Morphological and Biochemical Changes In The Leaves of Cajanus cajan and Amaranthus paniculatus Under Foliar Application of Aqueous Sulphur Dioxide

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ABSTRACT: The impact of elevated aqueous SO₂ (0, 10, 20, 30, 40, 50, 100 and 250 ppm) on morphological and biochemical changes in the leaves of pigeonpea (Cajanus cajan (L.) Millsp. cv. PDM1), a C₄ plant and amaranth (Amaranthus paniculatus L. a local cultivar), a C₄ plant leaf discs under light and dark conditions has been studied. The visible leaf injury symptoms of SO₂ such as chlorosis and necrotic lesions developed on the leaves of both pigeonpea and amaranth in response to foliar application of higher concentrations of SO₂ such as 50, 100 and 250 ppm. However, leaf curl symptom appears only in amaranth indicating its sensitivity to SO₂ than pigeonpea. The per cent injury index of SO₂ was associated more with amaranth than with pigeonpea. The acceleration of SO₂ induced leaf senescence was expressed more in amaranth. The degree of membrane damage appears to be more in amaranth than in pigeonpea as revealed by leachate analysis. The nitrate reductase activity increased initially at the lower aqueous SO₂ concentrations followed by a decrease at the higher SO₂ concentrations. The photosynthetic parameters such as Hill reaction activity as affected by aqueous SO₂ indicated the relative sensitivity of amaranth, a C₄ plant to SO₂ compared to pigeonpea, a C₃ plant.

Keywords: Amaranth, aqueous sulphur dioxide, Hill reaction activity, nitrate reductase activity, pigeonpea, per cent injury index.

I. INTRODUCTION

Sulphur dioxide is a common air pollutant of industrial activity. The combustion of fossil fuels such as coal, petroleum and burning of sulphur ores liberate SO₂. Sulphur dioxide enters plants mainly through the stomata of leaves. Within the leaf tissue, SO₂ is converted into products of reactivity which include HSO₃⁻, SO₃²⁻ and SO₄²⁻ that affect plant metabolism leading to plant damage. Sulphur dioxide affects plant composition, structure and morphology manifesting in decreased growth and productivity. In spite of its wide spread occurrence and deleterious effects, the mechanism of SO₂ phytotoxicity is not clear and needs further investigation.

Nitrate reductase is a substrate inducible enzyme and is influenced by environmental factors such as light, water, nitrogen status, temperature and pollution (Beever and Hageman, 1969; Sinha and Nicholus, 1981; Reed and Canvin, 1982; Chopra, 1983; Prakash et al., 1989; Riens and Heldt, 1992; Huber et al., 1992; Qifu and Murray, 1993). Nitrate reductase is prominently present in the leaves and is associated with the cytoplasm of leaf cells (Hewitt et al., 1976; Beever and Hagemann, 1980). The C₄ plants have been reported to use nitrate more efficiently than the C₃ plants (Moore and Black, 1979). It was also found that the two enzymes nitrate and nitrite reductases are present only in mesophyll cells of leaves (Moore and Black, 1979). The greater efficiency of C₄ plants to use nitrate when compared to C₃ plants is due to the spatial separation of nitrate and nitrite reduction from the site of CO₂ reduction via calvin cycle in bundle sheath cells. The nitrate and nitrite reductases in mesophyll cells act as nitrogen reduction traps (Moore and Black, 1979; Salisbury and Ross, 1986).

Sulphur dioxide also affects the activity of chloroplasts. Chloroplasts isolated from needles of lodge pole pine when treated with low concentrations of aqueous SO₂ (10-50 ppm) stimulated the Hill reaction activity. However, high concentrations of aqueous SO₂ (500-1000 ppm) inhibited the activity. The decrease in Hill reaction activity was accompanied by swelling and disintegration of thylakoid membranes (Malhotra, 1976). From the studies of how plants respond to severe stresses, we learn more about metabolism, its flexibility, its limits, and its diversity (Bohnert et al., 1995). Though certain direct effects of SO₂ on plant growth and metabolism are available, its differential effects on different plant species and its impact on plant cellular structures and related physiological processes and biochemical events still needs further investigation.

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Therefore, in the present investigation, to understand the negative effects of SO$_2$ pollution on morphological and biochemical changes of different plant species of pigeonpea and amaranth.

II. MATERIALS AND METHODS

Preparation of aqueous sulphur dioxide

Sulphur dioxide was prepared in the laboratory by reacting sodium metabisulphite with concentrated H$_2$SO$_4$ and the generated gas was collected into distilled water. Aqueous SO$_2$ concentration was determined titrimetrically according to the method of Vogel (1961). Fresh stock solution of 1000 ppm concentration was prepared and from it the various concentrations of SO$_2$ were prepared by diluting with distilled water. The pH was adjusted to 6.9 by adding dilute NaOH. It was reported that 1 ppm SO$_2$ in air gives 1000 ppm in aqueous solution (Puckett et al., 1973; Saunders and Wood, 1973; Malhotra, 1977).

Plant material

Seeds of pigeonpea (Cajanus cajan (L.) Millsp. cv. PDM1), a C$_3$ plant is an important pulse crop and amaranth (Amaranthus paniculatus L. a local cultivar), a C$_4$ plant is popular green leafy vegetable consumed all over India were selected for present study.

Effect of aqueous SO$_2$ incubation of leaf discs under light and dark conditions

Seeds were washed with distilled water and surface sterilized with 0.01 M mercuric chloride and were raised in earthen pots filled with soil containing farm yard manure and soil in the ratio of 1:3. The plants were watered on alternate days. The plants were grown under a natural photoperiod of approximately 12 h and average day temperatures of 31 ± 2 ºC and 21 ± 1 ºC at night at Andhra university experimental farm. The aqueous SO$_2$ at concentrations of 0, 10, 20, 30, 40, 50, 100 and 250 ppm was supplied as foliar spray at 8.00 a.m on every third day starting from five days after germination and continued up to one month. The zero SO$_2$ concentration treatment was called as control. The data were collected at weekly intervals starting from the day of foliar spray. Fully expanded third leaves from top of 1-month old pigeonpea and amaranth plants grown separately in earthenware pots for this purpose were harvested from 20 plants at 8.00 a.m. Discs of 1.0 cm diameter were cut from the leaves and floated with abaxial surface downwards in petri dishes containing 0, 10, 20, 30, 40, 50, 100 and 250 ppm aqueous SO$_2$. The petri dishes were covered with glass lids and sealed with siliccan grease. Some sets of leaf discs were exposed to light of 195 µ mol m$^{-2}$ s$^{-1}$ and other sets of leaf discs were wrapped in aluminum foil to obtain dark conditions. All the leaf discs were exposed to a temperature of 30 ± 2 ºC. The leaf discs were allowed to incubate 24 h in light and dark conditions. The leaf discs exposed to zero SO$_2$ concentration were termed as controls. The leaf disc samples were collected at 6, 12, 18 and 24 h of incubation, washed twice with distilled water to remove traces of aqueous SO$_2$ and used for analysis.

Leaf injury index

The per cent necrosis was calculated with reference to total leaf area. The necrotic areas of the affected leaves were determined by tracing the necrotic zones on a transparent paper and superimposing the tracings on a mm graph paper. The percentage of leaf area which was necrotic on damaged leaves (A) was assessed in each plant as was the percentage of injured leaves in the total leaf sample (L). The following injury index formula was then applied (Murray and Wilson, 1988a).

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I = (AL)^{1/2}
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Membrane permeability

For studies on the effect of SO$_2$ on membrane permeability the reducing sugars and amino acids were determined in the leachates form the leaf discs. The reducing sugars were estimated according to the phenol sulphuric acid method of Dubois et al. (1956) as followed by Smyth and Dugger (1980) and amino acids were determined according to the method of Moore and Stein (1948).

Nitratreductase (in vivo) activity (E.C.1.6.6.1)

Nitratreductase (in vivo) activity of control and treated leaf disc samples were estimated according to the method of Jaworski (1971) as modified by Dykstra (1974). Five hundred mg of material was chopped into pieces and placed in a 5 ml of incubation medium consisting of 0.1 M K$_2$HPO$_4$, 0.2 M KNO$_3$, 0.5% w/v PVP (polyvinyl pyrrolidone) and 5% (v/v) isopropanol having pH of 7.5. The material along with incubation medium was kept in dark for 2 h at room temperature. Then the reaction was stopped by adding 1 ml of 0.02% (w/v) N, 1-naphthylethenediamine-2HCl and 1 ml of 1.0% (w/v) sulphanilic acid in 1.5 N HCl. After 20 min the absorbance of the solution was read at 540 nm on Milton Roy Spectronic 1201 UV spectrophotometer. The nitrate reductase activity was expressed as µ moles of nitrate formed per g tissue per hour. The standard curve was prepared by using analar NaNO$_2$. 

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Hill reaction activity

Hill reaction activity of the isolated chloroplasts was determined according to the method of Trebst (1972). Five grams of washed and surface moisture blotted leaf discs were cut into small pieces. These pieces were ground in a chilled mortar with about 60 ml of chilled 0.4 M sucrose, 0.05 M KH₂PO₄-Na₂HPO₄ buffer (pH 7.2) and 0.01 M KCl. The homogenate was filtered through eight layered cheese cloth, and centrifuged at about 200 x g for 2 min. The supernatant was discarded and the pellet (chloroplast) was resuspended in about 10 ml of the buffer solution. All the preparations were carried out at 0 °C. The reduction of 2,6-DCIP was measured by adding 5 ml of the chloroplast suspension and 0.5 ml of 5x10⁻⁴ M DCIP (0.145 mg DCIP/ml water) enough of buffer solution was used to bring the final volume to 10 ml. The test tubes containing the above reaction mixture were exposed to 1000 watt bulb at a distance of 15 cm. To serve as the dark control, one tube was covered with aluminum foil to avoid light. All the tubes were put in a large beaker of water to maintain the desired temperature (usually about 20 °C.). The disappearance of the blue colour is measured as a change in absorbance at 600 nm as a consequence of the reduction of DCIP by the electrons that are obtained from the oxidation of water by the operation of photosystem II of photosynthesis was determined using 150-20 UV-VIS spectrophotometer (Hitachi, Japan). The concentration of chlorophyll was determined by the method of Arnon (1949). The Hill reaction activity of chloroplasts was expressed as micromoles of DCIP reduced per milligram chlorophyll per hour.

III. RESULTS

Leaf symptoms of SO₂ injury

In response to increasing concentrations of SO₂, pigeonpea and amaranth exhibited the visible symptoms of tip burn, interveinal and marginal necrosis and chlorosis. The intensity of leaf injury symptoms increased with increasing SO₂ concentration and leaf age in both the pigeonpea and amaranth. The leaf symptoms of SO₂ toxicity were more conspicuous at 100 and 250 ppm SO₂ in amaranth plants at the end of the experimental period. The mature leaves exhibited more injury than the young and immature leaves in both the plant species (Plate-1 a,b and 2 a,b). Sulphur dioxide induced early leaf drop at higher concentrations in both the pigeonpea and amaranth plants. The leaf drop of mature leaves of pigeonpea was started from the third week in 100 and 250 ppm SO₂ treated plants (Plate-3 and 4 a,b). However, the leaf drop of amaranth initiated from the second week, at the concentrations of 20, 30, 40, 50, 100 and 250 ppm aqueous SO₂ (Plate- 5 b and 6 a,b).

Plate-1: The effect of foliar application of 250 ppm aqueous SO₂ on the leaf morphology of 4-week old pigeonpea. a - Leaves showing chlorotic and small necrotic lesions with different patterns. b - A single leaf showing vein clearing.

Plate-2: The effect of foliar application of 250 ppm aqueous SO₂ on the leaf morphology of 4-week old amaranth. a - Leaves showing tip burn, chlorosis, large necrotic lesions and leaf curl. b- Twig showing more injury symptoms on the mature leaves than on immature leaves.
Figure-1: The effect of foliar application of aqueous SO$_2$ on per cent injury index of pigeonpea (a) and amaranth (b) leaves (vertical lines represent S.E).
- 100 ppm; ■ - 250 ppm; □ - 50 ppm; ■ ■ - 100 ppm; ■ ■ ■ - 250 ppm.

The leaf injury in response to SO$_2$ was also measured as per cent injury index in both the pigeonpea and amaranth. A continuous increase of the per cent leaf injury index was noted in higher SO$_2$ concentrations with increasing age (Fig. 1a,b). In pigeonpea the per cent injury of leaves increased from 1.76 to 25.15 in 100 ppm and 10.27 to 40.44 in 250 ppm aqueous SO$_2$ from the first week to the fourth week of plant growth. In amaranth on the other hand the injury was noted in the first week of 250 ppm SO$_2$ treated plants only. However, the injury was also conspicuous in 50 and 100 ppm aqueous SO$_2$ treatments with age. The per cent injury index increased gradually with age. The per cent injury of amaranth increased from 2.86 to 5.68 in 50 ppm, 4.87 to 30.86 in 100 ppm and 25.34 to 50.08 in 250 ppm aqueous SO$_2$ from the second week to the fourth week of plant growth.

Plate-3: The effect of foliar application of aqueous SO$_2$ on the growth pattern of pigeonpea. a. 1 - week old plants; b. 2 - week old plants. A - 0 ppm; B - 10 ppm; C - 20 ppm; D - 30 ppm; E - 40 ppm; F - 50 ppm; G - 100 ppm; H - 250 ppm.
Plate-4: The effect of foliar application of aqueous SO$_2$ on the growth pattern of pigeonpea. a. 3 - week old plants; b. 4 - week old plants. A - 0 ppm; B - 10 ppm; C - 20 ppm; D - 30 ppm; E - 40 ppm; F - 50 ppm; G - 100 ppm; H - 250 ppm.

Plate-5: The effect of foliar application of aqueous SO$_2$ on the growth patterns of amaranth. a. 1 - week old plants; b. 2 - week old plants. A - 0 ppm; B - 10 ppm; C - 20 ppm; D - 30 ppm; E - 40 ppm; F - 50 ppm; G - 100 ppm; H - 250 ppm.

Plate-6: The effect of foliar application of aqueous SO$_2$ on the growth patterns of amaranth. a. 3 - week old plants; b. 4 - week old plants. A - 0 ppm; B - 10 ppm; C - 20 ppm; D - 30 ppm; E - 40 ppm; F - 50 ppm; G - 100 ppm; H - 250 ppm.
Membrane Permeability

The release of solute from the SO$_2$ treated leaf discs into the incubation medium is considered as a measure of loss of membrane stability. If more solute was leached into the incubation medium over the controls, it may be considered that the membrane stability is affected by the treatments. The leaching experiments exhibited an increased in total soluble sugar and total free amino acid leakage with increasing SO$_2$ concentration and duration of incubation. The leaching was not conspicuous at lower concentrations and at early periods of SO$_2$ exposure. Leaching increased with increasing concentration of SO$_2$. Further, with increasing duration of exposure, the leaching increased even under low concentrations of SO$_2$. In between pigeonpea and amaranth, the later seems to leak out more solutes into the incubation medium than the former (Fig. 2 a,b,c,d and 3 a,b,c,d).

![Graphs showing the effect of aqueous SO$_2$ on soluble sugar content of the leachates of pigeonpea and amaranth leaf discs (Vertical lines represent S.E.). a and b - Pigeonpea; c and d – Amaranth, ---- under light; — under dark ○- 0 ppm; □-10 ppm; ×-20 ppm; □-30 ppm; □-40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm]
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Figure-3: The effect of aqueous SO$_2$ on free amino acid content of the leachates of pigeonpea and amaranth leaf discs (Vertical lines represent S.E.). a and b - Pigeonpea; c and d – Amaranth. ---- under light; — under dark
○- 0 ppm; □-10 ppm; ×-20 ppm; □-30 ppm; □-40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm

Nitrate reductase activity

The leaf discs of controls of both pigeonpea and amaranth showed an increase in vivo nitrate reductase activity continuously from 6 to 24 h incubation both under light and dark conditions. Three fold increase was noticed in amaranth at 24 h where as it was 1.65 fold in pigeonpea at the same period. The nitrate reductase was more active in light rather than in dark. Sulphur dioxide incubation initially stimulated nitrate reductase activity in lower concentrations with increasing time but it decreased in higher concentrations. The greatest nitrate reductase activity was registered at 10 ppm SO$_2$ at 24 h in both the plants. Higher SO$_2$ concentrations conspicuously inhibited the enzyme activity over the controls at 24 h SO$_2$ treatment. More activity of nitrate reductase was noticed in amaranth in response to SO$_2$ (Fig. 4a,b,c,d).
The Hill reaction activity continuously declined in control leaf discs of both pigeonpea and amaranth form 6 to 24 h of incubation. The SO$_2$ treatment initially enhanced the activity at lower SO$_2$ concentrations up to 50 ppm in pigeonpea and 40 ppm in amaranth followed by a decline thereafter. An increase of 2.36 fold in pigeonpea over the respective controls was noticed at 50 ppm aqueous SO$_2$ concentration under light. In amaranth an increase of 1.12 fold over the respective control was noticed at 30 ppm aqueous SO$_2$ concentration under light. The Hill reaction activity was continuously declined in 100 and 250 ppm SO$_2$ treatments from 6 to 24 h of incubation in both the plant species. The maximum reduction of Hill reaction activity was observed in 250 ppm SO$_2$ treatment in both pigeonpea and amaranth. The Hill reaction activity was much affected in amaranth than in pigeonpea (Fig.5 a,b,c,d).
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IV. DISCUSSION

Exposure of pigeonpea and amaranth to higher concentrations of aqueous SO$_2$ such as 50, 100 and 250 ppm showed visible injury to leaves. The typical SO$_2$ injury symptoms of both the pigeonpea and amaranth include tip burn, browning the interveinal areas, chlorosis and necrotic lesions (Plates-1 and 2). These symptoms were more expressed in the leaves of amaranth than in pigeonpea. Interestingly in amaranth the chlorotic and necrotic lesions led to leaf curling which indicated its sensitiveness to SO$_2$ (Thomas and Hendricks, 1956). Further mature leaves were more effected than immature leaves (Plates-3 to 6 a,b). The symptom development in leaves in response to SO$_2$ exposure was a consequence of physiological, biochemical and anatomical alterations in leaves. Usually leaves are the first to be affected by SO$_2$ since they are the main organs of SO$_2$ absorption (Geburek and Scholz, 1992; Taylor and Bell, 1992; Tripathi and Tripathi, 1992; Khan and Khan, 1993, 1994).

The per cent injury index increased gradually with age in response to SO$_2$. The per cent injury index was more in amaranth than in pigeonpea. Further the threshold concentration of aqueous SO$_2$ to leaf injury also varied between the two species (Fig. 1 a,b). A possible explanation of these differences in relative tolerance to SO$_2$ injury between pigeonpea and amaranth may be due to the differences in their habit, leaf surface morphology and leaf anatomical variations in addition to metabolic differences, such as carbon metabolism associated with them. More pubescent leaf surface of pigeonpea when compared to amaranth may provide a sort of avoidance mechanism through the increase of aerodynamic resistance for the entry of pollutant into the leaf, which may also act as an extra surface sink for the pollutant to avoid its toxic effect at the first instance (Murray and Wilson, 1988a,c; 1991; Taylor and Bell, 1992; Muthuchelian et al., 1993).

The increased solute leakage is generally considered as an index of membrane damage or deterioration (Simon, 1974; Khan and Malhotra, 1977; Farooq and Beg, 1980). Aqueous SO$_2$ enhanced considerably the levels of soluble sugars and amino acids in the incubation medium in both pigeonpea and amaranth (Fig. 2 and 3 a,b,c,d). The release of soluble sugars and amino acids into the incubation medium increased with increasing SO$_2$ concentration and duration of exposure in both the plant species. The loss of soluble sugars and amino acids into the incubation medium was more in amaranth than in pigeonpea. Further dark exposed leaf discs exhibited more loss of soluble sugars and amino acids than the light exposed ones in both plants. The data suggest the loss
of semi permeable property of cell membranes in the leaf discs of pigeonpea and amaranth in response to SO₂. Further the leaching was more in amaranth, indicating its relative sensitivity to SO₂.

Nitratreductase is an important enzyme of nitrogen metabolism. It is substrate inducible and influenced by environmental factors such as light, nitrogen status and temperature (Beever and Hegeman, 1969; Chopra, 1983). The in vivo nitrate reductase activity increased initially at the lower concentrations and was followed by a decline at higher concentrations of SO₂ in the leaf discs of both the plant species. The nitrate reductase activity was considerably more in light than in dark exposed leaf discs (Fig. 4a,b,c,d). Surprisingly amaranth leaf discs registered more nitrate reductase activity than pigeonpea. De kok et al. (1986a) observed increased in vivo nitrate reductase activity up to five fold in sulphite treated spinach leaves under aerobic conditions. They suggested that the increased nitrate reductase activity was due to the inhibition of respiration resulting in the inhibition of NADH oxidizing enzymes in the presence of sulphide, which altered the competition for NADH in favour of nitrate reductase. Though SO₂ stimulated the nitrate reductase activity of wheat, initially at lower concentrations it however, inhibited the activity at higher concentration and increasing duration of exposure (Huang et al., 1993). In SO₂ exposed oak plants, nitrate reduction was involved in the neutralization of protons generated by SO₂ uptake and therefore needs increased activity of nitrate reductase (Thomas Runge, 1992; Peuke and Rudolf, 1994). Thus, it is presumed that the initial increase in the nitrate reductase activity of the leaf discs of pigeonpea and amaranth may be an adaptive response to neutralize the increased protons generated in response to SO₂. The differential responses of nitrate reductase activity in response to SO₂ concentration may be attributed to the availability of photosynthesates and nitrate. However, the detailed mechanism of SO₂ inhibited nitrate reductase activity of the leaves remains to be elucidated (Klumpp et al., 1989; Hung et al., 1993). Greater nitrate reductase activity in amaranth than in pigeonpea leaf discs may be attributed to their differences in nitrogen use efficiency. The C₂ plants exhibit a partial division of labour between mesophyll and bundle sheath cells. The NO³⁻ and NO²⁻ reductases of mesophyll acts as nitrogen reduction traps in an analogous fashion to phosphoenolpyruvate carboxylase acting as a CO₂ trap of C₄ photosynthesis (Moore and Black, 1979). The decreased nitrate reductase activity in the dark may be to prevent the accumulation of toxic nitrite in the cells (Riens and Hans, 1992).

Aqueous SO₂ affects the structural organization of chloroplasts and thus effects all the reactions associated with photosynthesis (Silvius et al., 1975; Malhotra, 1976). An initial rise of Hill reaction activity at lower concentrations followed by a continuous decline with increasing concentration and duration of SO₂ exposure was registered in both the plant species (Fig. 5 a,b,c,d). The decline of Hill reaction activity was more in amaranth than in pigeonpea. Further the Hill reaction activity was more under light than under dark exposed leaf discs (Fig. 5 a,c). The stimulatory effect of lower concentrations of SO₂ on Hill reaction activity was in agreement with the studies of Malhotra (1976) and Veeranjaneyula et al., (1991). Based on ultrastructural and biochemical activities of chloroplasts of Pinus contorta, Malhotra (1976) suggested, a good correlation between disorganization of chloroplast membranes and the declining activity of Hill reaction of SO₂. The involvement of SO₂ derived anions sulphite and sulphate in inhibiting light reactions in SO₂-exposed leaves was well illustrated by Beauregard (1991, 1992). The SO₂-derived anions, sulphite and sulphate directly effect the chlorophyll-protein complex responsible for water cleavage and reduction of electron carriers in the photosynthetic membrane systems. Therefore SO₂ directly effects the Hill reaction activity by inhibiting the oxygen evolving complex. The differential response of Hill reaction activity to SO₂ in the leaf discs exposed to light and dark conditions was related to the pH levels in chloroplast. Thus the pH of the intrathylakoid space and its control by light play an important role in SO₂-derived anion toxicity. Hence it is presumed that when chloroplasts are in darkness, the pH equilibrates to a nearly neutral value in all compartments and the oxygen evolving protein complex is more liable to SO₂ injury. Thus SO₂-derived anions inhibit the Hill reaction instantly. On the other hand, under steady state illumination, the pH in the thylakoid space is acidic and the oxygen evolving complex proteins are more stable and the intensity of the inhibition of Hill reaction activity of SO₂ was relatively low (Beauregard, 1991). Furthermore, the generation of free radicals in the leaf cells to SO₂ may also affect thylakoid membranes mostly through lipid peroxidation. This may also contribute a decrease in Hill reaction activity in both the plant species (Libera et al., 1975; Peiser et al., 1982; Covello et al., 1989).

V. CONCLUSIONS
The per cent injury index of foliage increased with increasing aqueous SO₂ concentration and age. However, the SO₂ concentrations, which induced injury differed in pigeonpea and amaranth. SO₂-induced injury symptoms were appeared on the foliage of both pigeonpea and amaranth but the symptoms including tip burn, intraveinal bronzing, leaf curl and necrosis were more pronounced in amaranth leaves indicating the relative sensitiveness of amaranth to SO₂ compared to pigeonpea. Increased leaching of soluble sugars and amino acids
probably due to the damage of biomembrane intactness by SO₂. The nitrate reductase activity exhibited an increase at the lower and a decrease at the higher concentrations of SO₂ in both the plant species. Interestingly the nitrate reductase activity was more in amaranth than in pigeonpea. The nitrate reductase activity was more conspicuous in light than in dark. Hill reaction activity was affected by aqueous SO₂ exposure of both the plant species. The Hill reaction activity recorded higher values in lower concentrations and lower values at higher concentrations of SO₂. The Hill reaction activity was always registered lower values under dark conditions.

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