Effect of Foliar Application of Aqueous Sulphur Dioxide on Whole Plant Growth in Cajanus Cajan and Amaranthus Paniculatus

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Abstract:
The effect of elevated aqueous SO₂ (0, 10, 20, 30, 40, 50, 100 and 250 ppm) on different parameters of whole plant growth of pigeonpea (Cajanus cajan (L.) Millsp. cv. PDM1), a C₃ plant and amaranth (Amaranthus paniculatus L., a local cultivar), a C₄ plant has been studied. The percent seed germination and seed viability of both pigeonpea and amaranth decreased in response to aqueous SO₂ exposure. The foliar application of aqueous SO₂ interferes with several components of plant growth. The plant lengths including root and shoot lengths reduced in both the plant species in response to SO₂. Comparatively the root growth was more affected than the shoot growth in amaranth. Leaf dry weight was reduced in both pigeonpea and amaranth in response to SO₂ exposure. Comparatively the reduction was more in amaranth than in pigeonpea. The fresh and dry weights of the whole plant and its parts decreased more in amaranth than in pigeonpea. The shoot to root ratio increased with an increase in SO₂ concentration in both pigeonpea and amaranth. It was expressed more in pigeonpea than in amaranth. A reduction in root nodule number and viability of guard and adjacent epidermal cells declined in response to SO₂ exposure. Net primary productivity was increased in pigeonpea and decreased in amaranth in response to SO₂ application.

Keywords: Amaranth, aqueous sulphur dioxide, pigeonpea, whole plant growth, reduction, shoot to root ratio.

I. INTRODUCTION
Sulphur dioxide at low atmospheric levels may be beneficial to plants when the sulphur supply to the roots is limited (De Kok, 1990). However, at higher concentrations, SO₂ affects the growth and morphology of plants, specially the leaves (Ziegler, 1975). Most of the studies on the effect of SO₂ on plants are related to symptoms of foliar damage and chlorophyll degradation. Its impact on various physiological processes, metabolism and related growth are gaining more importance in recent years and needs further investigation. The studies of Hallgren and Huss (1975), Griffith and Campbell (1987), have shown that biological nitrogen fixation decreased significantly in response to aqueous solutions of NaHSO₃, NaHSO₄ and SO₂ exposure. The decrease of nitrogen fixation activity was attributed to the direct action of SO₂ on nitrogenase. Further, reduced photoassimilate supply under SO₂ exposure depletes the root nodules of necessary energy required for nitrogen fixation (Borland and Lea, 1991). Plant species, their different growth phases and parts exhibit marked differences in their sensitivity to SO₂. Hence, such differing sensitivities to SO₂ may provide a valuable tool for identifying the mechanisms of its phytotoxicity. Sulphur dioxide penetrates plants mainly through stomata and to a considerable extent through the epidermal surface of leaves (Ziegler, 1975; Garsed, 1981). Within the leaves, SO₂ is converted to products of reactivity which affect leaf metabolism. Therefore the present study was concerned with the effect of SO₂ on seed germination, plant growth and leaf metabolism of pigeonpea and amaranth in order to understand the mechanisms of phytotoxicity of SO₂.

II. MATERIALS AND METHODS
Preparation of aqueous sulphur dioxide
Sulphur dioxide was prepared in the laboratory by reacting sodium metabisulphite with concentrated H₂SO₄ and the generated gas was collected into distilled water. Aqueous SO₂ 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 SO₂ were prepared by diluting with distilled water. The pH was adjusted to 6.9 by adding dilute NaOH. It was reported that 1 ppm SO₂ in air gives 1000 ppm in aqueous solution (Puckett et al., 1973; Saunders and Wood, 1973; Malhotra, 1977).

Effect of foliar application of aqueous SO₂ 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 temperature of 31 ± 2°C and 21 ± 1°C at night at Andhra university experimental farm. The aqueous SO₂ 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 SO₂ 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, stem 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.

Seed germination

Seeds of pigeonpea (Cajanus cajan (L.) Millsp. cv. PDM1) and amaranth (Amaranthus paniculatus L. a local cultivar) were selected and placed separately in petri dishes lined with Whatmann No. 1 filter papers containing 0, 10, 20, 30, 40, 50, 100 and 250 ppm aqueous SO₂ solutions. The seeds were allowed to incubate for 6 and 24 h. After incubation the seeds were washed and placed in petri dishes lined with filter paper containing distilled water and allowed to germinate at 30 ± 2°C in the dark. After 24 h, the per cent germination was determined based on the emergence of 5 mm radicle from the seeds.

III. TTC Viability test

The seed viability as affected by different aqueous SO₂ were also determined following TTC (2,3,5-Triphenyl Tetrazolium Chloride) viability test. The quantitative study of the reduction product, 2,3,5-Triphenyl formazan was expressed as per cent reduction in absorbance at 520 nm. Five seeds of each lot of aqueous SO₂ treated and control seeds of pigeonpea and amaranth were selected randomly for tetrazolium viability test. The seeds were placed in 0.1% aqueous tetrazolium solution in the dark for 24 h. The excess tetrazolium solution was decanted off and the seeds were washed with distilled water thrice. Later they were immersed in 5 ml methanol. Then the test tubes were placed in a boiling water bath to extract the reduced TTC. The methanol was completely evaporated and the test tubes were removed from the bath and cooled to room temperature. Then 3 ml of methanol were added to each tube and vortexed. The reduction was estimated by reading the absorbance of the solution at 520 nm in a schimadzu (UV-240) spectrophotometer (Ghosh et al., 1981).

Plant length

Plant height, shoot and root length measurements were taken at weekly intervals and mean growth rates were ascertained by measuring mean height of 15 plants.

Fresh weight

Samples of 15 plants per pot were taken for estimation of fresh weights at weekly intervals. Roots, stems and leaves were separated and their fresh weights were determined.

Dry weight

After separation, immediately fresh weights of roots, stems and leaves were taken and were kept in a hot air oven maintained at 80°C for 48 h, by which time constant dry weights were obtained.

Root nodules

Root nodules of pigeonpea were recorded for second week, third week and fourth week only. The mean value of 15 plants were expressed in the data.

Epidermal cell survival

The viability of guard and adjacent epidermal cells was studied using epidermal strips removed from the lower surface of the third leaf of control and aqueous SO₂ treated 3-week old plants only. The epidermal strips (5 x 5 mm) thus obtained were placed in 0.1% solution of neutral red dye. Then the strips were incubated for 20-30 min in the solution and the strips were examined under compound microscope for the red colour intensity.

Shoot to root ratio (Dry weight)

Shoot to root ratio were determined by the procedure of Evans and Hughes (1961) as presented in the following formula

\[
\text{Shoot to root ratio (dry weight)} = \frac{\text{Mean shoot dry weight in mg}}{\text{Mean root dry weight in mg}}
\]

Net primary productivity (NPP)

Net primary productivity was determined according to the method of Sharma and Prakash (1991) by using the following formula

\[
\text{NPP} = \frac{\text{Dry matter accumulation of the plant}}{\text{Plant age}}
\]

RESULTS

Control seeds exhibited nearly cent per cent germination in both the plants of 6 h and 24 h of treatment. The germination percentage was gradually declined with increasing concentration of SO₂ treatment. The decline was more sharp in the 24 h treated seeds than the 6 h treated seeds. Among all the SO₂ treated seeds, the 250 ppm SO₂ treated seeds recorded lower seed germination percentage in both pigeonpea and amaranth. In pigeonpea, germination percentage values of 250 ppm treated seeds were 62.25 and 44.80 for the 6 and 24 h treated seeds respectively. The amaranth seeds recorded 51.65 and 31.33 per cent germination for 6 and 24 h, 250 ppm SO₂ treated seeds respectively. The amaranth seeds exhibited greater inhibition of seed germination than the pigeonpea seeds in all aqueous SO₂ concentrations (Figs.1a,b and 2 a,b).

Figure- 1: The effect of aqueous SO₂ on per cent seed germination of pigeonpea (vertical lines represent S.E).  a - 6 h treatment;  b - 24 h treatment

- 0 ppm;  - 10 ppm;  - 20 ppm;  - 30 ppm;  - 40 ppm;  - 50 ppm;  - 70 ppm;  - 100 ppm;  - 250 ppm.
Figure-2: The effect of aqueous SO$_2$ on per cent seed germination of amaranth (vertical lines represent S.E).

a - 6 h treatment;  b - 24 h treatment

- 0 ppm; - 10 ppm; - 20 ppm; - 30 ppm; - 40 ppm; - 50 ppm; - 100 ppm; - 250 ppm.

The seed viability as affected by different aqueous SO$_2$ treatments for the periods of 6 and 24 h was determined following the tetrazolium viability test based on the quantitative estimation of reduced tetrazolium, the formazan by colorimetry. The control seeds of pigeonpea and amaranth exhibited higher values of tetrazolium reduction than the SO$_2$ treated seeds in all the concentrations and of both the 6 and 24 h incubations (Figs. 3 a,b and 4 a,b). The tetrazolium reduction activity of SO$_2$ treated seeds showed a gradual decline with increasing SO$_2$ concentration by both pigeonpea and amaranth seeds. The greater decline was noted in both the 6 and 24 h aqueous 250 ppm SO$_2$ treated seeds. In pigeonpea the 24 h SO$_2$ incubated seeds exhibited tetrazolium reduction per cent values of 87.09, 82.26, 77.42, 75.16, 69.35, 66.13 and 56.45 for the corresponding 10, 20, 30, 40, 50, 100 and 250 ppm SO$_2$ incubated seeds respectively. Corresponding to the above stated aqueous SO$_2$ incubation concentrations, the amaranth recorded 81.25, 75.00, 71.88, 66.87, 61.50, 50.00 and 43.75 tetrazolium reduction per cent respectively.

IV. Plant length

Continuous increase in total plant length with age was observed from the first week to the fourth week in the control plants of both pigeonpea and amaranth. In pigeonpea, the increase was from 32.50 to 65.50 cm from the first to fourth week respectively. The amaranth recorded 10.27 cm on the first week and 30.28 cm on fourth week. Though the SO$_2$ treated plants showed a similar trend in total plant length, the values were always remained lower when compared to the corresponding control plants. In SO$_2$ treated plants the total plant length declined with increasing SO$_2$ concentration in both the plant species (Plates-1a,b,c,d and 2 a,b,c,d). The lowest values of plant length among the treatments were noted in 250 ppm aqueous SO$_2$ treated plants of pigeonpea and amaranth (Figs.5 a,b,c and 6 a,b,c).

Plate-1: The effect of foliar application of aqueous SO$_2$ on the growth pattern of pigeonpea exposed to 0 and 250 ppm is shown here in order to have a close comparison.
a. 1 - week old plants; b. 2 - week old plants; c. 3 - week old plants; d. 4 - week old plants. A - 0 ppm; B - 250 ppm.

Plate-2: The effect of foliar application of aqueous SO$_2$ on the growth pattern of amaranth exposed to 0 and 250 ppm is shown here in order to have a close comparison.

a. 1 - week old plants; b. 2 - week old plants; c. 3 - week old plants; d. 4 - week old plants. A - 0 ppm; B - 250 ppm.

Figure-5: The effect of foliar application of aqueous SO$_2$ on the plant length of pigeonpea. (Vertical lines represent S.E.). a - Whole plant length; b - Root length; c - Shoot length ○○ - 0 ppm; □□ - 10 ppm; •• - 20 ppm; ΔΔ - 30 ppm; ×× - 40 ppm; ●● - 50 ppm; ■■ - 100 ppm; ▲▲ - 250 ppm

The shoot and root length when considered separately also showed a trend similar to whole plant length in both the plants at all treatments (Figs. 5 b,c and 6 b,c).

Figure-6: The effect of foliar application of aqueous SO$_2$ on the plant lengths of amaranth. (Vertical lines represent S.E.). a - Whole plant length; b - Root length; c - Shoot length ○○ - 0 ppm; □□ - 10 ppm; ×× - 20 ppm; ΔΔ - 30 ppm; ×× - 40 ppm; ●● - 50 ppm; ■■ - 100 ppm; ▲▲ - 250 ppm

The shoot and root length when considered separately also showed a trend similar to whole plant length in both the plants at all treatments (Figs. 5 b,c and 6 b,c).

Fresh weights
The fresh weights of both pigeonpea and amaranth plants increased with increasing age. However, the values of SO$_2$ treated plants remained lower than the controls throughout the investigation. Among the aqueous SO$_2$ treatments 250 ppm treated plants showed lowest values in both pigeonpea and amaranth.

The control plant of pigeonpea showed an increase in the fresh weight from 0.43 g on the first week to 2.22 g on the fourth week. The fresh weight values of 250 ppm SO$_2$ treated plant were 0.28 g on the first week and 1.38 g on the fourth week (Fig. 7). The fresh weights of control plant of amaranth recorded 0.21 g on the first week and 2.26 g on the fourth week and the 250 ppm SO$_2$ treated plant registered 0.12 g on the first week and 1.24 g on the fourth week (Fig. 7).
The effect of foliar application of aqueous SO$_2$ on whole plant fresh weight of pigeonpea and amaranth. The leaf, stem and root fresh weights of both the plant species separately also showed a reduction with increasing SO$_2$ concentration. The reduction in fresh weight of roots were more pronounced in pigeonpea than in amaranth (Figs. 8 a,b,c and 9 a,b,c).

The dry weight accumulation of control plants increased with increasing age in both pigeonpea and amaranth plants. The SO$_2$ treated plants also exhibited an increase in dry matter accumulation, but their values were always remained lower than their controls. The reduction of dry matter accumulation of the whole plant and its parts was more conspicuous with increasing SO$_2$ concentrations. Among all SO$_2$ treatments more reduction of dry matter was observed in 250 ppm treated plants of both pigeonpea and amaranth (Fig. 10).

The individual parts of stem, root and leaves were also exhibited a trend similar to whole plants throughout the period of observation. The dry matter accumulation of roots were high and sharp in the control and in all the treatments of amaranth when compared to the respective control and treatments of pigeonpea (Figs. 11 and 12 a,b,c).

Dry weight
The dry weight accumulation of control plants increased with increasing age in both pigeonpea and amaranth plants. The SO$_2$ treated plants also exhibited an increase in dry matter accumulation, but their values were always remained lower than their controls. The reduction of dry matter accumulation of the

Figure-7: The effect of foliar application of aqueous SO$_2$ on whole plant fresh weight of pigeonpea and amaranth. (Vertical lines represent S.E.). ○○ -0 ppm; □□-10 ppm; ××-20 ppm; ΔΔ- 30 ppm; ××-40 ppm; ●●-50 ppm; ■■-100 ppm; ▲▲-250 ppm

Figure-8: The effect of foliar application of aqueous SO$_2$ on Leaf (a), Root (b) and Stem (c) fresh weights of pigeonpea. (Vertical lines represent S.E.). ○○ -0 ppm; □□-10 ppm; ××-20 ppm; ΔΔ- 30 ppm; ××-40 ppm; ●●-50 ppm; ■■-100 ppm; ▲▲-250 ppm

Figure-9: The effect of foliar application of aqueous SO$_2$ on Stem (a), Leaf (b) and Root (c) fresh weights of amaranth. (Vertical lines represent S.E.). ○○ -0 ppm; □□-10 ppm; ××-20 ppm; ΔΔ- 30 ppm; ××-40 ppm; ●●-50 ppm; ■■-100 ppm; ▲▲-250 ppm

Figure-10: The effect of foliar application of aqueous SO$_2$ on whole plant dry weights of pigeonpea and amaranth. (Vertical lines represent S.E.). ○○ -0 ppm; □□-10 ppm; ××-20 ppm; ΔΔ- 30 ppm; ××-40 ppm; ●●-50 ppm; ■■-100 ppm; ▲▲-250 ppm
Figure-12: The effect of foliar application of aqueous SO$_2$ on Leaf (a), Root (b) and Stem (c) dry weights of amaranth. (Vertical lines represent S.E.). ○-○ - 0 ppm; □-□ - 10 ppm; ×-× - 20 ppm; Δ-Δ - 30 ppm; ×-× - 40 ppm; ●-● - 50 ppm; ■-■ - 100 ppm; ▲-▲ - 250 ppm

Root nodules
Sulphur dioxide affected the root nodule number in pigeonpea, a legume plant (Plate-3 a,b). The SO$_2$ treated plants registered lower number of root nodules than the controls and it was more in 250 ppm aqueous SO$_2$ treatment (Fig.13).

Figure-13: The effect of foliar application of aqueous SO$_2$ on root nodule number of pigeonpea. (Vertical lines represent S.E.). ○-○ - 0 ppm; □-□ - 10 ppm; ×-× - 20 ppm; Δ-Δ - 30 ppm; ×-× - 40 ppm; ●-● - 50 ppm; ■-■ - 100 ppm; ▲-▲ - 250 ppm

Plate-4: The effect of foliar application of aqueous SO$_2$ on the epidermal cell survival of pigeonpea (a,b,c,d) and amaranth (e,f). Abaxial epidermal strips were isolated from the leaves of 3-week-old plants and were stained immediately with neutral red.

Epidermal cell survival
The per cent survival of guard and adjacent epidermal cells in relation to the degree of the intensity of neutral red dye were calculated as percentage over their respective controls. Aqueous SO$_2$ even at 10 ppm concentration caused a reduction in per cent survival of both guard and epidermal cells. The effect becomes more conspicuous at both higher SO$_2$ concentrations. The effect of SO$_2$ was seen more on the guard cells than on the epidermal cells of both pigeonpea and amaranth (Plate-4 a,b,c,d,e,f).

Plate-3: The effect of foliar application of aqueous SO$_2$ on root nodules of pigeonpea.

Shoot to root ratio
The shoot to root ratios of pigeonpea and amaranth increased with increasing age in all the treatments. However, the shoot to root ratios of SO$_2$ treated plants recorded higher values in pigeonpea, whereas in amaranth the SO$_2$ treated plants registered lower values than their respective controls. On the other hand, the shoot to root ratios of amaranth showed a sharp increase from the first to the second week followed by almost constant values up to the fourth week. The increase of shoot to root ratio was very high in pigeonpea than in amaranth (Fig.14).
amaranth seeds (Fig. 1 and 2 a,b). However, the decline of seed germination was more in amaranth than in pigeonpea. Earlier studies of seed germination in response to sulphur dioxide also indicated a decline in seed germination of Sorghum vulgare (Boralkhar and Chaphekar, 1979), radish, mustard and pearl millet (Banerjee and Chaphekar, 1980). The study of seed viability based on 2,3,5-triphenyl tetrazolium chloride reduction was conducted in the SO$_2$ treated seeds. The TTC reduction test was used to obtain a rapid estimation of potential germination capacity and viability of seeds (Moore, 1973). The TTC reduction test showed a progressive loss of its reduction ability in SO$_2$ treated seeds, of both pigeonpea and amaranth with increasing SO$_2$ concentration and duration of exposure (Figs. 3 and 4 a,b). The reduction of TTC measures the enzyme activity primarily associated with the mitochondria (Steponkus, 1971 and Towill and Mazur, 1974). The loss of seed viability in SO$_2$ treated seeds was associated with the decline of seed germination percentage in both the plants. Further the loss of viability was more in amaranth than in pigeonpea. The TTC reduction test may be used to detect the loss of activity of certain respiratory enzymes affected by SO$_2$ treatment of the seeds. Therefore it can be used as a sensitive test in the determination of differential responses of plant species to changing environments. Foliar application of aqueous SO$_2$ affected the total plant length. The total plant length declined gradually with increasing SO$_2$ concentration and age in both pigeonpea and amaranth (Figs. 5 a and 6 a). Reduction of both root and shoot lengths were exhibited by both the plant species, (Figs. 5 and 6 b,c). Exposure of pigeonpea and amaranth to higher concentrations of aqueous SO$_2$ such as 250 ppm showed visible injury to the plant growth. Further mature leaves were more effected than immature leaves (Plates-1 and 2). The reduction of both root and shoot lengths in SO$_2$ treatments were more pronounced in amaranth than in pigeonpea. Perhaps SO$_2$ might have impaired the photosynthesis and other metabolic activities leading to reduced plant growth (Murray and Wilson, 1991; Khan and Khan, 1993). The data presented in figures 7 to 9 indicate that SO$_2$ adversely affects the fresh weights of whole plant and their different parts, the stems, roots and leaves of both pigeonpea and amaranth. The fresh weight of root was effected more and it was conspicuous in pigeonpea (Fig.8b). Similar results were observed in cucumber (Mejstrik, 1980), Avena sativa (Subash Chand and Narendra Kumar, 1987) and Lycopersicum esculentum (Sharma and Prakash, 1991).

Aqueous SO$_2$ also reduced the dry weight of whole plant and their different parts, the leaves, stems and roots of both pigeonpea and amaranth (Figs. 10 to 12). The reduction becomes more conspicuous with increasing SO$_2$ concentration and age in both pigeonpea and amaranth. Though slight variation appeared in the rate of biomass accumulation at the root level in between pigeonpea and amaranth, at the whole plant level the biomass decline was more in amaranth. Lolium perenne (Ashenden, 1978), Nicotiana tabaccum and Cucumis sativus (Mejstrik, 1980), birch clones (Wright, 1987), Medicago sativa (Murray and Wilson, 1991) wheat and barley (Murray et al., 1992), and Plentago major (Taylor and Bell, 1992) also showed reduction in biomass accumulation in response to SO$_2$ exposure. Dry matter accumulation remained lower in SO$_2$ treated plants than controls. Among all concentrations more reduction of dry matter was observed in 250 ppm treated plants of both pigeonpea and amaranth. At the whole plant level the biomass decline was

**Figure-14**: The effect of foliar application of aqueous SO$_2$ on shoot to root ratio of pigeonpea and amaranth.

- ○ - 0 ppm; □ - 10 ppm; ×× - 20 ppm; Δ- Δ- 30 ppm; ×× - 40 ppm; ● - 50 ppm; ■ - 100 ppm; ▲ - ▲ - 250 ppm

**Net primary productivity**

The net primary productivity increased continuously with age in both the control and SO$_2$ treated plants of pigeonpea. The increase was more conspicuous between second and third week of plant growth. The values of SO$_2$ treated plants were always remained lower than the controls (Fig.15a). The control and SO$_2$ treated plants of amaranth showed a steep increase in the net primary productivity up to the second week followed by a slight decline on the third week and thereafter again recorded an increase on the fourth week. The net primary productivity was always registered lower values in SO$_2$ treated plants than the controls (Fig.15b).

**Figure-15**: The effect of foliar application of aqueous SO$_2$ on net primary productivity (NPP) of pigeonpea (a) and amaranth (b).

- ○ - 0 ppm; □ - 10 ppm; ×× - 20 ppm; Δ-Δ- 30 ppm; ×× - 40 ppm; ● - 50 ppm; ■ - 100 ppm; ▲ - ▲ - 250 ppm

**V. DISCUSSION**

Sulphur dioxide effects seed germination. A gradual decline of seed germination with increasing aqueous SO$_2$ concentration and duration of exposure was exhibited by both pigeonpea and

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more in amaranth. The reduction may be considered as a direct effect of SO\textsubscript{2} on photosynthetic activity. The reduced nitrogen fixation and its metabolism in response to SO\textsubscript{2} application probably would have indirectly affected the dry weight of both the plants. It is interesting to note the effect of foliar application of SO\textsubscript{2} on root nodules of pigeonpea. Pigeonpea, a legume plant exhibited a reduction in root nodule number in response to SO\textsubscript{2} exposure. The number of root nodules decreased gradually with increasing SO\textsubscript{2} concentration and age (Fig. 13; Plate-3). Sulphur dioxide is known to affect root nodule number and nitrogen fixation capabilities (USEPA, 1976). Reduction of root nodule number was also observed in soybean cultivars (Qifu and Murray, 1993) and in Dolichos lablab (Banerjee and Chaphekar, 1980). The reduction in root nodule number in response to SO\textsubscript{2} may be considered mostly due to reduced assimilate availability at the sites of nodule initiation (Qifu and Murray, 1993). Stomatal responses to foliar application of aqueous SO\textsubscript{2} various with its concentration, duration, age of the plant and the plant species studied. The effect of SO\textsubscript{2} on the stomata of intact leaves were studied in the third leaves of both pigeonpea and amaranth. The leaves were collected from the 3-week old SO\textsubscript{2} exposed plants. The intensity of guard cell damage increased with increasing SO\textsubscript{2} concentration, in both pigeonpea and in amaranth as indicated by the complete disappearance of neutral red dye in some of the guard cells exposed to 250 ppm aqueous SO\textsubscript{2} both in pigeonpea and in amaranth (Plate-4). Structural alteration of guard cells was also observed in amaranth in response to 250 ppm SO\textsubscript{2} application (Plate-4). The reduction in the viability of guard cells and the alteration of guard cell chloroplast to SO\textsubscript{2} was noticed Vicia faba (Black and Black, 1979 a,b). Any factor that affect the turgor of guard and adjacent epidermal cells, will affect stomatal conduction (Black and Black, 1979a) and more so by SO\textsubscript{2} (Kimmer and Kozlowski, 1981).

Sulphur dioxide affects the biomass accumulation in plants (Murray, 1985a). Pigeonpea showed a continuous increase in shoot to root ratio with increasing SO\textsubscript{2} concentration and age. The relative rate of increase in shoot to root ratio was very high in pigeonpea than in amaranth under SO\textsubscript{2} exposure. Amaranth also exhibited an increase in shoot to root ratio in response to SO\textsubscript{2} (Fig. 14). However maximum increase was observed in amaranth from the first week to second week followed by a slow pace of increase in the later stages. A number of studies have shown that SO\textsubscript{2} can alter the pattern of assimilate allocation favouring shoot growth at the expense of root growth (Mejstrik, 1980) leading to increased shoot to root ratio (Darrall, 1989; Qifu and Murray, 1991, 1993). The net primary productivity (NPP) increased in all the treatments with age (Fig. 15 a,b). However, the increase of NPP was more in control plants than in SO\textsubscript{2} treated plants. The higher values of NPP exhibited by pigeonpea relative to amaranth indicated the relative resistance of pigeonpea to SO\textsubscript{2} (Sharma and Prakash, 1991).

VI. CONCLUSIONS

The per cent seed germination of both pigeonpea and amaranth decreased with increasing aqueous SO\textsubscript{2} concentration. Aqueous SO\textsubscript{2} also decreased the seed viability as indicated by the tetrazolium reduction activity. The percentage decrease of seed germination and loss of seed viability was more in amaranth than in pigeonpea. The plant length was effected by SO\textsubscript{2} in both the plant species. The shoot length was more effected in amaranth than in pigeonpea. On the other hand, the root length was relatively more effected in pigeonpea than in amaranth. The fresh weights of whole plants and their parts decreased with increasing aqueous SO\textsubscript{2} concentration and age in both the plant species. Dry weight accumulation in the whole plants and their leaves, stems and roots increased with increasing plant age throughout the investigation period, both in control and SO\textsubscript{2} treated plants. However, SO\textsubscript{2} treated plants showed a decline in dry matter accumulation when compared to their respective controls in both pigeonpea and amaranth. The root dry weight was effected more than shoot dry weight. Interestingly the root dry weight decreased more in pigeonpea than in amaranth. A reduction of root nodule number in response to SO\textsubscript{2} exposure in pigeonpea. The viability of guard and adjacent epidermal cells declined with increasing SO\textsubscript{2} concentration. The shoot to root ratio increased with increasing SO\textsubscript{2} concentration and age in the both plant species. The relative rate of increase in shoot to root ratio was very high in pigeonpea than in amaranth. The increased shoot to root ratio indicated the altered pattern of assimilate allocation in response to SO\textsubscript{2} application favouring shoot growth perhaps at the expense of root growth. The increase of net primary productivity was more in SO\textsubscript{2} treated plants of pigeonpea than their respective controls. On the other hand, amaranth exhibited a decrease in net primary productivity. The reduction in plant growth might be due to the interference of SO\textsubscript{2} in photosynthesis and photosynthate partitioning. The studies on the effect of aqueous SO\textsubscript{2} on the morphology, physiology, and on the growth of pigeonpea and amaranth indicated that amaranth appears to be relatively more sensitive to SO\textsubscript{2} than pigeonpea.

VII. REFERENCES


