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EFFECT OF SHORT-TERM EXPOSURE OF TWO DIFFERENT CONCENTRATIONS OF SULPHUR DIOXIDE AND NITROGEN DIOXIDE MIXTURE ON SOME BIOCHEMICAL PARAMETER OF SOYBEAN (*GLYCINE MAX* (L.) MERR.)

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Abstract

The effect of elevated level of SO_2+NO_2 on some physiological and biochemical parameters of *Glycine max* were examined. Twelve days olds Soybean (*Glycine max*) seedling were exposed to two different (2% and 3%) levels of SO2+NO2 mixture. Fumigants were applied for 30 minutes per day, 3 days per week for 3 weeks duration. Aeration with enriched SO_2+NO_2 air resulted in higher phenolic content as compared to plants grown in control condition. However, reduction in leaf total carbohydrate, total protein and total chlorophyll were found in plants grown under elevated levels and the effect were more pronounced at 3% SO_2+NO_2 exposure as compared to 2% concentration.

Introduction

The presence of unaccepted level of foreign gaseous and particulate matters in the atmosphere is referred to as air pollution (Odigure, 1999). Air pollution is considered to be primarily an urban problem in Pakistan, as the rate of urbanization increases, air pollution levels are expected to increase significantly (Qadir, 2002). Air pollution is generally caused by automobiles, aircraft, industrial plants, power generation systems, construction projects and solid wastes (Javed *et al*., 2009). These sources add pollutant like sulfur dioxide, nitrogen oxides and dust, ash, soot, metals and various other chemicals (Javed *et al*., 2009).

Sulphur dioxide (SO_2) and nitrogen oxides (NO_2) are the most phytotoxic pollutants; these polluting gases enter leaves through stomata, following the same diffusion pathway as CO_2 (Zeiger, 2006). Sulfur dioxide occupies leading position as an air pollutant due to its potential hazard for vegetation as well as due to its wide distribution over the world has been reported to induce visible injury to leaves and leads to reduction in photosynthetic pigments inhibition of metabolic processes and suppression of growth and yield of plants of natural and agricultural ecosystems (Agrawal & Agrawal, 1991). In the cells, SO_2 dissolves to give bisulfite and sulfite ion Sulfite is toxic and at low concentrations it is metabolized by chloroplasts to sulfate, which is non toxic and at sufficiently low concentrations, bisulfite and sulfite are effectively detoxified by plants and SO_2 air pollution then provides a sulfur source for the plant (Zeiger, 2006).

Nitrogen-containing air pollutants (NO, NO_2 and NH_3) can affect vegetation indirectly, via chemical reactions in the atmosphere, or directly after being deposited on vegetation, soil or water (Anon., 2000). Nitrogen oxides result in growth stimulation at low concentration and growth reduction at higher concentration (Anon., 2000). NO_2 dissolves in cells and gives rise to nitrite ions (NO_2–, which are toxic at high concentrations) and nitrate ions (NO_3–) that enter into nitrogen metabolism as if they had been absorbed through the roots (Zeiger, 2006). Caporn & Mansfield (1976) reported that high concentration of NO and NO_2 (> 200 μg/m^3), show inhibition of photosynthesis.

Studies were conducted to determine whether combinations of SO_2+ NO_2 would elicit a synergistic effect on physiological and biochemical parameter (total carbohydrate, total chlorophyll, total protein and phenolic content) of *Glycine max*.

Material and Method

Experiments were conducted on Soybean (*Glycine max*). The seeds were obtained from local Market. Healthy seeds of *Glycine max* were selected and sterilized with 0.1% Mercuric chloride solution for 5 minutes followed by rinsing with tap and distilled water. Seeds were sown in 8cm diameter plastic pots containing 300gm of sterilized soil. Twelve days old seedlings of G*lycine max* were exposed to 2% and 3% levels of SO_2+NO_2 mixture in controlled environment chamber. Air was drawn into chamber by cylinder which were obtained from "The National Gas Limited Pakistan", enriched with SO_2 and NO_2, and blown through the chamber. Air enters near the bottom and flows out the open top. They provide SO_2 and NO_2 control at a fraction of the cost of free air SO_2 and NO_2 enrichment. Plants were fumigated with SO_2+NO_2 mixture for 30 minutes per days, 3 days per week for 3 weeks duration under artificial light condition. Non-treated plants served as control. There were there replicates for each treatment. Plants were watered according to requirement throughout experimental period.

The leaf samples from both control and treated plants were collected in early hours of morning and were kept in labeled sample bags. The plants samples were analyzed for following biochemical parameters.

Estimation of chlorophyll content: Chlorophyll were extracted from the leaves and estimated by the method of Maclachlam & Zalik (1963). For extraction 0.5g leaf samples were ground in 10ml of 80% (v/v) acetone and centrifuged at 1000rpm for 10 minutes to clear the suspension supernatant, which contained soluble pigment and was used for determination of chlorophyll. Absorbance of the extract was recorded at 663 and 645nm on spectrophotometer against 80% (v/v) acetone blank. The chlorophyll content was calculated using the formula given below and expressed in mg/g fresh weight.

$$\text{Chlorophyll "a" mg/g} = \frac{12.3D_{663} - 0.861D_{645}}{d \times 1000 \times w} \times V$$

$$\text{Chlorophyll "b" mg/g} = \frac{19.3D_{645} - 3.6D_{663}}{d \times 1000 \times w} \times V$$

where: D_{663} = absorbance at 663nm, D_{645} = absorbance at 663nm, W = fresh weigh of plant taken, d = length of light path in cm, V = volume of acetone.

Protein estimation: Estimation of Protein was done in plant extracts by the method of Lowry *et al*., (1951). For protein assays, 0.5g leaves were ground in 10ml of distilled water. The homogenates were centrifuged for 10 minutes at 3000rpm and the resulting supernatants were used for determination of protein content assays. To 0.2ml of plant extract, 3ml alkaline copper sulfate reagent was added. After 10 minutes 0.5 ml of Folin's reagent was added. The solution was mixed instantaneously and allowed to develop the color absorbance which was recorded at 750 nm. The total protein content was calculated from a standard curve of Bovine serum albumin and expressed as µg/ mg of leaf fresh weight.

Estimation of total phenolic compound: Total phenolic compounds were determined by the Folin-ciocalteu method as modified by Swain & Hillis (1959). A sample of 1gm leaves grinded in a mortar and pestle with 10ml of 1N HCl. The mixture was covered and incubated in boiling water bath for about half an hour. The extract was centrifuged at 1000 rpm for about 5 minutes. The supernatant was then dried on hot plate for 1.5 hour at medium heat (45°C). The resulted residue was dissolved in 1 ml of 80% ethanol (v/v).For the assay, 5 ml of distilled water, 0.2 ml of Folin-ciocalteu reagent1 /10 and 3 ml of Sodium carbonate (Na_2CO_3) 7.5% (w/v) was added to 0.5 ml of leaf extract. The mixture was then incubated at 35°C in a shaking water bath for 30 minutes. After cooling, the absorbance was measured at 660 nm. The total content of phenolic compounds was calculated from a standard curve of Gallic acid and expressed as µg/ mg of leaf fresh weight.

Estimation of carbohydrate content: Carbohydrate content was measured according to the method of Yemm & Willis (1954) using anthrone reagent. Leaves 1.0 gm was homogenized in 10 ml of distilled water and centrifuged at 500 rpm for 5 minutes. The supernatant was used for estimation of total carbohydrate content. The reaction mixture consisted of 0.5 ml of supernatant and 5 ml of anthrone reagent which was boiled at 100°C for 30 minutes. Absorbance was determined at 620nm. The carbohydrate content is expressed as µg/mg fresh weight.

Data were statistically analyzed by "SPSS" and "SIGMA PLOT" program was used for graphic presentation of the data.

Results

Carbohydrate content: The result obtained for the effect of different concentration of SO_2 + NO_2 mixture on total carbohydrate content of *Glycine max* leaves are shown in Fig. 1. Significant (**$p<0.01$) decrease in total carbohydrate content was observed in treated samples as compared to control samples throughout experimental period.

Chlorophyll content: Different concentrations of SO_2 + NO_2 mixture showed Significant (*$p<0.05$) changes in the chlorophyll content of *Glycine max* leaves (Fig. 2). In all treatment decrease in chlorophyll content was observed as compared to control.

Protein content: Decrease in total protein content was observed in leaf of *Glycine max* at all treatment as compared to control (Fig. 3) and the result obtained was significant (***$p<0.001$). In all treatment decrease in total protein content continued till the end of the experimental period.

Total Phenolic compounds: Different concentration of SO_2 + NO_2 mixture adversely affected the phenolic content. The phenolic content of *Glycine max* showed a sharp increase with the increase in the concentration of gases (SO_2 + NO_2) mixture. The increase was significantly high (*$p<0.05$) over the control and this increase continued till the end of experiment (Fig. 4).

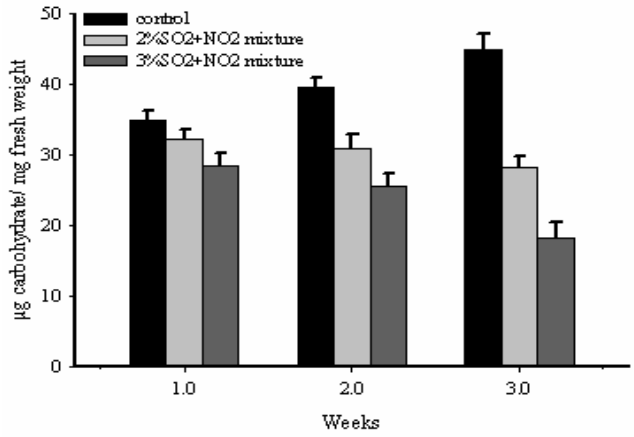

Fig. 1. Changes in the total carbohydrates of *Glycine max* after fumigation with different concentration of mixture of SO2+NO2.

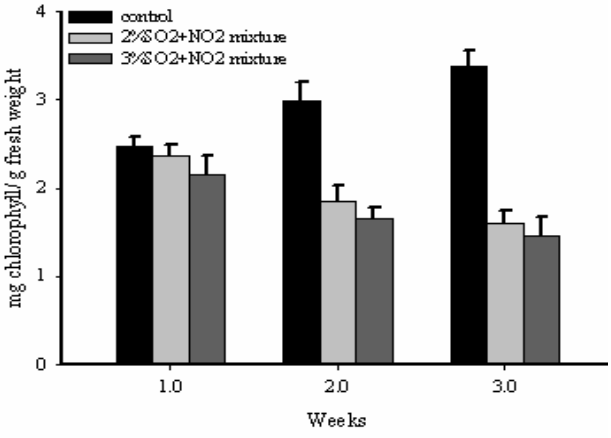

Fig. 2. Changes in the total carbohydrates of *Glycine max* after fumigation with different concentration of mixture of SO2+NO2.

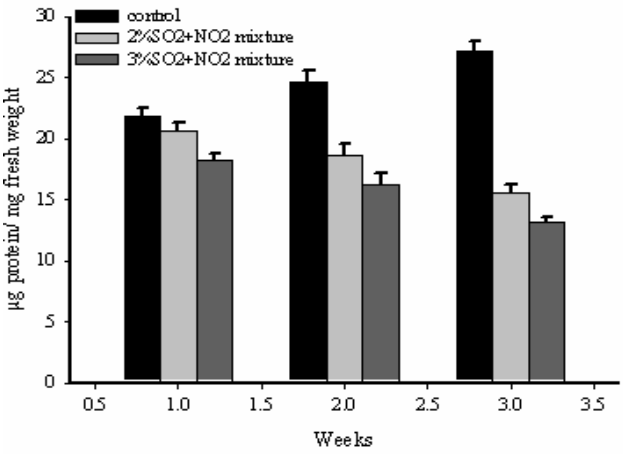

Fig. 3. Changes in the total protein of *Glycine max* after fumigation with different concentration of mixture of SO2+NO2.

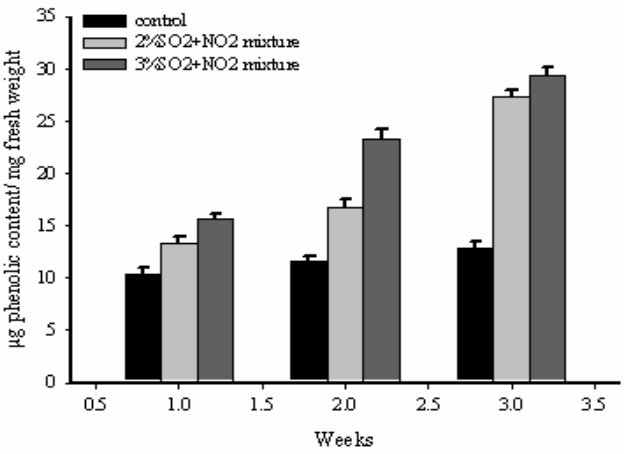

Fig. 4. Changes in the total phenolic content of *Glycine max* after fumigation with different concentration of mixture of SO2+NO2.

Discussion

SO_2 and NO_2 are commonly occurring gaseous air contaminants which arise from urban and industrial activities (Elkiey & Ormord, 1981). The concentrations of soluble carbohydrates were greatly reduced in leaf of *Glycine max* treated with different concentration of mixture of SO_2+ NO_2 gases. The decrease in total sugar content of damaged leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate (Tzvetkova & Kolarov, 1996). Likewise, Bucker & Ballach (1992) found the level of soluble carbohydrates decreased due to fumigation with mixture of O_3, SO_2 and NO_2 both in young and mature leaves, this decrease in soluble sugars may be a consequence of increased metabolic consumption of energy under stress conditions.

Chlorophyll content could be a useful indicator for the evaluation of injury induced by pollutants (Knudson *et al*., 1977). Therefore, the variation in chlorophyll content has been used in many studies in order to investigate the effects of pollutants on plants. In the present investigation, SO_2 + NO_2 fumigation reduces the total chlorophyll content in leaves of soybean plants. The decrease in chlorophyll content is associated with the development of injury symptoms in leaves. Agrawal *et al*., (1982) reported that exposure of rice plants to low concentration of SO_2 showed foliar injury at different levels.

Similarly, Chun-Yan *et al*., (2007) explained that exposure to moderate dose of NO_2 had a favorable effect on plants, whereas the exposure to high NO_2 concentration caused a reduction in total chlorophyll content. Knudson *et al*., (1977) suggested that air pollution affects chlorophyll molecules by impairing the synthesis of new molecules. Likewise, Beckerson *et al*., (1979) elucidate that SO_2 affect both cellular and chloroplast structure and levels of chlorophyll in it.

Decrease in the total protein content is evident in leaves of soybean after fumigation with elevated levels of SO_2+ NO_2 mixture. Yamazoe & Mayumi (1977) observed the leaf damage and decrease in the protein in *Zea mays* after NO_2 fumigation. Ito *et al*., (1984) after NO_2 fumigation observed change in amino acid composition of *Vicia faba*. In another report decline in total protein content after fumigation with sulphur dioxide has been reported for a number of plants (Godzik & Linskens, 1974). Agrawal & Deepak (2003) determined that SO_2 enrichment results in diminish leaf protein levels by 13%. Zeiger (1975) suggested that such decrease could be attributed to break down of existing protein and reduction in synthesis.

In contrast to effects on the primary metabolic pathways, exposure of high concentration of SO_2 + NO_2 mixture can also alter the secondary metabolism. In the present study SO2+NO2 fumigation affect the phenolic content in leaves of soybean (*Glycine max*). Phenolic content of two cultivars of wheat (*Triticum aestivum*) has shown an increase in response to SO2 fumigation (Agrawal & Deepak, 2003). Pasqualini *et al*., (2003) reported an increase in total phenol content with exposure to sulphur dioxide and a reduction with exposure to nitrogen oxide pollution, they also found an increase in *p*-Coumaric acid, syringic acid and 4-hydroxybenzoic acid concentrations with exposure to nitrogen oxide pollution, whereas Gallic acid and vanillin was decreased in the presence of sulphur dioxide and ozone.

Increased phenolic content in plants is related to impaired growth and accelerated leaf senescence which is indicated by enhanced autumn leaf yellowing and lower chlorophyll and Mg content. The change in carbon allocation towards defensive phenolics at the expense of growth was greater in the sensitive species as compared to tolerant species (Ammar *et al*., 2001).

Conclusion

Exposure of the different concentration of SO_2 + NO_2 mixture on *Glycine max* show adverse affects on physiological, biochemical functions and intermediary metabolism.

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