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Anatomical Changes In the Leaf Cells Of *Cajanus cajan* (L.) Millspaugh And *Amaranthus paniculatus* (L.) By Foliar Application of Aqueous Sulphur Dioxide

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Abstract: *Atmospheric sulphur has always been present throughout the course of Earth's history. The effect of aqueous* SO_2 *(0, 10, 20, 30, 40, 50, 100 and 250 ppm) on water content, transpiration rate and leaf anatomy of third leaf of 3-week old plants of pigeonpea (Cajanus cajan (L.) Millsp. cv. PDM) and amaranth (Amaranthus paniculatus L.) were studied. The water content and transpiration rate gradually decreased with increasing* SO_2 *application in both the plant species. The water content and transpiration rate in response to* SO_2 *was decreased more in amaranth than pigeonpea. The leaf tissue damage was associated mostly with spongy tissue of pigeonpea and bundle sheath cells and vascular tissue of amaranth with increasing concentrations of* SO_2*. This differential effect of* SO_2 *on the leaf tissue of pigeonpea, a* C_3 *plant and amaranth, a* C_4 *plant may be attributed to the differences in sulphur metabolism associated with the different tissue.*

Keywords: *Amaranth, aqueous* SO_2*, damage, leaf tissue, pigeonpea, transpiration rate, water content.*

I. Introduction

Sulphur dioxide (SO_2) is a common air pollutant of industrial activity. Many of the metropolitan cities in India are ranked amongst the top few cities of the world for air pollutants concentrations (Baldasano *et al*., 2003). The combustion of fossil fuels such as coal, petroleum and burning of sulphur ores liberate SO_2. In addition to the normal sulphur uptake in the form of sulphate from the soil, plants may receive additional sulphur through leaves from the atmosphere. SO_2, normally 0.05-0.5 ppm in the urban areas and up to 2.0 ppm or more around point sources of air pollution, is the major source of atmospheric sulphur (Khan and Khan, 2000; Khan *et al*., 2006). Physiological processes such as photosynthesis, respiration, stomatal activity, transpiration and translocation are reported to be adversely affected by SO_2 (Darrall, 1989; Agrawal and Deepak, 2003). Alterations in various physiological functions were ascribed to changes in permeability of plasma membrane (Legge and Krupa, 2002), by interfering with enzymatic activities, and altering metabolic functions and nutrient uptake and water relations (Li *et al*., 2007).

Black and Unsworth (1980) reported that low concentration of SO_2 stimulated stomatal conductance in Vicia faba L. within 15 mins of exposure, which persisted for several days. This has been attributed to the destruction of epidermal cells adjacent to stomata and accumulation of sulphur within guard cells. Larger stomatal apertures not only allow ingress of the damaging pollutant, but also enhance water loss due to unrestricted transpiration. Once SO_2 enters through stomata, the route to the surface of a nearby subsidiary or epidermal cell is very short and therefore, the cells of the epidermis are more susceptible. The detrimental effects of SO_2 occur due to reactions under liquid phases after their uptake in the plants. SO_2 readily dissolves in the apoplastic water to produce mainly sulphite (SO_3^2), bisulphite (HSO_3) and H^+ ions, reducing the pH of the medium (Legge and Krupa, 2002). The phytotoxicity of SO_2 due to SO_3^{2-} and HSO^{3-} ions (DeKok, 1990). Most leaves have the capacity to detoxify, sulphite and bisulphite, if the concentrations are not excessively high, by oxidizing them to less toxic sulphate ion (Rao, 1992). Several studies have shown that the disturbances caused by SO_2 to biochemical functions (Li *et al*., 2007) and cell structure (Jutawong and Suwanwarer, 1997; Tripathi and Gautam, 2007) of plants appear before visual symptoms or growth reductions.

SO_2 absorption by shoots may lead to a decrease in sulphate uptake by roots and sulphate transport from roots to shoots (Herschbach, 1992). Leaves experience the maximum brunt of exposure and accordingly undergo structural and functional alterations with changing habitat environment. On reaching the mesophyll through stomata, SO_2 combines with water to form sulphurous acid, which, on dissociation, produces phytotoxic sulphite and bisulphite ions (Rennenberg and Polle, 1994; Rennenberg and Herschbach, 1996). SO_2 can cause foliar injury, disturb stomatal behaviour and transpiration, inhibit photosynthesis and reduce the final yield (Yunus and Iqbal, 1996; Dhir *et al*., 2001; Wali *et al*., 2004). Sulphur dioxide effect the internal structure of the leaves (Stewart *et al*., 1973). The first cells to be injured were either immediately beneath the epidermis, nearer to the stomata, or else closes to the vascular tissue and near the intercellular spaces. The injury later spreads to the inner mesophyll cells (Soikkeli, 1981). This makes the palisade tissue to lose their turgidity and to become flaccid. Later, the spongy tissues also collapse and the intercellular spaces become more or less obliterated (Pande and Oates, 1986).

Alteration in plant growth patterns often involves changes in physiological and anatomical characteristics, which in turn have a bearing on the quality of plant products. Pigeonpea (*Cajanus cajan* (L.) Millsp. cv. PDM), a C_3 plant is an important pulse crop of India. The seeds of pigeonpea are rich in protein content and are commonly used as source of vegetable protein in daily dietary intake of Indians. Being a legume, it fixes nitrogen and enriches soil fertility. And also it is profitable crop in India. Amaranth (*Amaranthus paniculatus* L.), a local cultivar), a C_4 plant is popular green leafy vegetable consumed all over India. Inspite of such an importance of these crops, there is no much information on their water content, transpiration rate and leaf anatomy under the influence of SO_2 stress and therefore selected for present investigation.

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 and effect of foliar application of aqueous SO_2 on whole plant growth

Seeds of pigeonpea and amaranth 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 $31 \pm 2^{o}C$ and $21 \pm 1^{o}C$ 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. The plants were separated into leaves, stems and roots prior to each analysis. The data were expressed on whole plant, per part and on unit fresh weight and/or dry weight basis. The contents expressed for the whole plants were obtained by summation of the individual parts.

Water content

The amount of water content in different plant parts was obtained by subtracting the values of dry weights from the respective fresh weights. The difference will be the water content present in the plant parts.

Transpiration rate

The rate of transpiration was determined according to the method followed by Devi *et al*. (1976). Uniform control and treated plants were used. Two graduated tubes connected by rubber tubes were filled completely with freshly boiled and cooled distilled water. Into one of the free ends of the tube, the root system of the seedlings was introduced using a one-holed stopper. To the other free end of the tube a drop of olive oil is added to avoid evaporation. The whole set was checked for air tightness and supported by a burette stand. The results were expressed as loss of water in mg per cm^2 per hour.

Leaf anatomy

Third leaf of 3-week old control and treated plants were excised, fixed in formalin-acetic acid-alcohol (FAA) and dehydration, infiltration and embedding of the leaf material were carried out according to the method of Sass (1958). Transverse sections of 10 µ thickness were cut using rotary microtome. Stain combinations of safranin and fast green were used to stain the leaf tissue.

III. Results And Discussion

Water content

The water content of both the control and SO_2 treated whole plants and their parts of pigeonpea and amaranth showed an increase with increasing age. However, the SO_2 treated plants showed lower values than controls. The difference between the controls and the treatments increased with increasing concentration of SO_2 in both plant species (Figure-1a,b,c,d and Figure-2a,b,c,d).

The water content of the control plant and its parts registered higher values than the SO_2 treated plants of both pigeonpea and amaranth. The degree of decrease in water content of treated plants becomes more conspicuous with increasing SO_2 concentration in both the plant species (Figure-1 and 2). However, the decrease was more in amaranth. The decrease in water content of SO_2 treated plants was closely associated with the decrease in plant growth and leaf area (Mattas and Pauli, 1965; Barrs, 1968).

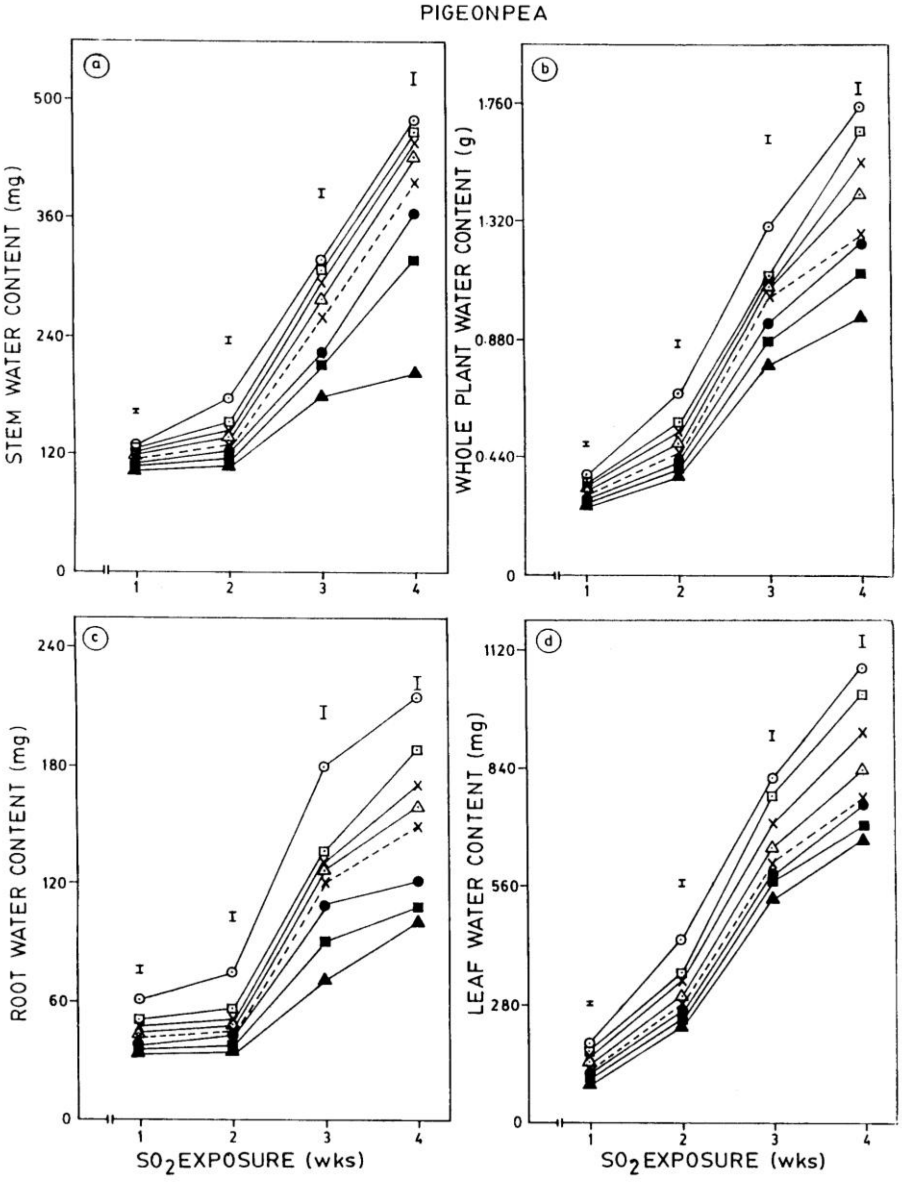

Figure-1: The effect of foliar application of aqueous SO_2 on stem (a) whole plant (b) root (c) and leaf (d) water content of pigeonpea (vertical lines represent S.E.)
○- 0 ppm; □-10 ppm; ×-20 ppm; Δ-30 ppm; ×--- 40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm.

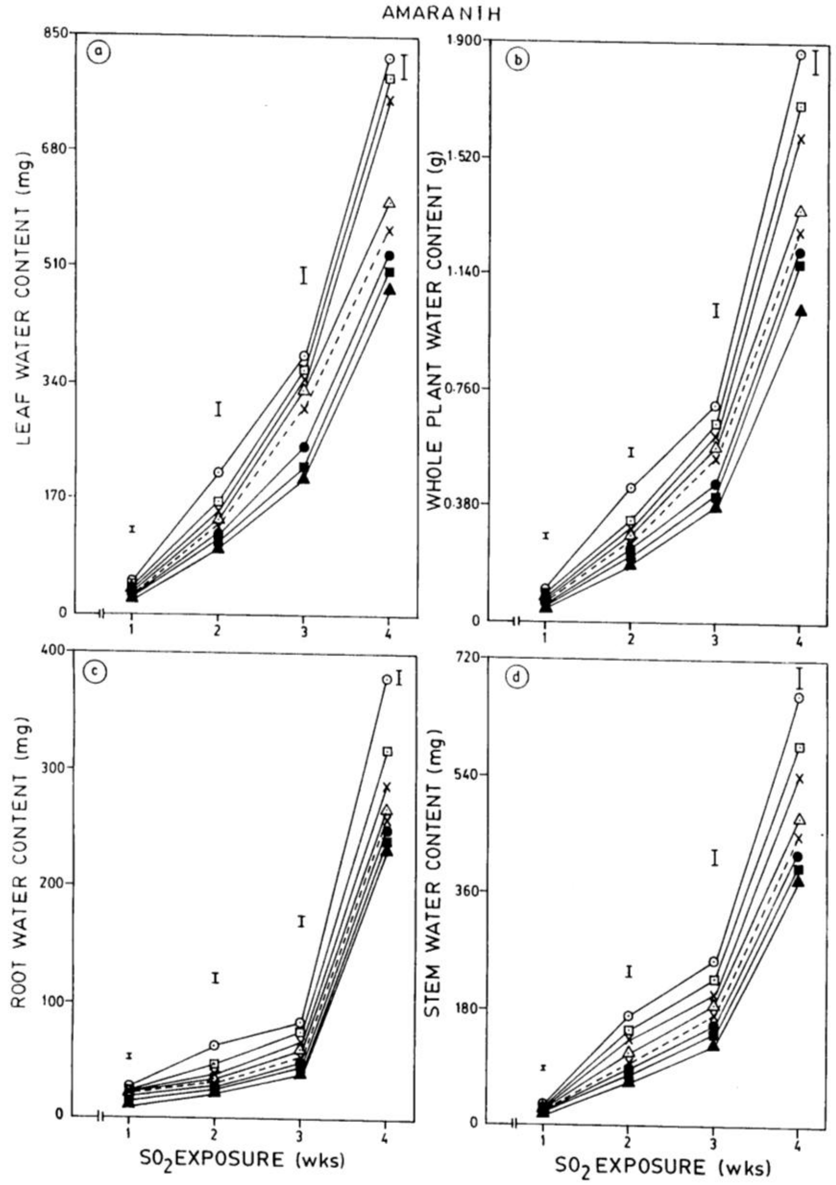

Figure-2: The effect of foliar application of aqueous SO_2 on leaf (a) whole plant (b) root (c) and stem (d) water content of amaranth (vertical lines represent S.E.)
○- 0 ppm; □-10 ppm; ×-20 ppm; Δ-30 ppm; ×--- 40 ppm; ●-50 ppm; ■-100 ppm; ▲-250 ppm.

Transpiration rate

The control plants of pigeonpea and amaranth exhibited grater transpiration rate (mg $H_2O/cm^2/h$) than the SO_2 treated plants. The decline of transpiration rate in SO_2 treated plants was commensurate with increasing SO_2 concentration. Marked decline of transpiration rate was noticed in 250 ppm treated plants in both pigeonpea and amaranth (Figure-3 a, b).

The transpiration rates of control plants of both pigeonpea and amaranth exhibited higher values than SO_2 treated plants. The transpiration rate gradually decreased with increasing SO_2 application in both the plant species (Figure-3 a,b). The decrease of transpiration in response to SO_2 application has been demonstrated in various plant species such as beans (Black and Unsworth, 1980; Taylor *et al*., 1981), peas and *Allium ursinum* (Olszyk and Tibbitts, 1981; Steubing and Fangmeier, 1987; Kaiser *et al*., 1993). The decreased transpiration in response to SO_2 application may be due to the reduced leaf area or may be due to the damage of stomatal complex or both (Black and Unsworth, 1980).

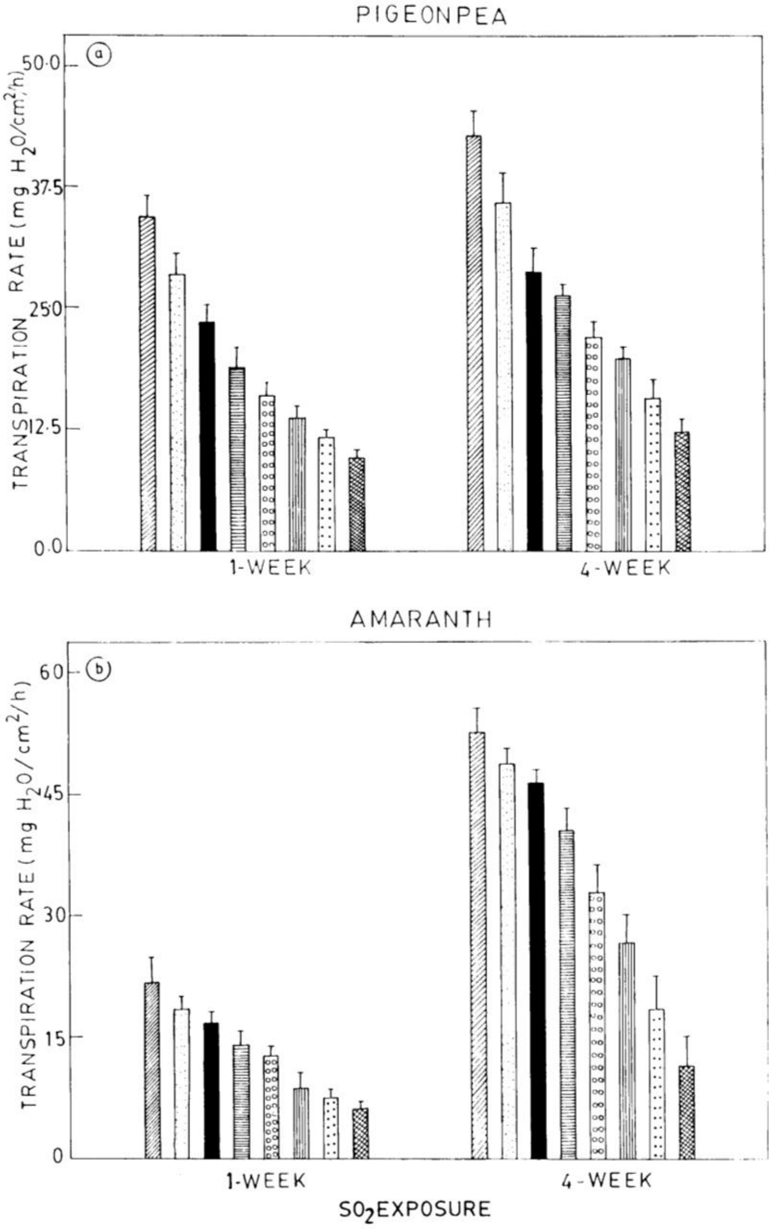

Figure-3: The effect of foliar application of aqueous SO_2 on transpiration rate of pigeonpea (a) and amaranth (b) (vertical lines represent S.E.)

▨ - 0 ppm; ░ - 10 ppm; ■ - 20 ppm; ▤ - 30 ppm; ◌ - 40 ppm; ▥ - 50 ppm; ⁙ - 100 ppm; ▩ - 250 ppm.

Leaf anatomy

Plate-1a,b,c,d represents the transverse sections of middle leaflet of the trifoliate leaf of three week old plants of pigeonpea. The leaf section of the control exhibited the normal dicotyledonous leaf features with upper and lower epidermis and palisade and spongy parenchyma (Plate-1a). The leaves exposed to 50 ppm aqueous SO_2 showed some damage of the spongy parenchyma cells (Plate-1b). The damage becomes conspicuous at 100 ppm SO_2 concentration even effecting the epidermal layers (Plate-1c). 250 ppm SO_2 was characterized by the total collapse of the spongy parenchyma specially at the necrotic areas (Plate-1d).

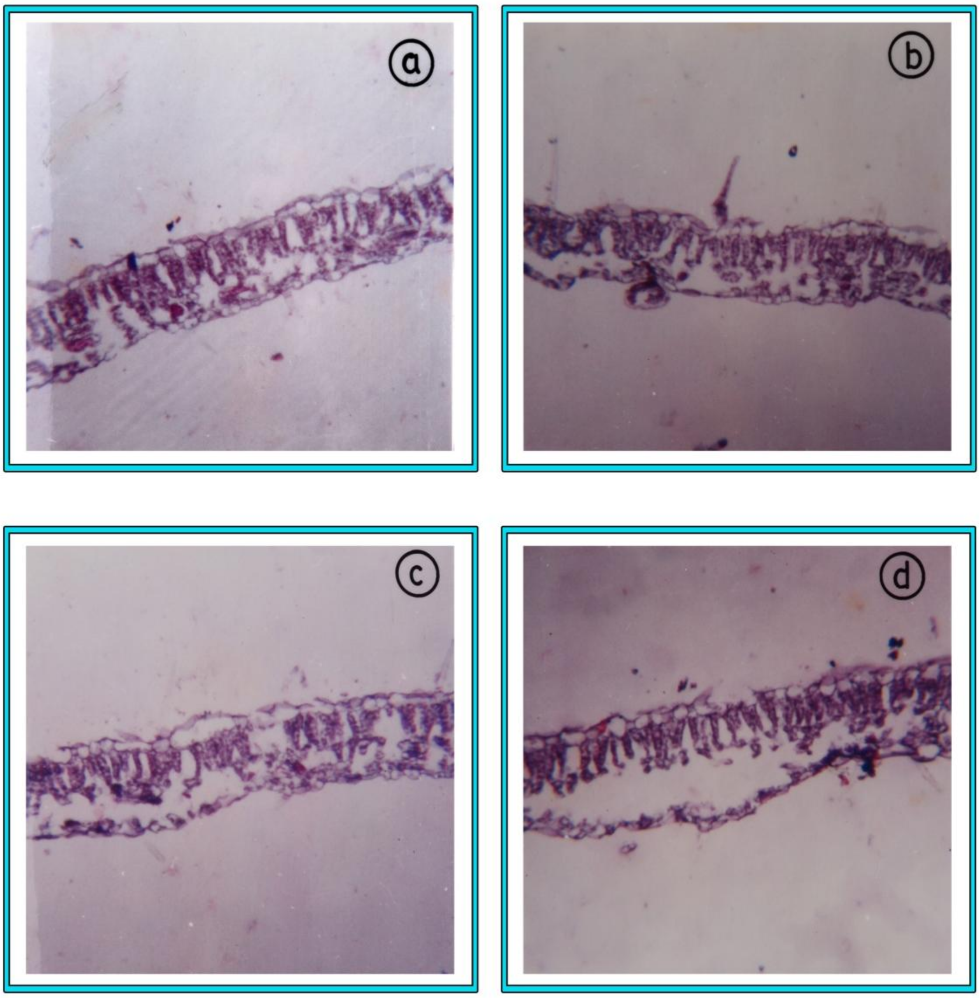

Plate-1: Transverse section of leaves of 3-week old pigeonpea plants, showing the effect of foliar application of aqueous SO_2 on leaf internal morphology.
a - 0 ppm (10x100); b - 30 ppm (10x100); c - 100 ppm (10x100); d - 250 ppm (10x100).

Plate-2a illustrates the transverse section of control leaf of amaranth, which shows a series of vascular bundles, arranged closely adjacent to each other. The mesophyll cells are arranged radially with two to three rows of parenchyma cells in between the upper and lower epidermis. The vascular tissues were surrounded by bundle sheath cells containing chloroplasts showing a typical ‘kranz’ anatomy of a C_4 plant. Plate-2b represents the transverse section of the leaf exposed to 50 ppm aqueous SO_2 in which the gap between vascular bundles increases. In 100 ppm SO_2 treated leaves of amaranth both upper and lower epidermal layers, and the mesophyll becomes enlarged with relatively thin walls (Plate-2c). The 250 ppm SO_2 led to the collapse of the mesophyll cells and the vascular bundles to a great extent. It also effected the epidermal cells. The condition becomes more severe at the necrotic areas (Plate-2d).

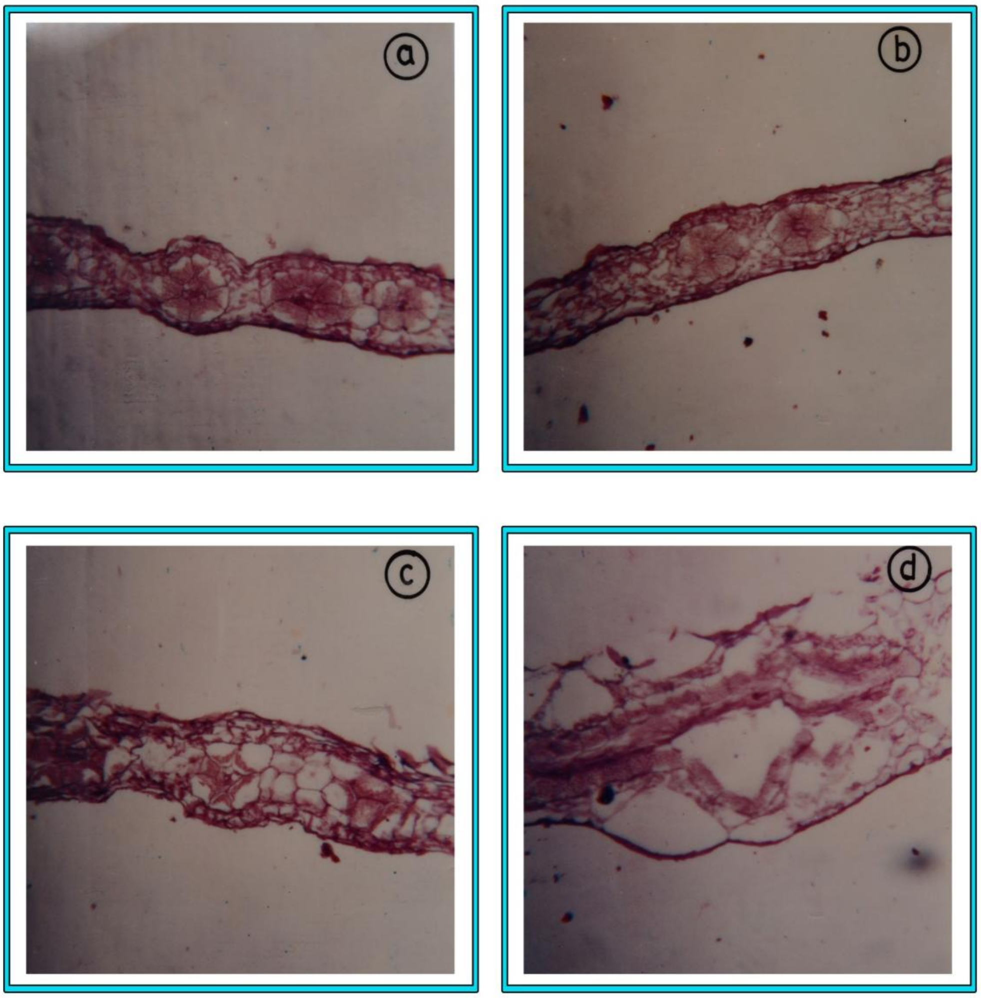

Plate-2: Transverse section of leaves of 3-week old amaranth plants, showing the effect of foliar application of aqueous SO_2 on leaf internal morphology.
a - 0 ppm (10x100); b - 30 ppm (10x100); c - 100 ppm (10x100); d - 250 ppm (10x100).

Sulphur dioxide effects the internal morphology of the leaves (Pande and Oates, 1986). The leaf tissue damage increased with increasing concentrations of SO_2 both in pigeonpea and amaranth. However, some differences were noted between pigeonpea and amaranth leaf tissue damage. In pigeonpea leaves, SO_2 damage was more conspicuous in spongy parenchyma whereas in amaranth SO_2 conspicuously affected bundle sheath cells and vascular tissue (Plate-1 and 2). This differential effect of SO_2 on the leaves of pigeonpea and amaranth may be related to their differences in leaf anatomy, carbon metabolism and spatial differences of sulphur metabolism, (Grewick *et al*., 1980; Salisbury and Ross, 1986) probably in mesophyll cells of pigeonpea a C_3 plant and bundle sheath cells of amaranth a C_4 plant.

IV. Conclusions

The water content and transpiration rate decreased in both the plant species as a consequence of foliar application of aqueous SO_2. The degree of decrease was more in amaranth than pigeonpea. The decreased rate of transpiration was associated with the reduced content of water in both pigeonpea and amaranth. The mesophyll tissue damage of leaf was noticed in both pigeonpea and amaranth in response to foliar application of aqueous SO_2. Spongy tissue was more affected in pigeonpea whereas in amaranth bundle sheath cells and vascular tissues were affected more. This preferential affect of SO_2 on the leaves of pigeonpea and amaranth was related to their differences in leaf anatomy and spatial differences in sulphur metabolism.

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