International Journal of Engineering Inventions
e-ISSN: 2278-7461, p-ISSN: 2319-6491
Volume 5, Issue 8 (Sep 2016 PP: 36-47)

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

B. Priyadarshini, B. Sujatha*, Ch. Umamahesh, M.V.V.P. Kumar, L.B. Divya Jyothi, A. Satya Gowri Parvathi and J. Saraswathi
Department of Botany,Andhra University,Visakhapatnam-530003, A.P., India.
**Corresponding author*

ABSTRACT:- The impact of elevated aqueous SO_2 (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 C_3 plant and amaranth (*Amaranthus paniculatus* L. a local cultivar), a C_4 plant leaf discs under light and dark conditions has been studied. The visible leaf injury symptoms of SO_2 such as chlorosis and necrotic lesions developed on the leaves of both pigeonpea and amaranth in response to foliar application of higher concentrations of SO_2 such as 50, 100 and 250 ppm. However, leaf curl symptom appears only in amaranth indicating its sensitiveness to SO_2 than pigeonpea. The per cent injury index of SO_2 was associated more with amaranth than with pigeonpea. The acceleration of SO_2 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 SO_2 concentrations followed by a decrease at the higher SO_2 concentrations. The photosynthetic parameters such as Hill reaction activity as affected by aqueous SO_2 indicated the relative sensitivity of amaranth, a C_4 plant to SO_2 compared to pigeonpea, a C_3 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 SO_2. Sulphur dioxide enters plants mainly through the stomata of leaves. Within the leaf tissue, SO_2 is converted into products of reactivity which include HSO_3^-, SO_3^{2-} and SO_4^{2-} 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 SO_2 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 (Beevers 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; Beevers and Hagemann, 1980). The C_4 plants have been reported to use nitrate more efficiently than the C_3 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 C_4 plants to use nitrate when compared to C_3 plants is due to the spatial separation of nitrate and nitrite reduction from the site of CO_2 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 SO_2 (10-50 ppm) stimulated the Hill reaction activity. However, high concentrations of aqueous SO_2 (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 SO_2 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.

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_2SO_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 silican 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).

$$I = (AL)^{1/2}$$

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).

Nitrate reductase (*in vivo*) activity (E.C.1.6.6.1)

Nitrate reductase (*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_2HPO_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-napthylethylenediamine-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 nitrite formed per g tissue per hour. The standard curve was prepared by using analar $NaNO_2$.

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 KH_2PO_4-Na_2HPO_4 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 collected and centrifuged at about 1000 x g for 10 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 ºC. 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 ºC.). 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 SO_2 injury

In response to increasing concentrations of SO_2, 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 SO_2 concentration and leaf age in both the pigeonpea and amaranth. The leaf symptoms of SO_2 toxicity were more conspicuous at 100 and 250 ppm aqueous SO_2 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 SO_2 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 SO_2 (Plate- 5 b and 6 a,b).

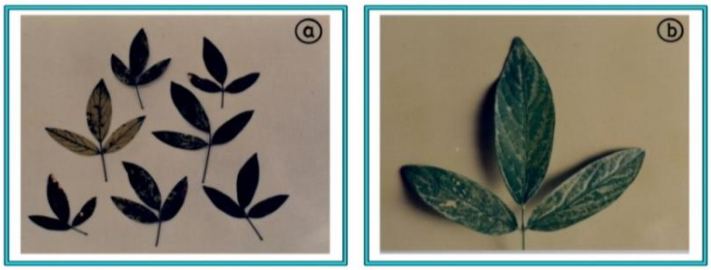

Plate-1:The effect of foliar application of 250 ppm aqueous SO_2 on the leaf morphology of 4-week old pigeonpea. a - Leaves showing chlorotic and small necrotic lesions with different patterns. b - A single leaf showing vein clearing.

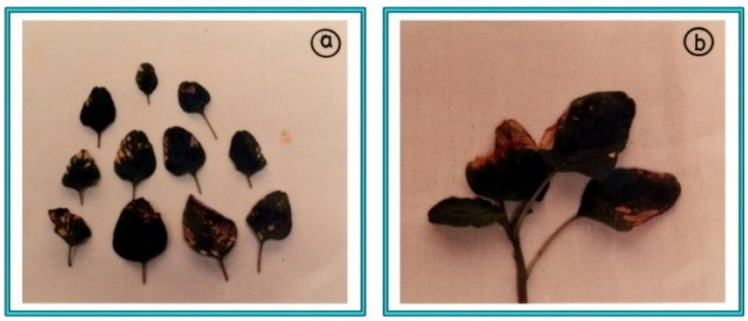

Plate-2: The effect of foliar application of 250 ppm aqueous SO_2 on the leaf morphology of 4-week old amaranth. a - Leaves showing tip burn, chlorosis, large necrotic lesions and leaf curl. b- Twig showing more injury symptoms on the mature leaves than on immature leaves.

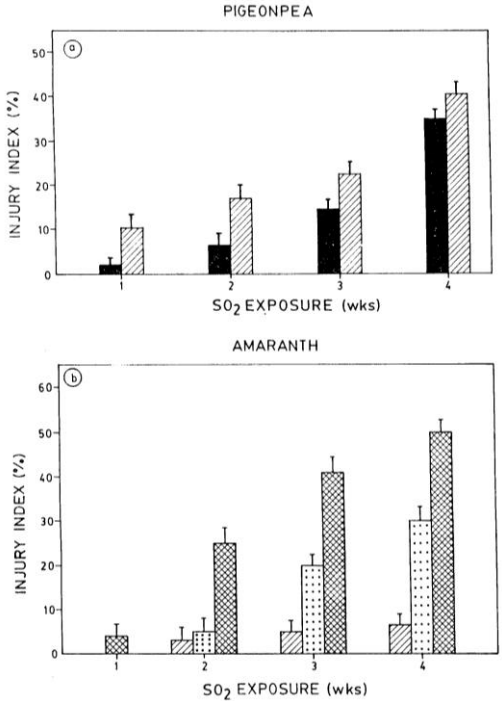

Figure-1 : The effect of foliar application of aqueous SO_2 on per cent injury index of pigeonpea (a) and amaranth (b) leaves (vertical lines represent S.E).
■- 100ppm; ▨ - 250 ppm; ▨ - 50 ppm; ⁝⁝ - 100 ppm; ▩ - 250 ppm.

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.

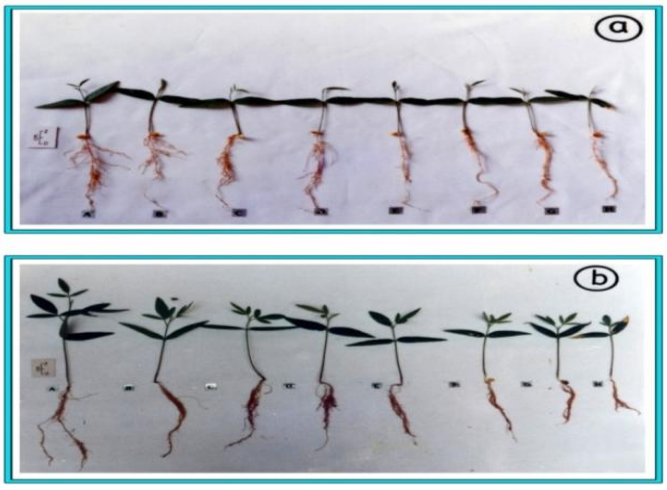

Plate-3: The effect of foliar application of aqueous SO_2 on the growth pattern of pigeonpea. a. 1 - week old plants; b. 2 - week old plants. A - 0 ppm; B - 10 ppm; C - 20 ppm; D - 30 ppm; E - 40 ppm; F - 50 ppm; G - 100ppm; H - 250 ppm.

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 - 100ppm; H - 250 ppm.

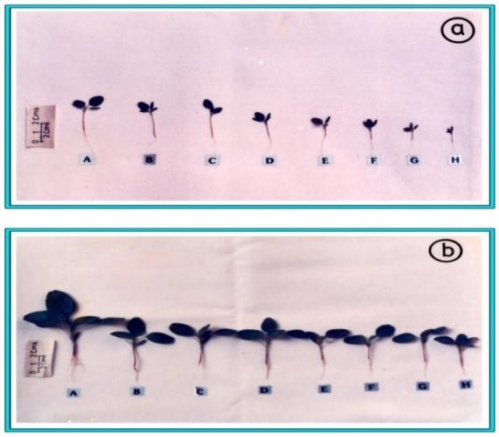

Plate-5: The effect of foliar application of aqueous SO_2 on the growth patterns of amaranth. a. 1 - week old plants; b. 2 - week old plants. A - 0 ppm; B - 10 ppm; C - 20 ppm; D - 30 ppm; E - 40 ppm; F - 50 ppm; G - 100ppm; H - 250 ppm.

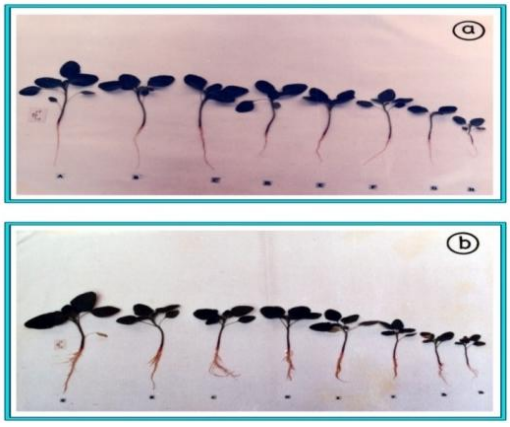

Plate-6: The effect of foliar application of aqueous SO_2 on the growth patterns of amaranth. a. 3 - week old plants; b. 4 - week old plants. A - 0 ppm; B - 10 ppm; C - 20 ppm; D - 30 ppm; E - 40 ppm; F - 50 ppm; G - 100ppm; H - 250 ppm.

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).

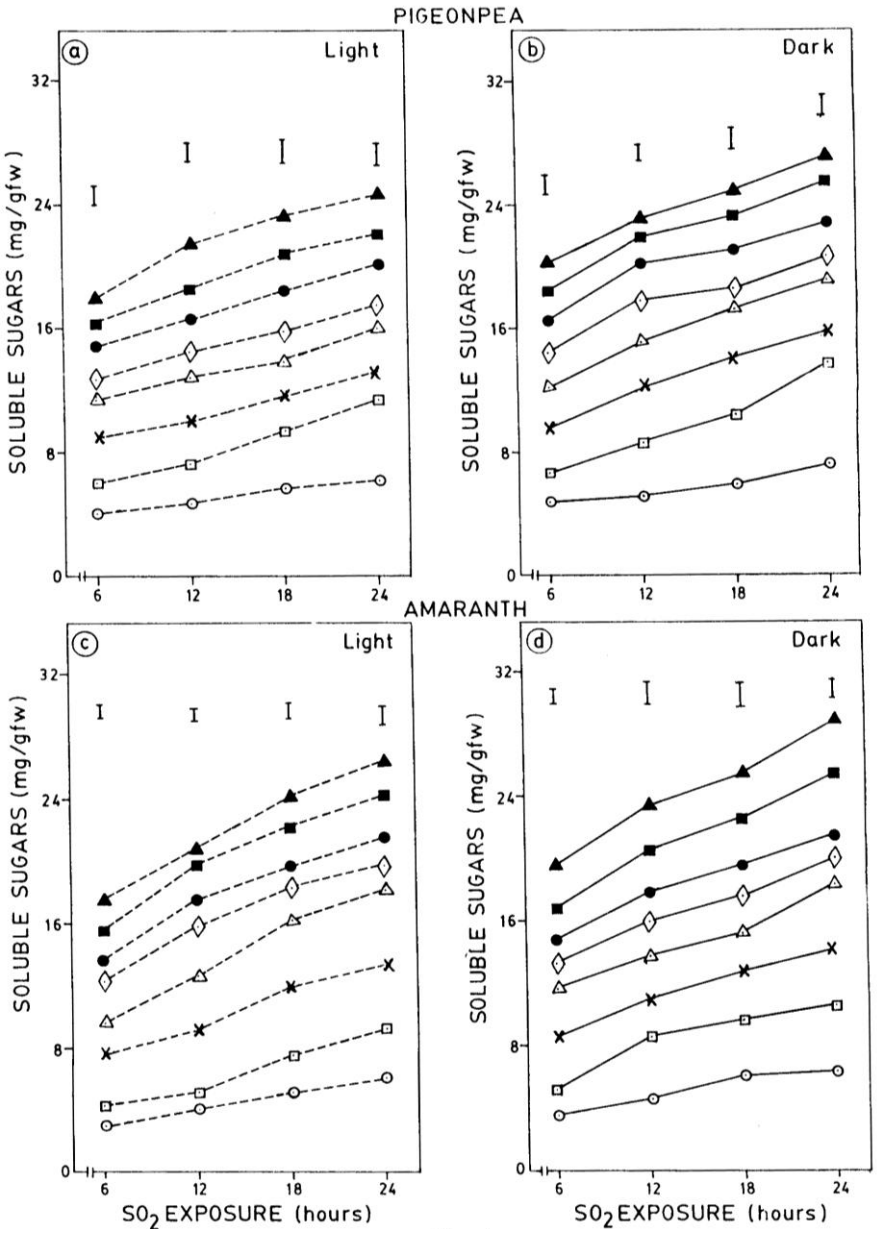

Figure-2: The effect of aqueous SO_2 on soluble sugar content of the leachates of pigeonpea and amaranth leaf discs (Vertical lines represent S.E.). a and b - Pigeonpea; c and d – Amaranth, ---- under light; — under dark ○- 0 ppm; □-10 ppm; ×-20 ppm; □-30 ppm; □-40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm

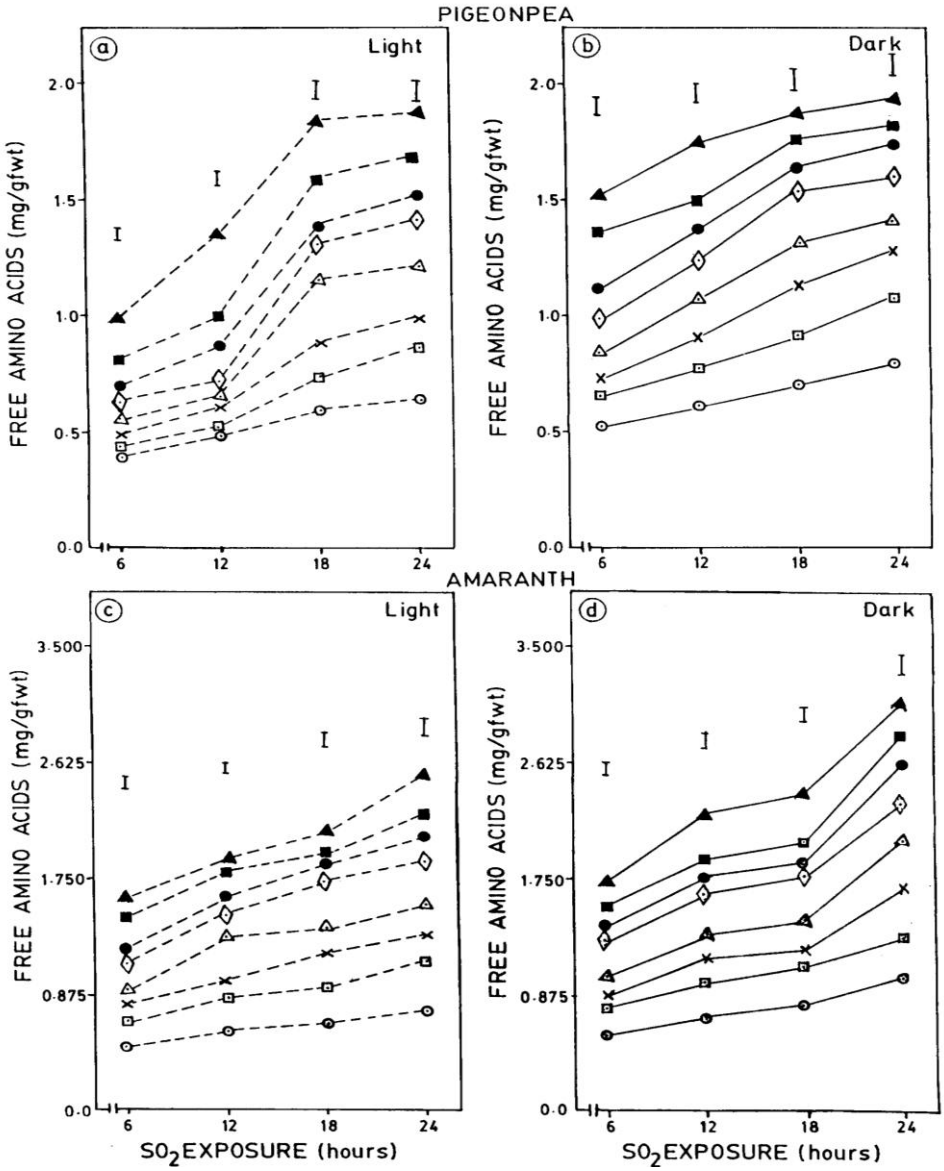

Figure-3: The effect of aqueous SO_2 on free amino acid content of the leachates of pigeonpea and amaranth leaf discs (Vertical lines represent S.E.). a and b - Pigeonpea; c and d – Amaranth, ---- under light; — under dark

○- 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).

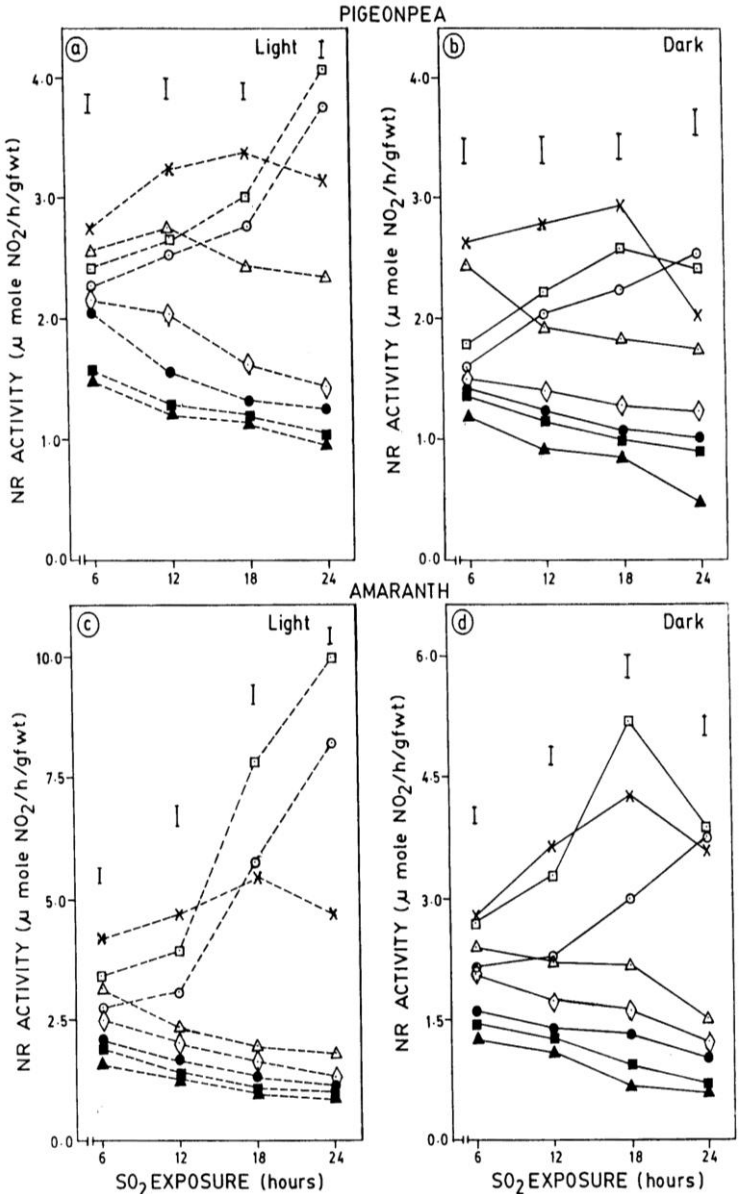

Figure-4: The effect of aqueous SO_2 on the activity of nitrate reductase of pigeonpea and amaranth leaf discs (Vertical lines represent S.E.). a and b - Pigeonpea; c and d – Amaranth, ---- under light; — under dark ○- 0 ppm; □-10 ppm; ×-20 ppm; □-30 ppm; □-40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm

Hill reaction

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).

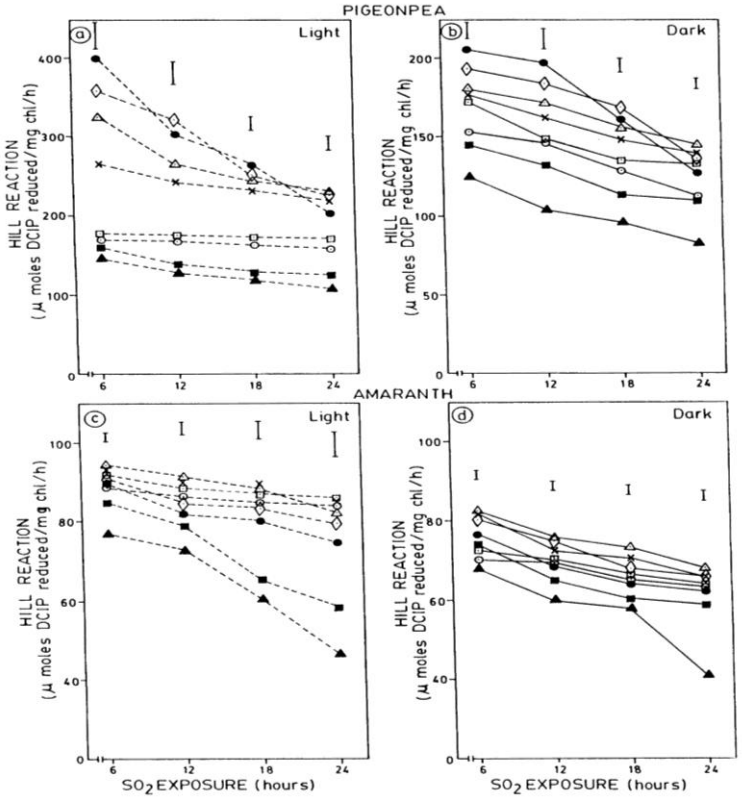

Figure-5: The effect of aqueous SO_2 on Hill reaction activity in pigeonpea and amaranth leaf discs (Vertical lines represent S.E.). a and b - Pigeonpea; c and d – Amaranth, ---- under light; — under dark
○- 0 ppm; □-10 ppm; ×-20 ppm; ◇-30 ppm; ◻-40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm

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 SO_2. Further the leaching was more in amaranth, indicating its relative sensitivity to SO_2.

Nitrate reductase is an important enzyme of nitrogen metabolism. It is substrate inducible and influenced by environmental factors such as light, nitrogen status and temperature (Beevers 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 SO_2 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 SO_2 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 SO_2 exposed oak plants, nitrate reduction was involved in the neutralization of protons generated by SO_2 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 SO_2. The differential responses of nitrate reductase activity in response to SO_2 concentration may be attributed to the availability of photosynthates and nitrate. However, the detailed mechanism of SO_2 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 C_4 plants exhibit a partial division of labour between mesophyll and bundle sheath cells. The NO^{3-} and NO^{2-} reductases of mesophyll acts as nitrogen reduction traps in an analogous fashion to phosphoenolpyruvate carboxylase acting as a CO_2 trap of C_4 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 SO_2 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 SO_2 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 SO_2 on Hill reaction activity was in agreement with the studies of Malhotra (1976) and Veeranjaneyulu *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 SO_2. The involvement of SO_2 derived anions sulphite and sulphate in inhibiting light reactions in SO_2-exposed leaves was well illustrated by Beauregard (1991, 1992). The SO_2-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 SO_2 directly effects the Hill reaction activity by inhibiting the oxygen evolving complex. The differential response of Hill reaction activity to SO_2 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 SO_2-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 SO_2 injury. Thus SO_2-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 SO_2 was relatively low (Beauregard, 1991). Furthermore, the generation of free radicals in the leaf cells to SO_2 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 SO_2 concentration and age. However, the SO_2 concentrations, which induced injury differed in pigeonpea and amaranth. SO_2-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 SO_2 compared to pigeonpea. Increased leaching of soluble sugars and amino acids

probably due to the damage of biomembrane intactness by SO_2. The nitrate reductase activity exhibited an increase at the lower and a decrease at the higher concentrations of SO_2 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 SO_2 exposure of both the plant species. The Hill reaction activity recorded higher values in lower concentrations and lower values at higher concentrations of SO_2. The Hill reaction activity was always registered lower values under dark conditions.

REFERENCES

[1] Arnon DI., 1949. Copper enzymes in isolated chloroplasts:polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*., **24**:1-15.

[2] Beauregard M., 1991. Involvement of sulfite and sulfate anions in the SO_2-induced inhibition of the oxygen evolving enzyme photosystem II in chloroplast : A review. *Environ. Exp. Bot*. **31**:11-22.

[3] Beauregard M., 1992. Modelling of the photosystem II, 33 KDa protein structure function and possible sulfate-sensitive sites derived from sequence - encoded information. *Environ. Exp. Bot*., **32**:411-423.

[4] Beevers L and Hageman RH., 1969. Nitrate reduction in higher plants. *Ann. Rev. Plant. Physiol*., **20**:495-522.

[5] Beevers L and Hageman RH., 1980. Nitrate and nitrite reduction. In: The Biochemistry of Plants.Vol. 5. (Ed. BJ Miffilin), New York. Pp. 115-168.

[6] Bohnert HJ, Nelson DE and Jensen RG., 1995. Adaptations to environmental stresses. *The Plant Cell*, **7**:1099-1111.

[7] Chopra RK., 1983. Effect of temperature on the *in vivo* assay of nitrate reductase in some C_3 and C_4 species. *Ann. Bot*., **51**:617-620.

[8] Covello PS, Chang A, Dumbrof EB and Thompson JE., 1989. Inhibition of photosystem II proceeds thylakoid membrane lipid peroxidation in bisulfate-treated leaves of *Phaseolus vulgaris*. *Plant Physiol*., **90**:1492-1497.

[9] De kok LJ, Stulen I, Bosma W and Hibma J., 1986a. The effect of short term H_2S fumigation on nitrate reductase activity in spinach leaves. *Plant Cell Physiol*., **27**(7):1249-1254.

[10] Dubios M, Gilles KA, Hamilton JK, Rebers PA and Smith F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem*., **28:**350-356.

[11] Dykstra GF., 1974. Nitrate reductase activity and protein concentration of two populous clones. *Plant Physiol*., **53**:632-634.

[12] Farooq M and Beg MU., 1980. Effect of aqueous sulphur dioxide on the membrane permeability of common Indian tree leaves. *New Botanist*, **VII**:213-217.

[13] Geburek TH and Scholz F., 1992. Response of *Picea abies* (L.) Karst. Provenance to sulphur dioxide and aluminium: A pilot study. *Water, air and soil pollution*, **62**:227-232.

[14] Hewitt EJ, Hucklesby DP and Notton BA., 1976. Nitrate metabolism. In: Plant Biochemistry (Eds. J.Bonner and JE Varner). 3rd Ed. Academic Press, New York. Pp. 633-681.

[15] Huang L, Murray F and Yang X., 1993. Responses of nitrogen metabolism parameters of sublethal SO_2 pollution in wheat (*Triticum aestivum* cv. Wilgoyne (Ciano/Gallo) under mild NaCl stress. *Environ. Expt. Bot*., **33**:479-493.

[16] Huber SC, Huber JL, Campbell WH and Margaret GR., 1992. Comparative studies of light modulation of nitrate reductase and sucrose-phosphate synthatase activities in spinach leaves. *Plant Physiol*., **100**:706-712.

[17] Jaworski EG., 1971. Nitrate reductase assay in intact plant tissue. *Biochem. Biophys. Res. Commum*., **43**:1274-1279.

[18] Khan MR and Khan MW., 1994. Single and interactivity effects of O_3 and SO_2 on tomato. *Environ. Expt. Bot*., **34**(4):461-469.

[19] Khan AA and Malhotra SS., 1977. Effects of aqueous sulphur dioxide on pine needles glycolipids. *Phytochemistry*., **16**:539-543.

[20] Khan MR and Khan MW., 1993. The interaction of SO_2 and root-knot nematode on tomato. *Environ. Pollut*., **81**:91-102.

[21] Klumpp A, Kuppers K and Guderian R., 1989. Nitrate reductase activity of needles of Norway spruce fumigated with different mixtures of ozone, sulfur dioxide and nitrogen dioxide. *Environ. Pollut*., **58**:261-271.

[22] Libera W, Ziegler I and Ziegler H., 1975. The action of sulfite on the HCO_3^- fixation and the fixation pattern of isolated chloroplasts and leaf tissue slices. *Z Pflanzen Physiol*., **74**:420-423.

[23] Malhotra SS., 1976. Effects of sulphur dioxide on biochemical activity and ultrastructural organization of pine needle chloroplasts. *New Phytol*., **76**:239-245.

[24] Malhotra SS., 1977. Effects of aqueous sulphur dioxide on chlorophyll destruction in *Pinus contorta*. *New Phytol*., **78**:101-109.
[25] Moore R and Black CC., 1979. Nitrogen assimilation pathways in leaf mesophyll and bundle sheath cells of C_4 photosynthesis plants formulated from comparative studies with *Digitaria sanguinalis* (L) Scop. *Plant Physiol*., **64**:309-313.
[26] Moore S and Stein WH., 1948. Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem*., **211**:908-913.
[27] Murray F and Wilson S., 1988a. Effect of sulphur dioxide, hydrogen fluoride and their combination on three Eucalyptus species. *Environ. Pollut*., **52**:265-279.
[28] Murray F and Wilson S., 1988c. Joint action of sulfur dioxide and hydrogen fluoride on growth of *Eucalyptus tererticornis*. *Environ. Expt. Bot*., **28**:343-349.
[29] Murray F., Wilson S and Monk R., 1991. The effects of SO_2 on final growth of *Medicago truncatula.* *Environ. Expt. Bot*., **31**: 319-325.
[30] Muthuchelian K, Nedunchezlian N and Kulandaivelu G., 1993. Effect of simulated acid rain on $^{14}CO_2$ fixation, ribulose-1,5-bisphosphate carboxylase and nitrate and nitrite reductases in *Vigna sinensis* and *Phaseolus mungo*. *Photosynthetica*, **28**:361-367.
[31] Peuke AD and Rudolf T., 1994. The effects of SO_2 fumigation on the nitrogen metabolism of aseptically grown spruce seedlings. *Environ. Pollut*., **83**:371-377.
[32] Pieser GD, Lizada C and Yang SF., 1982. Sulfite-induced lipid peroxidation in chloroplasts as determined by ethane production. *Plant Physiol*., **70**:994-998.
[33] Prakash TR, Murthy RS and Swamy PM., 1989. Influence of thiobencarb on nitrate reductase and DCPIP Photoreduction in rice and *Echinochloacrus-galli* (L.) (barnyard grass). *Weed Res*., **19**:427-432.
[34] Puckett KJ, Niebor E, Flora WP and Richardson DHS., 1973. Sulphur-dioxide: Its effect on photosynthetic ^{14}C fixation in lichens and suggested mechanisms of phototoxicity. *New Phytol*., **72**:141-154.
[35] Qifu M and Murray F., 1993. Effects of SO_2 and salinity on nitrogenase activity, nitrogen concentration and growth of young soybean plants. *Environ. Expt. Bot*., **33**:529-537.
[36] Reed AJ and Canvin DT., 1982. Light and dark controls of nitrate reduction in wheat (*Triticum aestivum* L.) protoplasts. *Plant Physiol*., **72**:573-577.
[37] Riens B and Hans WH., 1992. Decrease of nitrate reductase activity in spinach leaves during a light-dark transition. *Plant Physiol*., **98**:573-577.
[38] Salisbury BF and Ross CW., 1986. Assimilation of Nitrogen and Sulfur. In:Plant Physiology. CBS publishers and Distributors, pp. 251-265.
[39] Saunders PJW and Wood CM., I973. SO_2 in the environment, its production, dispersal, and fate. In: Air Pollution and Lichens (Ed. by B. W. Ferry, M. S. Baddeley, and D. L. Hawksworth), pp. 6. Athlone Press, London.
[40] Silvius JE, Ingle M and Bear CH., 1975. Sulfur dioxide inhibition of photosynthesis in isolated spinach chloroplasts. *Plant Physiol*., **56**: 434-437.
[41] Simon EW., 1974. Phospholipids and plant membrane permeability. *New Phytol*., **73**:377-420.
[42] Sinha SK and Nicholas DJD., 1981. Nitrate reductase. In: Physiology and Biochemistry and drought resistance in plants. (Eds.L.G.Paleg and D. Aspinall), Academic Press, New York. Pp. 145-168.
[43] Smyth DA and Dugger WM., 1980. Effects of boron deficiency on ^{86}rubidium uptake and photosynthesis in the diatom *Cylindrotheca fusiformis.* *Plant Physiol*., **65:**692-695.
[44] Taylor HJ and Bell JNB., 1992. Tolerance to SO_2, NO_2 and their mixture in *Plantago major* L. Populations. *Environ. Pollut*., **76**:19-24.
[45] Thomas FM and Runge M., 1992. Proton neutralization in the leaves of English oak (*Quercus robur* L.) exposed to sulfur dioxide. *J. Expt. Bot*., **43**:803-809.
[46] Thomas MD and Hendricks RH., 1956. Effect of air pollution on plants. In:Air Pollution Handbook (Eds. P.L.Magill, F.R.Holden, Charles Ackley), Mc Graw Hill Book Company, Inc. New York, Tornoto, London. Pp. 9.1-9.9.
[47] Trebst A., 1972. Measurement of Hill reaction and photoreduction. *Methods Enzymol*., **24**:146-165.
[48] Tripathi BD and Tripathi A., 1992. Foliar injury and leaf diffusive resistance of rice and white bean in response to SO_2 and O_3 singly and in combination. *Environ. Pollut*., **15**:265-268.
[49] Veeranjaneyulu KN, Soukpoe-Kossi CN and Le Blanc RM., 1991. SO_2 effect of photosynthetic activity of intact sugar maple leaves as detected by photo acoustic spectroscopy. *Plant Physiol*., **97**:50-54.
[50] Vogel AI., 1961. A text book of quantitative inorganic analysis including elementarary instrumental analysis. The English language book society and longman. pp.370.