ,(1984), Hamid and Jawaid, (2009), Rai et al., (2011), Singh et al., (2012), Brahmachari and Kundu, (2017),and Lee et al., (2017) have reported a general decrease in protein content in SO_2-fumigated plants in several cultivated and wild species. Reduction in protein content can be attributed to a) enhanced sulphitolysis, b) free radical reactions, and c) reduction in *de novo* protein synthesis, as suggested by Lendzian and Unsworth, (1983). Production of HSO_3^- and SO_3^- ions induces cleavage of disulphide linkages in proteins giving thiols and sulphonates. According to Shimazaki et al., (1980) sulphitolysis of the disulphide bond causes unfolding of protein molecules. Free radicals generated during SO_2 oxidation to $SO4^{-2}$, may induce lipid peroxidation, which constitutes an important mechanism of membrane deterioration in SO_2 -fumigated plants, as has been shown by Irigoyen et al., (1992). In addition, SO_2-induced free radicals also inactivate proteins by modifying their amino acid residues (Wolff et al., 1986), thereby rendering many vital enzymes inactive. A distinct relationship seems to have been established regarding the effects of SO_2 on total proteins and the concentration of antioxidant enzymes. Peroxidases and SOD have been shown to play a vital role in SO_2 tolerance, as reported by Chauhan, (1989b), Singh et al., (2012), Brahmachari and Kundu, (2017),and Lee et al., (2017). *Vigna* and *Zea mays*, which show high SOD and peroxidase content, recorded less lipid peroxidation (Chauhan 1989b, 2015). Reduction in total leaf protein content after SO_2 exposure was also less in these two species as compared to tomato, which incidentally had low activities of these two enzymes. In addition, the emission of volatiles like ethylene, ethane, acetaldehyde and ethanol from SO_2-treated plant tissues increase dramatically prior to the appearance of visible injury symptoms. Chauhan, (1990) reported that the spurt in volatile emissions in indicative of lipid peroxidation due to sulphate oxidation-induced free radical generation.

Interestingly, recent investigaions, as reviewed by Li et al., (2022) also tend to implicate SO_2 as a vital signalling molecule. SO_2 fumigation is now thought to reprogramme the expression of many genes thereby modulating physiological processes like seed germination, stomatal action, fruit-keeping and response to environmental stress.

TABLE 1. EFFECT OF SO_2 TREATMENTS ON TOTAL LEAF PROTEINS IN *Vradiata*

Period of Fumigation (Days)	15		30		45	
TREATMENT Conc. (ppm) Time (h)	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction
C-1 (0×4)	9.42±0.40	7.5	11.73±0.754	14.06	14.06±1.00	16.57
T-1 (0.05×4)	8.711±1.14		10.08±0.4012		11.73±0.686	
C-2 (0×2)	7.15±0.547	12.56	9.2±0.628	15.20	10.95±0.374	22.10
T-2 (0.10×2)	6.26±0.282		7.8±0.249		8.53±1.13	
C-3 (0×1)	7.37±0.426	18.04	9.6±1.13	20.80	15.02±0.756	39.34
T-3 (0.20×1)	6.04±0.764		7.68±0.453		9.11±1.457	

Mean (SD) of 5 replicates C-1, C-2, C-3 : Controls [air time (h)];
T-1, T-2, T-3 : Treatments [Conc. of SO2 (ppm) Exposure time (h)]

SIGNIFICANCE OF FACTORIAL EFFECTS : *V. radiata*

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)	17	3022.50	77.30	2.34**	17	4620.89	42.9	1.51*	17	7325.4	96.3	1.09*
Exposure Time (h)	27	2964.50	19.40	0.60	27	4629.54	51.64	1.81**	27	7399.83	170.67	1.94**
SO_2 Conc. Exposure Time	53	3074.96	129.76	3.93**	53	4700.2	123.0	4.32*	53	7583.90	354.74	4.04*
Error	10		32.96		10		28.46		10		87.7	

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

TABLE 2. EFFECT OF SO2 TREATMENTS ON TOTAL LEAF PROTEINS IN *S. esculentum*

Period of Fumigation (Days)	15		30		45		60	
Treatment Conc. (ppm) Time (h)	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction
C–1 (0×4)	7.4±0.432		9.28±0.730		11.33±1.247		11.75±1.224	
T–1 (0.05×4)	6.73±0.066	9.0	7.17±0.733	22.73	8.6±0.588	24.09	8.82±1.290	30.21
C–2 (0×2)	6.40±0.821		7.82±1.28		9.46±0.653		9.51±1.158	
T–2 (0.10×2)	5.57±0.871	12.96	5.57±0.475	28.77	4.22±0.553	55.57	4.02±14.74	57.94
C–3 (0×1)	6.4±0.821		7.82±1.28		9.46±0.653		9.51±1.589	
T–3 (0.20×1)	5.08±0.472	20.63	5.24±0.691	33.00	3.76±0.4618	59.84	3.55±0.2948	62.68

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 : *S.esculentum*

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)	17	2018.38	9.60	0.43	17	2796.52	31.62	0.69*	17	3415.33	126.85	2.4**	17	3598.32	145.52	1.93
Exposure Time (h)	26	2033.19	21.40	0.96	26	2837.01	72.11	1.58**	26	3570.47	281.99	5.34**	26	3729.33	276.53	3.66**
SO_2 Conc. Exposure Time	53	2064.96	53.18	2.38	53	2914.28	149.38	3.27**	53	3750.12	461.64	8.74**	53	3950.28	497.48	6.59**
Error	10		22.17		10		45.65		10		52.8		10		75.43	

TABLE 3. EFFECT OF SO_2 TREATMENTS ON TOTAL LEAF PROTEINS IN *Z. mays*

Period of Fumigation (Days)	15		30		45		60	
TREATMENT Conc. (ppm) Time (h)	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction	Total Leaf Protein Content (mg/g f wt.)	Percent Reduction
C–1 (0×4)	7.84±0.1666		14.86±0.914		17.04±0.514		19.8±0.2.42	
T–1 (0.05×4)	7.55±0.2962	3.6	13.60±0.4618	8.4	15.71±0.412	7.89	16.7±0.50	14.14
C–2 (0×2)	6.0±1.2247		11.55±1.0		12.11±1.09		16.0±0.92	
T–2 (0.10×2)	5.77±1.238	3.83	10.26±1.52	11.6	10.33±0.422	14.69	13.0±0.845	18.75
C–3 (0×1)	8.13±0.4000		15.0±1.33		16.38±1.94		14.22±2.19	
T–3 (0.20×1)	7.75±0.8931	4.32	13.28±1.19	1..46	13.25±0.390	19.10	11.11±0.993	21.87

Mean (± SO) 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 : *Z.mays*

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)	17	2835.0	52.9	2.08*	17	9398.15	126.0	2.06**	17	10665.18	251.30	1.070*	17	12656.90	296.00	2.18**
Exposure Time (h)	26	2783.3	1.2	0.04	26	9300.60	28.0	0.50	26	10504.37	91.0	0.38	26	12488.48	127.58	0.94*
SO_2 Conc.X Exposure Time	53	2861.6	79.5	3.12**	53	9487.56	215.96	3.54**	53	10990.69	576.8	2.46	53	12919.60	558.70	4.12
Error	10		25.4		10		61.0		10		234.8		10		135.52	

Levels of significance : ** P < 0.50 ; * P < 0.25 – 0.1

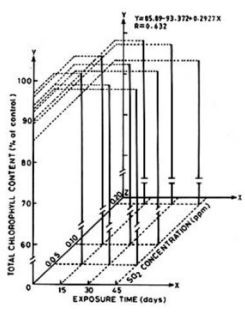

Fig.1 . *V.radiata*

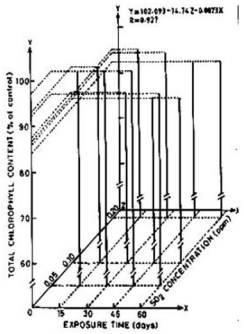

Fig.2. *S.esculentum*

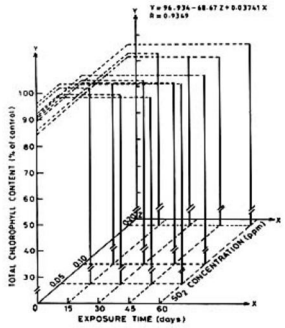

Fig.3. *Z.mays*

Figs 1-3. Significance of Factorial Effects

IV. ACKNOWLEDGEMENT

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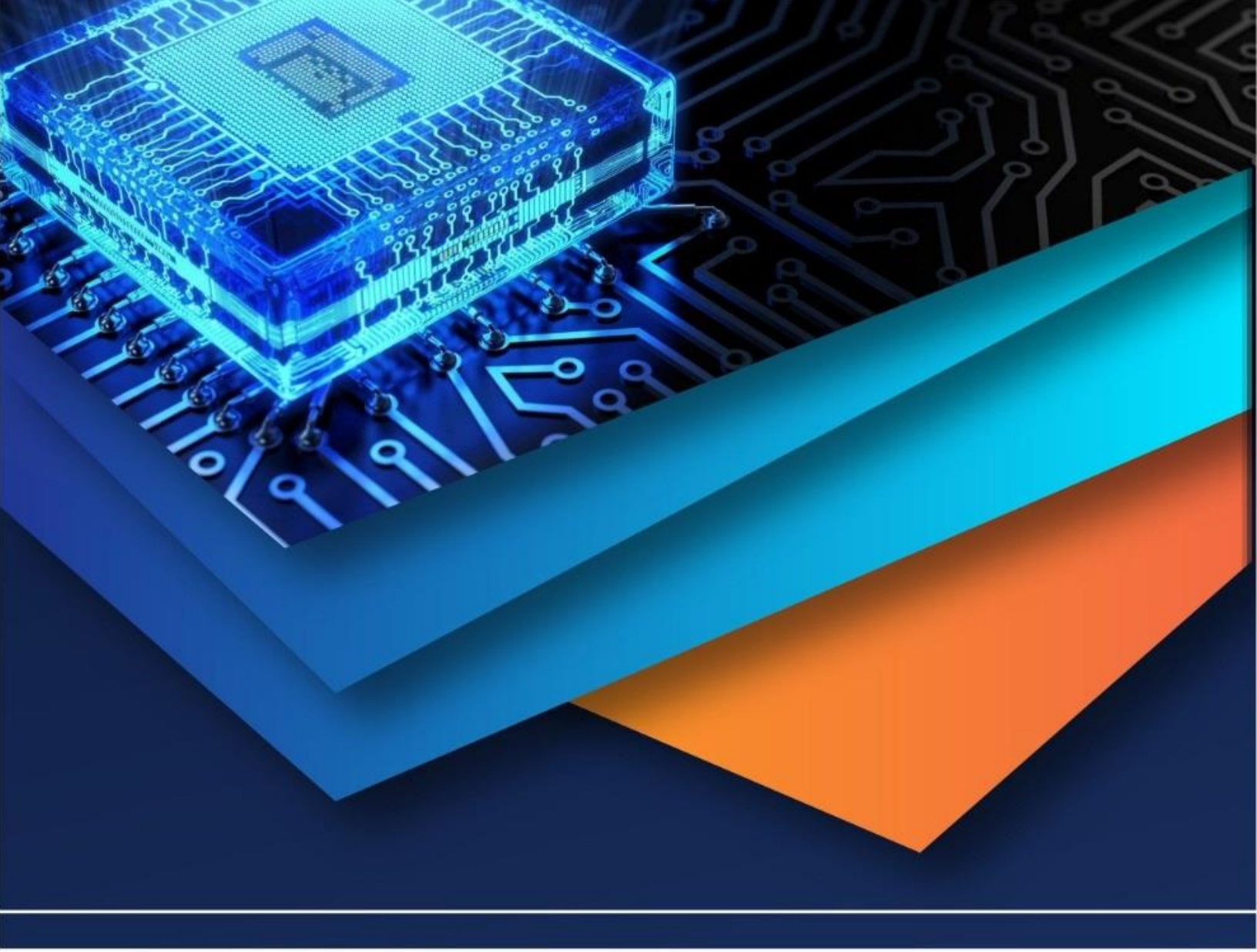

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