EFFECT OF OZONE AND SULPHUR DIOXIDE POLLUTANTS SEPARATELY AND IN MIXTURE ON CHLOROPHYLL AND CAROTENOID PIGMENTS OF *Oryza sativa*

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Abstract. Exposure of rice plants to low concentrations of O_3 and SO_2 singly and in combination showed foliar injury of different levels. The maximum leaf injury was noted in case of O_3 + SO_2 treated plants and the minimum in O_3 treated ones. Also the reductions in chlorophyll *a*, *b* and total chlorophyll and carotenoid contents in leaves exposed to O_3 + SO_2 mixtures were higher than the reduction noted in case of each individual pollutant. Thus the results suggest a synergism existing between O_3 and SO_2 regarding plant injury, especially with respect to chlorophyll and carotenoid contents of rice *(Oryza sativa)*.

Introduction

In the vicinity of urban and industrial areas, crop plants are frequently exposed to gaseous pollutants. The ecological inplications of pollutants are quite serious for crop yield. Ozone and SO_2 are a likely combination in many areas. These two gases both singly and in combination, disrupt various metabolic processes and consequently affect growth and development of plants (Menser and Heggestad, 1966; Tingey *et al.*, 1971).

Pigment concentration in leaves is an important parameter for determining the photosynthetic efficiency of plants. Chlorophyll content, an index of photosynthetic potential of plants, is highly susceptible to pollutant action. Pigment interaction with pollutants leads to destruction of photosynthetic leaf areas and development of characteristic foliar symptoms. Both O_3 and SO_2 individually are known to reduce the chlorophyll (Rao and LeBlanc, 1966; Adedipe *et al.*, 1973; Knudson *et al.*, 1977), and carotenoid contents (Chang and Heggestad, 1974; Bauer and Grill, 1977) in plants. But the information regarding the effect of a mixture of O_3 + SO_2 on chlorophyll and carotenoids is meagre.

2. Materials and Methods

2.1. Experimental plant

Rice plants (*Oryza sativa* L. cv Ratna) were raised in a nursery and when 20-days old were transplanted 10 cm apart into well manured 1.5 × 1.5 m^2 plots.

2.2. Fumigation process

When 40-days old, the plants were exposed separately to 0.08 ppm O_3, 0.5 ppm SO_2, and 0.04 + 0.25 ppm O_3 + SO_2, after enclosing them in a transparent polyethelene

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chamber of 1.5 × 1.5 × 1.5 m^3 for 1.5 h daily for 20 days (between 40 and 60-days old). They were then allowed to recuperate between 60 and 70-days old and again exposed to the pollutants between 70 and 80-days old. Control plants were subjected to the same conditions but no pollutant was added to the chamber.

2.3. Generation of Pollutants

Ozone was produced by an ozone generator using an ultra violet lamp. Ozone concentration within the exposure chambers, was determined by the method of Byers and Saltzman (1958). Sulphur dioxide was produced by bubbling air into 1% aqueous sodium metabisulphite solution and the desired concentration within the chamber was achieved by dilution with carrier air. The gas concentration into the chamber was measured using the method of West and Gaeke (1956). For the pollutant mixture, O_3 and SO_2 were added simultaneously within the chamber.

2.4. Sampling of Plants

Random samplings of plants were done at 40 (prior to fumigation), 50, 60, 70, 80, and 90-days old. They were analyzed with respect to foliar symptoms, percent leaf area damage, chlorophyll *a*, *b* and total chlorophyll and carotenoid contents.

2.5. Analysis

The plants were visually examined for the presence of foliar symptoms. Foliar injury was expressed in terms of percent leaf area damaged as compared to control.

For the estimation of chlorophyll and carotenoid contents, 0.25 g leaf was homogenized in cold 80% acetone and centrifuged at 3000 G for 15 min. The final volume of the supernatant was made to 25 ml by adding 80% cold acetone. The optical densities of the leaf extracts were measured at 480 and 510 nm wavelengths for carotenoids and 645 and 663 nm wavelengths for chlorophyll, in spectronic 20 spectrophotometer. The amount of chlorophyll *a* and *b* was calculated by using the formulae of Maclachlan and Zalik (1963), and total chlorophyll content was obtained by adding the two. Carotenoid contents were calculated by the formulae of Duxbury and Yentsch (1956).

The data of Table II were statistically treated using students '*t*' test.

3. Results

After 7 days of treatment, O_3 treated plants showed chlorotic spots on either surface of leaves. Later on such chlorotic spots turned brown in color. Those treated with SO_2 showed chlorotic interveinal streaks on either leaf surface within 3 days of fumigation, which gradually turned necrotic. However, in case of $O_3 + SO_2$ exposed plants, interveinal chlorisis was visible on the next day after initial fumigation. Such symptoms were produced nearer the leaf tip and gradually changed into dark brown necrotic areas.

The maximum leaf area damage at 90-days old plant was 71.5% in case of $O_3 + SO_2$-treated plants, followed by SO_2-treated (58.0%) and O_3-treated (28.0%) (Table I).

Maximum reduction in total chlorophyll content was observed in case of $O_3 + SO_2$-

TABE I

Percent leaf area damage in O_3-, SO_2-, O_3 + SO_2-treated rice plants at different stages of their growth

Plant age (day)	Leaf area damage (%)		
	O_3	SO_2	$O_3 + SO_2$
40	–	–	–
50	7.20	20.62	31.66
60	12.26	32.76	45.05
70	15.66	40.33	56.20
80	26.50	53.66	68.33
90	28.00	58.00	71.50

TABLE II

Chlorophyll *a*, chlorophyll *b* and total chlorophyll (mg g^{-1} dry leaf) of control, O_3-, SO_2 and O_3 + SO_2-treated rice plants at different stages of their growth (values are mean of three replicates ± standard deviation)

Plant age (day)	Chlorophyll	mg g^{-1} dry leaf			
		Control	O_3	SO_2	$O_3 + SO_2$
40	*a*	2.09 ± 0.04	2.09 ± 0.04	2.09 ± 0.04	2.09 ± 0.04
	b	3.08 ± 0.02	3.08 ± 0.02	3.08 ± 0.02	3.08 ± 0.02
	Total	5.17 ± 0.07	5.17 ± 0.07	5.17 ± 0.07	5.17 ± 0.07
50	*a*	2.34 ± 0.02	1.96 ± 0.09^a	1.84 ± 0.08^b	1.75 ± 0.00^b
	b	3.47 ± 0.09	2.65 ± 0.06^b	2.44 ± 0.25^a	2.14 ± 0.00^b
	Total	5.82 ± 0.12	4.62 ± 0.15^a	4.29 ± 0.33^a	3.89 ± 0.00^b
60	*a*	2.09 ± 0.32	1.75 ± 0.18	1.59 ± 0.03	1.73 ± 0.00
	b	4.10 ± 0.45	2.25 ± 0.27^a	2.28 ± 0.03^a	1.72 ± 0.62^a
	Total	6.79 ± 0.73	4.00 ± 0.19^a	3.87 ± 0.06^a	3.46 ± 0.57^a
70	*a*	3.04 ± 0.00	1.73 ± 0.02^b	1.67 ± 0.07^b	1.53 ± 0.09^b
	b	5.35 ± 0.00	2.66 ± 0.08^b	2.28 ± 0.03^b	1.88 ± 0.01^b
	Total	8.39 ± 0.00	4.39 ± 0.05^b	3.96 ± 0.37^b	3.42 ± 0.10^b
80	*a*	3.35 ± 0.00	2.18 ± 0.14^b	2.15 ± 0.03^b	1.65 ± 0.08^b
	b	5.82 ± 0.00	3.28 ± 0.35^b	3.17 ± 0.01^b	2.26 ± 0.05^b
	Total	9.17 ± 0.00	5.47 ± 0.50^b	5.33 ± 0.02^b	3.91 ± 0.07^b
90	*a*	3.00 ± 0.04	2.21 ± 0.12^b	2.05 ± 0.18^b	1.82 ± 0.21^b
	b	5.23 ± 0.21	3.62 ± 0.28^a	3.29 ± 0.39^a	2.26 ± 0.05^b
	Total	8.23 ± 0.24	5.83 ± 0.41^b	5.33 ± 0.57^a	4.09 ± 0.22^b

Level of significance: ^{a} $p > 0.01$; ^{b} $p > 0.001$.

treated plants and minimum in case of O_3 treated plants (Table II). The reductions in total chlorophyll content were 20.56 to 47.64% for O_3, 26.30 to 52.80% for SO_2, and 33.17 to 59.28% for O_3 + SO_2-treated plants. The reductions in the contents of chlorophyll *a* relative to control were maximum in O_3 + SO_2-treated leaves, i.e., 17.17 to 50.79% followed by SO_2-treated leaves by 21.32 to 44.99% and O_3 treated leaves by

16.07 to 43.18%. The reductions were directly proportional to the cumulative pollution dose.

The contents of chlorophyll *b* also decreased. With respect to control, reductions in chlorophyll *b* contents were 23.50 to 50.18% for O_3, 29.66 to 57.26 for SO_2, and 38.17 to 64.77% for O_3 + SO_2 exposed plants. However, at any given age the reduction of chlorophyll *b* was more than chlorophyll *a*.

Carotenoid content also decreased in treated leaves. The reductions were 17.88 to 29.68% for O_3, 20.26 to 36.01% for SO_2, and 21.91 to 49.96% for O_3 + SO_2-treated leaves (Figure 1).

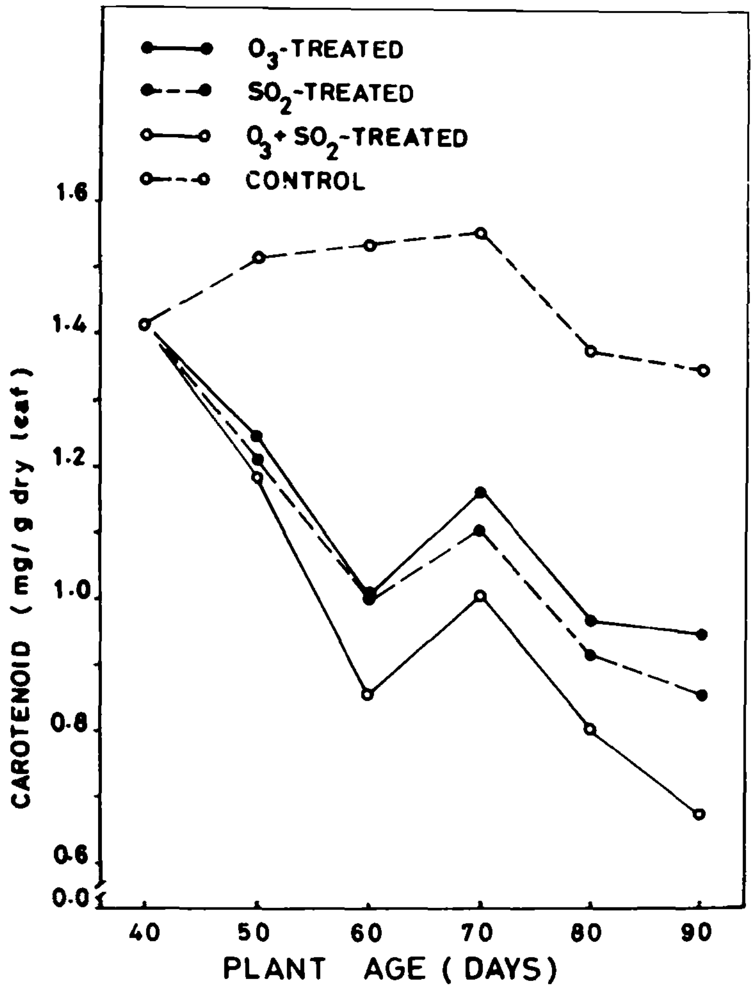

Fig. 1. Changes in carotenoid contents of control, O_3-, SO_2-, and O_3 + SO_2-treated rice plants at different stages of their growth.

4. Discussion

The decrease in chlorophyll content associated with the development of injury symptoms in leaves may inhibit photosynthesis in O_3 and SO_2 treated plants (Miller *et al.*, 1969; Tanaka *et al.*, 1974; Sij and Swanson, 1974; Koziol and Jordon, 1978). More reduction in chlorophyll *b* in O_3-treated leaves is in good agreement with the finding of Nobel (1974). It has been suggested that O_3 by itself affects chlorophyll molecules or it impairs synthesis of new molecules (Knudson *et al.*, 1977; Adedipe *et al.*, 1973). It can also affect both cellular and chloroplast structure and levels of chlorophyll in it (Beckerson and Hofstra, 1979). It has been seen that SO_2 interacts with chlorophyll forming phaeophytin (Rao and LeBlanc, 1966; Malhotra, 1977), which occurs mostly at high concentration of SO_2. So at low SO_2 concentration, one may expect the inhibition of photosynthesis and not phaeophytin formation (Nieboer *et al.*, 1976; Malhotra, 1977). The decrease in chlorophyll induced by $O_3 + SO_2$ mixture followed a pattern different from that noted in case of either SO_2 or O_3. In the former case the loss of chlorophyll is rather quick and possibly accompanied with inhibition of the process of chlorophyll synthesis and not through phaeophytin formation (Beckerson and Hofstra, 1979).

The decrease in carotenoid content in pollutant treated leaves may be ascribed to impaired synthesis of pigment. It has been observed that pollutants inactivate carotenoids (Czuchajowska and Przybylski, 1978). Ozone or SO_2 alone may cause the carotene contents to decrease (Chang and Heggestad, 1974; Bauer and Grill, 1977). Coulson and Heath (1974) have reported O_3 – induced inhibition of photosystem I of photosynthesis in plants. As carotenoids are the constituents of light harvesting pigments of photosystem I, it is likely that a decrease in carotenoid content has direct bearing on photosynthesis. In the present study, maximum carotenoid reduction was noted in case of $O_3 + SO_2$ treated plants, which showed their synergistic mode of action.

Therefore, it may be concluded that plants exposed to O_3 and SO_2 both singly and in combination adversely affect the photosynthetic efficiency of *Oryza sativa*. The greater effect of $O_3 + SO_2$ on pigment concentration suggests their synergistic mode of action. And thus the pigment reductions and foliar injury may lead reduce photosynthate production leading to reduced growth and productivity of *Oryza sativa*.

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