RESPONSE OF SIX COMMON TREE SPECIES TO SULPHUR DIOXIDE FUMIGATION

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P R E F A C E

The research work embodied in this dissertation has been carried out in School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. The work is original and has not been submitted so far, in part or full, for any other degree or diploma of any university.

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CONTENTS

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R. MISHRA

EFFECT OF SO_2 ON PLANTS WITH SPECIAL REFERENCE TO TREES - A REVIEW

General

Sulphur dioxide is one of the major air pollutants besides nitrogen oxides, carbon monoxide, hydrocarbons, ozone, fluorides and flyash. SO_2 is released from fossil fuel combustion and smelting of some metal ores. The effects of SO_2 on terrestrial vegetation can be very spectacular specially near point sources. Growing concern with air pollution problems have greatly reduced the episodal discharges of sulphur dioxide, however, environmentalists are actively concerned about the complex ways in which sub-lethal concentrations of SO_2 affects plant growth.

Investigations on the effect of SO_2 on trees started with the studies of Schroer in 1873. He observed that coniferous needles are more resistant as compared to deciduous trees. Later he revised his opinion and suggested that coniferous trees may be more sensitive because of their needle longivity. In North America and western countries, most of the studies have been carried out with coniferous trees, e.g. Pinus strobus, Pinus contorta, Picea abies, Ulmus americana, Picea glauca, Abies balsamea etc. Some studies have been also carried out with few broad leaved-trees e.g. Betula sp', Populus sp', Larix sp', Prunus sp. etc. (Swannapinut and Kozlowski, 1980; Keller, Norby 1981). In all the cases it is reported that there is a gross simplification of the forests and high mortality of tree seedlings due to increasing stress of SO_2 in the atmosphere.

In India studies on the effect of air pollutants on plants started rather late and such studies have been carried out only at few institutions, namely, Banaras Hindu University, Jawaharlal Nehru University, Lucknow, Bombay, Ujjain, Baroda, Calcutta and Kanpur.

The effects of sulphur dioxide on trees depends on many factors, e.g. climatic (temperature, moisture and light intensity), edaphic and biotic. Freer-Smith (1985) studied the effects of SO_2 and NO_2 on Betula pendula and reported decrease in dry weight and premature leaf fall. Under poor light conditions, B. pendula leaf growth was favoured, and also showed greater phytotoxic effect of SO_2 and SO_2 and NO_2 was greater. In conditions of large PFD under high light conditions, and long days the toxic effects of SO_2 were lost. The results of this study suggest significant interaction between SO_2 effects and the photoenvironment. Genetic variability in the species is another important factor influencing the response of trees to SO_2 stress (Biggs and Davis, 1981; Genys and Heggested, 1978; Karosky and Steiner, 1981; and Ormorod (1972). For example, Dochinger and Jensen (1975) found that the growth responses of hybrid poplar (Populus deltoides Bartr x P. trichocarpa Torr and Gray)(P. pondderosax P. deltoides) clones were correlated with the degrees of foliar injury caused by chronic and acute exposures to SO_2.

Our knowledge on the effect of air pollution on trees is based on two types of studies, namely field studies and fumigation studies. Field studies are highly instructive but interpretation of the results are complicated because many other gaseous pollutants like NO_X, O_3, hydrocarbons and particulates are usually present besides sulphur dioxide. In order to overcome such problems experimental fumgiation studies with sulphur dioxide have been undertaken to access its impact on plants using tree saplings Kozlowski (1981), Suwannapinut (1980), Norby and Kozlowski, (1981), Keller (1980), Jones and Mansfield (1982), Garsed, Muller and Rutter (1982), Constantiniduou, Kozlowski and Jensen (1976), Varshney and Garg (1981), Sahare (1984), Varshney and Varshney (1981), Rao et al. (1980).

The field study of trees is important as it provides a broad idea of the impact of air pollutants. Fumigation of tree saplings with sulfur dioxide provides more reliable information on plant responses at morphological, physiological and bichemical levels.

Morphological Effects

The visible leaf injury symptoms in plants due to SO_2 can be considered in three general categories (1) leaf tissue collapse with necotic patches (2) chlorosis or other colour changes and growth alterations. One of the most common effects of SO_2 is the plasmolysis of the cells and final collapse of the tissue. The affected areas generally dry out leaving the necrotic patches characteristics of the toxicant.

Chlorosis, the loss or reduction of chlorphyll is a very common and non-specific symptom in plants. The loss of chlorophyll results in the yellowing of the leaves. Sometimes other colours develop from pigments already present but normally masked by chlorophyll. Reduced growth or lack of normal vigour has been reported in plants suffering from SO_2 pollution. Constantinidou, Kozlowski, Jensen (1976), Rao et al. (1981), Varshney and Varshney (1981). Premature leaf fall in fumigated plants has been also reported.

The initial disruption of the cellular integrity usually appears in the spongy parenchyamal cells. Subsequently, the palisade layer is affected. These areas first appear water soaked, then become dry and papery and usually bleach to a light ivory or tan colour. The final effect is the formation of pattern of light-coloured intervenial blotches.

The reduction in plant height, number of branches, nodes and leaves, phytomass stem diameter leaf diffusive resistance has been also reported by many workers (Ashenden, 1979; Mansfield, 1977; Constantinidon, Kozlowski and Jensen, 1976; Marshall and Furnier, 1981; Varshney and Varshney, 1979 and Rao et al., 1981.)

Foliar injury

The details of foliar injury in tree species are relatively scarce but many workers have reported from time to time, (Yunus and Ahmed, 1981; Kasad, 1982; Giridhar and Chaphekar, Srivastava et. al., 1980; Ghouse and Khan, 1983; Rao, 1972; Shetye, 1979;

Pawar and Dubey, 1983; Singh and Rao 1983; Kramer and Kozlowski, 1979; Scheffer and Hedgcock, 1955, Kercher, Axelrod and Bingham, 1980).

The visible injury symptoms include pre-mature defoliation, necrosis, leaf margin and leaf tip burns, chlorosis and bronzing. The typical leaf injury pattern due to so_2 i.e. injury to the marginal or interveinal tissue in broadleaved plants extending irregularly from margins and tips towards the midrib, has been reported by some. In monocots the leaf injury extends froms tip towards the base of the leaf. Injury to the soft tissue of pulvinus leads to defoliation. It has been observed that the extent of foliar injury is a function of dose (concentration x time) of pollutants to which a plant is exposed. Many instances of foliar injury are due to episodal emission. In the latter case, a cloud of pollutants causes devastation of all plants in its path of travel, while the plants only a few meters away remain unharmed. Such observations of damage were made in Bombay repeatedly (Chaphekar, 1970; Chaphekar et al., 1980), as well as in Delhi during gas emissions from Sri Ram Fertilizers Ltd., during 1985 (Varshney/unpublished).

Tree species growing under the SO_2 stress show differences in the micromorphological features of leaves. Stomatal index, stomatal density, size of epidermal cells and trichomes, opening of stomatal apertures, cuticular striations, have been reported to be affected in plants of polluted areas. Yunus and Ahmed (1981) while working on P. guava reported that stomatal index and

stomatal density was high but epidermal cell and trichomes were smaller in trees growing in non-polluted sites at Lucknow. In case of Tabernaemontana coronaria (Srivastava et. al., 1980) stomatal size was smaller but stomatal frequency was higher in P. pinnata while the epidermal cells were smaller.

A good amount of work has been done on Mangifera indica L. Leaf injury was found to be proportional to pollution stress at the site (Rao, 1972; Shetye, 1979 and Giridhar, 1981). Reduced fruiting has also been assigned to air pollution.

Many workers have reported reduction in tree growth due to SO_2 pollution, Kramor and Kozlowski (1979); Scheffer and Hedgcock (1955)' Kercher. Axelrod amd Bimgham (1980); Ayazloo and Bell (1981), Garsed and Rutter (1982); Horsman, Roberts and Bradshaw (1979), Pawar and Dubey (1983), Rao et al. ((1972); Varshney and Garg (1982).

At present there is widespread concern and anxiety about the forest decline due to air pollution in North America and all over Europe. Since SO_2 affects tree growth, hence, the reduction in tree wealth may be an important biological indicator of pollution stress. Schroer (1973) pointed out that on the basis of short term fumigation studies conifer needles are more resistant to SO_2 than deciduous trees or herbs, but coniferous trees may still be more sensitive because of their needle longivity. In general, deciduous trees completely renew their photosynthesizing apparatus each year whereas confiers keep their needles for several years. Thus in deciduous trees fumigated with SO_2

develop visible symptoms quickly, shed their foliage but may recuperate and form new leaves when brought to pure air. On the other hand confiers often do not exhibit any visible symptoms of injury for a long time, until they suddenly drop their needles.

Garsed and Rutter (1982) investigated the effect of 3.12 and 124.8 u mol m^{-3} SO_2 on conifer populations. They obtained different orders of sensitivity after 35 and after 67 days, and stated that the order obtained at 8,000 ug m^{-3} (125 u mol m^{-3}) was virtually the reverse of that at 200 ug m^{-3} (31.3 u mol m^{-3}). They concluded that the relative sensitivity depends almost entirely on the concentration and duration of exposure and that "short-term fumigation at high SO_2 concentration cannot be used to predict responses to long term exposure to SO_2 in the field.

Garsed, Mueller and Rutter (1982) fumigated pine seedlings (initially 3 years old) for 65 days with two peak concentrations (4.68 and 11.7 u mol m^{-3}) or with a constant lower concentration (0.036 ppm or 1.50 u mol m^{-3}). The peak concentrations lasted either 5 or 21 hr., and were applied at intervals of 1 or 22 days respectively. The effect on growth expressed as excess of dry weight gain over that of the control; during fumigation, indicated that the short but frequent peaks no matter which of the two concentrations was used, depressed growth about as much as the constant and contineous fumigation.

Constatinidou and Kozlowski (1979) detected a slowing down of leaf expansion and a reduction in the number of emerging leaves in elm seedlings after fumigation for 6 hr at 2 ppm (83.2 u mol m^{-3}) of SO_2. It was also shown that an increase in temperature (Norby and Kozlowski, auther, year 1981) or in humidity during fumigation (Norby and Kozlowski, 1982) increases sensitivity. Jones and Mansfield (1982) have shown importance of light as the modifying factor, as in low light intansity in winter increases sensitivity of grasses.

Keller (1980) reported that SO_2 fumigation to Picea abies reduced CO_2 uptake, decreased ring width, finally caused a decrease of wood production. When root growth in fumigated seedlings of Picea abies was investigated in the subsequent year, these conifers exhibited a carry over effect. Root growth evidently reacted more strongly than shoot growth; just as in herbs. Fumigation with 0.1 ppm of SO_2 did not cause any visible injury symptoms to the shoot, but root growth was depressed by 50 percent.

Similarly, Suwannapinut and Kozlowski (1980) found in seedlings of two broad leaf species (Populus sp, and Betula sp) that the inhibition of dry weight increment was more sensitive indicator of susceptibility in roots than in shoots. Even a short SO_2 peak affected root dry weight of elm seedlings (Constantanidou and Kozlowski, 1979) or of red pine seedlings Norby and Kozlowski, 1981). Likewise, Jensen (1981) detected in fumigated poplar cuttings that available photosynthate was used more for shoot growth than for root growth on the other hand,

the investigation by Garsed, Rutter and Pelton (1981) indicated that increment losses in scots pine seedlings root were smaller than those in the shoots.

In Fagus sylvatica after a winter time fumigation many terminal buds failed to break in the following spring. This may produce bushy stem whereas silvicultural practice aims at producing straight stems with wood of high quality (Keller, 1978). In other beeches it was observed that the long term fumigation not only depressed ring width, but also made the young trees more vulnerable to bending by heavy snow (Keller and Bedaputa, 1981).

A classic example of SO_2 effect on plant community structure in shown by studies around an iron-sintering plant near Wawa, Ontario, Canada. Dominant species in the forest around this point source of pollution includied Picea glauca Picea marina Abies balsamea Pinus banksiana Thuja occidentalis Larix laricina and Pinus strobus Acer spicatum and Pyrus decora occurred often as understory species (Gordon and Gorham, 1963). Severe SO_2 injury to plants was primarily restricted to a narrow strip northeast from the point source, because southwest winds predominated. Gordon and Gorham (1963) found that SO_2 induced successive deterioration of tree, shrub, and microflora layers of the plant community. They reported that forest was "peeled off in layer's" as the smelter was approached from the north east. Pinus strobus was the most sensitive tree species. Seedling of this species were not observed, within 48 km from the sintering

plant, and seedling of Picea glauca, Picea mariana and Populus tremuloides were not recorded within 24 km.

Excessive production of SO_2 by a smelter in Ducktown, Tennessee, resulted in elimination of all trees and shrubs in a 27 m^2 area. Nickel and copper smelters in Sud bury, Ontario, Canada, released up to several thousand tons of SO_2 daily, resulting in gross simplification of the surrounding forest. Mortality of Pinus strobus was recorded through an 1,865 km^2 area of surrounding boreal forest. The populations of Quercus petraia and Fagus sylvatica are declining near iron ore roasting furance in Bierdorf, Germany (Guderian and Kueppers, 1980).

In India Rao (1972) Shetye (1979) and Giridhar (1983) while working on Mangifera indica reported reduction in growth and biomass. Pawar and Dubey (1983) have reported reduction in the length of terminal branches and number of leaves per branch in mango due to high level of SO_2 concentrations. Upto 100% reduction in flowering and fruiting has been reportedc by them. Developmental lag in new branches from vegetative buds of young twigs has also been recorded by Giridhar (1983) in mango and Asupala (Polyalthia longifolia) growing in polluted areas of Bombay, Melilotus alba growing near a power plant in Kashmir, had smaller leaf area, smaller roots, and shoots as well as less biomass as compared to the plants of the same species growing in relatively pollution free area (Ghouse and Khan , 1983).

Yunus and Ahmed (1979) reported that Dalbergia sissoo Psidium guava Terminalia arjuna Cassia fistuala Cedrela toona, and Syzygium cumini, are more sensitive to SO_2 pollution whereas Azadirachta indica Ficus religios Piinceolobium dulce and Calotrpis procera are more tolerant. Sahare (1984) while working working on some tree species viz. Bauhinia variegata Caesalpinia phulcherima Leucaena leucocephala Tabranaemontana coronaria Ficus benghalensis Polyalthia longofolia Morous indica and Putrangiva roxburghii, reported that after 180 days of SO_2 exposure the plant height, number of branches, number of nodes, number of leaves and biomass, were reduced drastically over control. The maximum leaf injury was observed in Bauhinia variegata the minimum was found in T. Coronaria. The degree of injury was shown to be dependent on leaf age, and also on the exposure period of the entire plant. The findings of Guderian (1970) supported this view. Sahare (1984) concluded that naturally growing trees in polluted area did no develop any injury while the transplanted tree seelings, exposed to the same level of SO_2, suffered from foliar injury, even at low concentration of SO_2 under field condition within a short span of exposure . In the same way height of the pine seedlings was found to be reduced by SO_2 (Riding and Boxer, 1983). Number of leaves were significantly reduced in Ulmus americana seedlings exposed to 2 ppm SO_2 for 6 hr. (Constatinidou and Kozlowski, 1979).

The root biomass was more affected as compared to stem in B. variegata F. bengalensis and P. longofolia (Sahare 1984; Rao et al., 1981) noticed that dry weight of leaf, stem and root in fumigated plants was reduced. Reduction in stem and root dry weight was observed by Constantinidou and Kozlowski (1979) in Ulmus americana after five weeks of fumigation with 2 ppm SO_2.

Davis and Gerhold (1976) have classified North American trees according to their relative susceptibility (Table I). Newpar A list of Indian trees has also been compiled in Table 2 according to their Air pollution Tolerance Index (APTI) as proposed by Singh and Rao (1983). According to Singh and Rao (1983) Air Pollution Tolerance Index (APTI) represents a synthetic value of four different biochemical parameteres, namely leaf extract PH, ascorbic acid (A), total chlorophyll (T) and relative water content (RWC) and the following formula was used to calculate APTI values $ATPI = \frac{[A \quad (T+P)] + R}{10}$

Table 1: Relative susceptibility of trees to SO_2

Sensitive	Intermediate	Tolerant
Acer negundo var. intesius	Abies balsamea	Abies amabilis
A melanchier alnifolia	Abies grandes	Abies concolor
Betula alleghaniensis	Acerglabrum	Acer plantanoides
Betula papyrifer	Acer negundo	Acer saccharinum
Betula pendula	Acer rubrum	Acer saccharum
Betula populifolia	Alnus tenuifolia	Crataegus douglasei
Fraxinus pennsynvanica	Betula occidentales	Ginkgo biloba
Larix occidentalis	Picea engelamannii	Juniperus occidentalis
Pinus banksiana	Picea glauca	Juniperus osteosperma
Pinus resinosa	Pinus contorta	Juniperus scopulorum
Pinus strobus	Pinus monticola	Picea pungens
Poplus gradidentata	Pinus nigra	Pinus edules
Populusnigra 'italica'	Pinus ponderosa	Pinus flexiles
Poplus tremuloides	Populus balsamifera	Platanns x acerifolia
Rhus typhina		Populus x canadensia
Salix nigra	Populus deltoides	Quercus gambelli
Sorbus sitchensis	Populus trichocarpa	Quercus palustris
Ulmus parvifalia	Prunus armeniace	Quercus rubra
	Prunus virginiana	Rhus glabra
	Pseudotsugamenziesii	Thuja occidentalis
	Quercus alba	Thuja plicata
	Sorbus aucuparia	Tilia cordata
	Syringa vulgaris	
	Tilia americana	
	Tsuga heterophylla	
	Ulmus americana	

Source: Davis and Gerhold (1976)

Rao et. al., (1983) have made an effort to categorieses the plant species on according to APTI Index, the APTI formula appeared to useful in providing guideline for classifying plants into sensitive, resistant and intermediate categories. However, an universally acceptable formula for categorisation of plants according to their sensitivity to air pollution is needed.

The effect of SO_2 have been studied in grasses and many dicot crops besides trees. Many workers have repored reduction in growth. Yield and productivity in grasses and other herbacious plants (Whit-more and Froeer - Smith; 1982; Crittenden and Read 1979; Ashanden and Mansfield 1979; Roberts, 1976; cowling and Lockyer, 1976; and Cowling and Lockyer, 1978).

Table 2: Relative susceptibility of some Indian trees to SO_2

Sensitive	Intermediate	Tolerant
Bauhinia variegata	Tamarindus indica	Ficus religiosa
Leucaena leucocephala	Psidium guava	Ficus glomerata
Delonix regia	Morus alba	Albizzia labbek
Dalbergia sissoo	Tabernae montana coronaria	Cassia fistula
Tectona grandis	Moringo aliefera	Pithecelobium dulce
Butea frondosa	Anthocephalus cadamba	Polyalthia logifolia
Bambusa bambos	Bombay ceiba	Ficus infectoria
Litchi chinensis	Madhuca indica	Nerium odorum
Nyctanthes asbortristics	Leucaena leucocephala	Eucalyptus citridora
Casuarina equisetifolia	Mangifera indica	Phyllanthus distichus
Grewia asiatica	Anona squamosa	Zizyphus jujuba
Alstonia scholaris	Syzigium jambolana	Azadirachta indica
Artocarpus heterophyllus	Acacia arabica	Phyllanthus emblica
Cordia myxa		Sapindus mukorossi
Feronica elephantanum		
Aegle marmelos		

Source: Singh and Rao (1983)

Grasses :

Impact of SO_2 pollution on grasses have been observed by many workers. Roberts (1976); Colvill (1983); Ashenden (1977); Ashenden and Mansfiedl (1979); Crittendedn and Read (1979); Bell, Rutter and Relton (1979) and Tingey (1979).

A ten month long investigation was conducted during 1978-79 and 1979-80 to determine the effect on the growth of ryegrass Lolium parenne L., by Colvill et. al., (1983) of both filtering polluted urban air and adding SO_2 to clean rural air. Four open top chambers and two unchambered plots were used at each of the two sides in NW England; St. Halens, Lancashire, a polluted urban site where on ambinet aire was Charcoal filtered in two of the chambes, and Ness Chesire a relatively less polluted rural site, where SO_2 was added to the ambinet air, in two of the chambers. There were no significant differences between the yields of grasses, gronw in an unfiltered (90 ugm SO_2) or filtered (35 ugm^{-3} SO_2) air at St. Halens. Reductions upto 29% in shoot yield was observed by Roberts et al. (1983) in rye grass (Lolium perenne L. V. 523) using open top chambers. For filtered and un filtered air in urban areas in north west England). The filtering capacity of these chambers produced a 56% reduction in SO_2

concentration over an 8-month study period and the reductions of urban SO_2 concentrations from 125 to 61 ug m^{-3} resulted in a significant 16% increase in shoot yield.

Crittenden and Read (1979); Bell, Rutter and Relton (1979) have that grass seedlings are much more susceptible than older plants to chronic SO_2 concentrations. Whitmore and Mansfiled (1983) have investigated this possibility by the simultaneous fumigation with 177 ug m^{-3} (2.77 u mol m^{-3}) of SO_2 for 7 months over winter or single, newly emerged seedlings and of 42 day old plants with three or four tillers, Poa pratensis Dactylis glomerata Lolium pernne (V 523, and CV 524 Eskimo) and Phleum pratense (V 548 and CV Eskimo). The plants were harvested in the spring, and it was found that Poa pratensis D. glomerata and Phleum pratense (v. 548 and CV Eskimo) were reduced in growth by SO_2 only if plants were fumigated from emergence. However, the two Loium cultivars showed the opposite effect, with significant reduction being limited to the older plants.

Whitomore and Freer-Smith (1982) demonstrated the effect of prolonged SO_2 fumigation. They fumigated Poa pratensis with 177 ug m^{-3} (2.77 u mol m^{-3}) of SO_2 in outdoor chambers for 11 months, starting in October and observed 64% reduction in dry weight from

January onwards upto March, but during the summer the adverse effect of SO_2 declined very much, and by the final harvest in September, it had been transformed into a significant stimulation of of 17% in comparison with controls. It is reported that low to moderate concentrations(.015 - 0.25) of SO_2 can increase the growth of sulfur deficient grasses (Cowling, Jones and Lockyer, 1973; Cowling and Lockyer, 1976; 1978).

Impact of SO_2 pollution on many shrubs have been studied by many workers. Roberts (1976); Dreisinger and Mc Govern (1970); Thompson et al. (1970); Taylor (1968): Tingey et. al., (1971), Varshney and Varshney (1981), Rao et. al., (1982).

The efacts of fumigation with 0.02 of SO_2 on Nicotiana tobaccum L. cv and Cuomia sativa L. cv. unikat were investigated for 4 weeks in fumigated chambers. There were significant reductions in the fresh weights of green leaves, shoots and roots, in the root/shoot ratio and leaf area (Mejarnik, 1980). Greater reduction was found in C. sativa than N. tobaccum. The reduction in fresh weight of root was 39% for C. sativus and 83% for N. tobaccum while reduction in leaf area was 5% and 46% respectively.

Tingey (1975) studied the effect of O_3 and SO_2 singly and in combination to examine the effect of gas mixtures on plant growth in Raphanus sativus, Nicotiana tobaccum and Madicago sativa. He observed that reduction in growth was equal to the additive effect of the two gases in N. tobaccum less than the additive effect in M. tobaccum and not different from effect of individual gases in R.sativus under chronic exposure.

Soybean (Glycine max) showed reduction in dry weight per seed (Reich et al., 1982) by the application of low dosage of O_3 and SO_2. Corresponding decrease in total dry weight per plant was also observed. Bleasdale (1973) has shown 16 and 57% reduction in weight in S23 ryegrass grown in polluted air over plants grown in similar air but first passed through a water scrubber. Plants which were treated for a part of each day with polluted air were heavier and had more leaves and tillars than plants grown continuously in either scrubbed or polluted air. No leaf lessions appeared but leaf senescence was acclerated by polluted air. Shoot may clog stomata and may produce necrotic spots if it is carried with a soluble toxicant such as excess acid, sulphuric acid, aerosols cause leaf spots. Milchunag and Lauenroth (1983). Significantly lower values for number of seeds

per cone were recored in white pine growing in the polluted area (Houstin, 1977). In red pines cone length, percentage of filled seed, seed germination, filled pollen pollen germination and pollen tube length were significantly lower in the plants of polluted area, in read pine.

Pandey (1983) made an attempt to assess the effect of Obra Thermal Power Plant on its surrounding area with special reference to vegetation. He observed chlorosis and necrosis in the leaves of a number of tree species. An increase in leaf weight and increase in the average photosynthtic areas per leaf and decrease in the percentage of injured leaf areas was observed as the distance from the power plant increases. He also observed a gradual increase in the calorific value of leaves, as one moves away from power plant.

Varshney and Varshney (1982) while working on Phaseolus radiatus L., Brassica nigra L., and Zea mays, observed after six weeks of SO_2 fumigation (3-10 pphm) , that initially with low concentration the leaf biomass increased by 0.3 percent, but on higher concentration the leaf biomass was decreased by 1-4.5 percent. Zea mays is relatively more resistant than P. radiatus and B. nigra.

Among angiosperms it can be broadly said that even at low concentrations and short periods of exposure herbs and grasses show injury symptoms. However, Cowling and Lockyer (1976) have reported So_2 can increase the growth of sulfur deficient grasses. However, short exposures of low concentration of pollutants do not produce such type of injury in trees. Prologed chronic exposure to even moderate concentration of pollutants, reduces growth in tree , but foliar injury appears very late (Taylor, Leninger and Hoard, 1980) . The response of tree sapling to pollutants is however, different from mature trees. Growth of tree seedlings has been shown to be affected even under short exposures to moderate concentrations, of pollutant! Prolonged exposure, to low concentrations of SO_2 affects the growth of tree salings drastically. Constantinidou, Kozlowski and Jensen (1976); Garsed, Rutter and Relton (1981); Gordon and Gorham (1963); Guderion and Kueppers (1980); Whitmore and Freer-Smith (1982); Le Blank and Rao (1979), Varshnbey et. al., (1981).

Physiological and Biochemical Effecgts :

Various physiological and biochemical activities of plants such as photosynthesis, respiration, transpiration, nitrogen fixation, reproduction and enzyme activity are adversely affected by SO_2 (Malhotra, 1977; Ma et al. 1973; Bull and Mansfield, 1974; Varshney and Varshney , 1984). Now it is well know that ecophysiological and biochemical changes are likely to occur much before the appearance of any visible changes. Plants exposed to sublethal concentrations of SO_2 may appear green and normal but their biochemical and physiological processes are subdued and they generally function at a reduced efficiency. The invisible changes caused by non-necrotic SO_2 exposure can can however, be detected by metabolic level by examining certain biochemical parameters.

Photosynthetic pigments

At ultrastructural level SO_2 can disrupt the chloroplast structure. Swelling of thylakoid membrance, reduction of grana lamellae, agranulation of chloroplast stroma, stretching of chloroplast envelope (Lebera et al., 1973; Fischer et al., 1973; Mlodzianowski and Bialobok, 1977; Soikkeli and Tuovenin, 1979; Soikkeli, 1981; Mausch et al., 1973; Phalichs, 1972). The most

important changes in chlorplasts is the reduction in both the photosynthetic pigments viz., carotenoids and chlorophyll. The reduction in these photosynthetic pigements finally reduces the photosynthetic activity of plants.

CHLORPHYLL

SO_2 exposure to plants reduces the cheorophyll content drastically. A 11.6% reduction in chlorophll content of Anacardia indica leaf, due to the effect of SO_2 was observed by Pol et. al; (1982). Reduction in Chlorophyll was observed by Garg and Varshney (1983) in Medicago sativa., Triticum aestivum and Zea mays . Risks and Williams (1975) showed higher chlorophyll a degradation in comparison with chlorophyll b, in the leaves of Quorcus oatraca grown under particulate pollution stress. Chlorophyll content reduction was observed by Sahare and Varshney (1984) in Bauhinia variegata, Polyathia longifolia, Cesalpinia phulcherima Tabernae montana coronora, Lencaena leucocphala, Morus indica and Putrangiva roxburghi. They exposed the seedlings for 180 days at polluted site of I.P. power plant.

Within the Chloroplasts, the Chlorophyll pigment may undergo several photo chemical reactions such as oxidation, reduction, phaeophytinization and reversible bleaching (Vernon and Seely, 1966). The Chlorophyll a have been found to be more affected than Chlorophyll b both under *in vivo* and *in vitro* studies (Bortitz, 1964, Katz and Shore, 1955). The conversion of Chlorophyll to Phaeophytin following the fumigation with SO_2 has been observed by Rao and Le-Blanc (1966).

Recent studies suggest that the SO_2 effect on pigment breakdwon , and the photosynthesis is very specific and is not only due to increased acidity. Malhotra et al. (1977) reported in *Pinus contorta* that below 100 ppm SO_2 in solution had no effect on Chlorophyll a or phaeophytin. But at lower concentrations of SO_2 (10-50 ppm), there was increase in Chlorophyllase activity and Chlorophyll b was converted to the corresponding Chlorophyllide b (the - ide indicates the porphyrin without the alcohol side chain). The chlorphyllase converts the Chlorophyll to Chlorophyllide by the removal of the phytol group. Willstatter and Stoll (1910) dicovered this enzyme and observed that it was intimately associated with Chlorophyll and its properties were also influenced by light.

Recently, Sugahara et al. (1980) observed that in vitro water-soluble protein complexes of chlorphyll and Chlorophyllide were stable and were not destroyed by even 40 mM SO_3^{-2}. The photo-conversion of the dark form of Chlorophyll a and Chlorophyllide, a protein complex (CP 668) to the illuminated form (CP 743) inhibited by SO_3^{-2}. The inhibition occurs apparently due to irreversible denaturation of protein complex. Probably caused by the destruction of disulphide bonds.

R-S-S-R + SO_3^{-2} + R - S SO_3^{-2} + R - S (Ceil and Mc. Phee, 1955) Peiser and Yang (1977; 1978) have demonstrated that rapid in vitro Chlorophyll destruction is caused by free radicals produced during the oxidation of HCO_3^- catalyzed decomposition of linoleic acid hydroperoxide.

Chlorophyll reduction has been reported by Gar and Varshney (1983) in Medicago sativa, Triticum aestivum and Zea mays. Dubey et al. (1982) evaluated the chlorophyll damage in North Betul Forest Division at three sites due to SO_2 pollution from Satpura Thermal Power Station, Sarni, M.P. In Adina cordifolia, Buchanania spreng and Dispyros melanoxylan. with the increasing concentration of SO_2 chlorophyll reduction was more in B. lanzan and A. cordifolia than in D. melanoxylon. Vij et al. (1981) have

reported chlorophyll reduction in Adina cordifolia Buchanania lanzan Diospyros melanoxylon Madhuca laifolia and epiphytic Vanda sp. in plants upto 3 to ten Km distance from the Sarni power plant.

Rao et al. (1983) fumigated twelve species of one year old tree saplings to SO_2 for 4 hr daily for 5 days a week for 5 months from February to July. They observed that the total chlorophyll levels in these plants were lower than the control and decreases with increase in SO_2 concentration. They observed Dalbergia sissoo and Madhuca indica as highly sensitive.

Carotenoids

Carotenoids have been found to be affected by sulfur dioxide fumigation. Many workers (Folk and Gigme, 1958; Rabe and Harris, 1963; Hocking and Hocking, 1977) have reported that sulphite ion formed in the plant cell under sulfur dioxide fumigation decreases the carotenoids pigments by oxidation.

In India, only a few studies have been conducted for studying the effects of SO_2 on carotenoids. Agrawal et al. (1982; 1983) while studying of SO_2 singly land in combination with ozone on Vicia faba, Panicum miliaceum solanum melongena, Cicer arietinum and Oryza sativa. Found reduction in carotenoids contents in plants fumigated with SO_2 and ozone alone as well as in combination. The carotenoid reduction was more in plants were fumigated with SO_2 and the effects of ozone was less servere than sulfur dioxide (Singh and Rao, 1982).

Photosynthesis :

Lebra, Ziegler and Ziegler (1973) observed that exposure of isolated spinach chlorplasts to low concentrations of sulphite (below > 1mM) produced a stimulation of carbon fixation. High levels of sulfite (upto 3) stimulated photosynthetic electron transport but inhibited carbon fixation. It has been observed that sulphur dioxide (>0.2 ppm) promote yield in Medicago sativa (Thomas et al. , 1943) and net photosynthsis in (Katz, 1949). But continuous exposure to low concentrations (0.15 - 0.45 ppm) of SO_2 are known to bring about premature senescence (Guderian, 1977). At high sulfite concentration the rate of carbon fixation was still high than the control, however, yield had dropped below the control plants. Miszalski and Ziegler (1979) showed

that exposure of whole spinach plants to 0.67 ppm (1.8 mg m^{-3}) sulphur dioxide for 1 hour enhanced thiol groups in chloroplast membranes and increased light activation of NADP-GPD. Paul and Bassham (1971) demostrated stimulation of carbon fixation by sulfite in isolated cells of the opium poppy (Papaver somniferum). Pierre (1977) and Pierre and Queiroz (1982) have shown that the activity of several enzymes present in the soluble phase of leaf extract of whole bean plant exposed to low concentrations of SO_2 (0.1 ppm) increased over control.

The sulphur dioxide affects photosynthesis in various ways and it can be discussed under two broad categoreis, viz. a) photochemical processes and b) biochemical processes. The following three aspects are concerned with the effects of sulphur dioxide on photochemical process.

1. Fluorescence
2. Hill reaction
3. Photosynthetic electron transport

Fluorescence: Fluorescence is the rapid emission of light from

excited molecule chlorophyll. Arndt (1974) stated that to study the effects of SO_2, in vivo, chlorophyll fluorescenc is one of the useful tool. The effects of sulphur dioxide on the variable fluorescence spectra have been studied by Hallgren et. al. (1978). At pH 8.0 and 1.0 mm SO_3^{-2} increases the fluorescence yield of spinach chloroplasts, but the opposite effect was observed at pH 6.2, where HCO_3^- dominates (Hallgren, 1978). Arndt (1974) has noticed both a slight SO_3^{-2} stimulation of fluorescence at low concentrations and a decrease at higher concentations (1 mM 10^{-3} M), indicating two oxidizing and reducing agents different modes of action of this compound on the electron transport chain in photosynthesis.

Hill reaction: Sulphur dioxide disrupts the thylakoids and grana. Such disruptions are likely to have important consequences on the activities of PSI and PSII. As PSI and PSII are both localized in the memberanes of chlorplasts (Boardman, 1968), the Hill reaction activity decreases (photoproduction of oxygen by chloroplasts).

$$A+H_2O \xrightarrow[\text{Chloroplasts}]{hv} AH_2 + 1/2\ O_2$$

The isolated chlorplasts from needles of Pinus contorta (Lodge pole pin) were treated with (50-100 ppm) of aqueous

sulphur dioxide showed that, at a low conentration , sulphur dioxide stimulated Hill reaction but 500 to 1000 ppm of SO_2 completely inhibited the Hill reaction activity.

3. Photosynthetic electron transport: Recent studies have shown that fumigation with SO_2 at 1 and 2 ppm for 1 hr produced no effect on 2.6 dichloro-indophenol (DCIP) photoreduction (Hill reaction). However, there was rapid inhibition following long exposures (for 3-6 hr at 1 and 2 ppm). Shimazaki and Sughara (1980) investigated the site of sulphur dioxide attack (at 2,0 ppm for 5 and 1.0 ppm SO_2 for 6 hr) in the electron transport systems by studying both photosystems. Electron flow from H_2O to DCIP was inhibited while from DCIP to NADP to the same degree as the electron flow H_2O to DCIP. These results, suggest that SO_2 inhibited the electron flow driven by PSII but not by PSI. A similar effect of SO_2 was observed on the photosystems of Latuca

sativa chlorplasts (Shimazaki and Sughara, 1980). Studies on isolated chloroplasts from SO_2 exposed leaves of Latuca sativa demonstrated that the site of sulphur dioxide action was located close to the oxidizing site rather than the reducing site of PSII (Shimazaki and Sughara, 1980).

In India, work related to effect of sulfur dioxide on photosynthesis has not been done so far. Recently Varshney I (1987) have studied the effect of SO_2 pollution on the rate of net photosynthesis of various tree species. Varshney (1988) observed that net photosynthesis in Lycopersicum esculentum reduces 2 to 50 percent on fumigation with 0.1 and 0.2 ppm of sulphur dioxide.

Ribulose bisphosphate carboxylase activity:

The enzyme ribulose diphosphate carboxylase catalyzes the covalaent insertion of CO_2 and simultaneous cleavage of the 5 carbon sugar ribulose, 1,5-diphosphate to form two molecules of 3-phosphoglycerate, one of which bears the isotopic carbon introduced as CO_2 in its carboxylase group. This enzyme has a very complex structure, a molecular weight of 550,000 and is located onthe outer surface of the thylakoids memberane. It makes up about 15% of the total chloroplast protein. Ribulose biphosphate carboxylase is the most abundant enzyme in the biosphere. It is the key enzyme in biomass production from CO_2 in the plant world.

RuBP carboxylase is a complex enzyme having two functional activities. It can either catalyse carboxylation of the substrate RuBP to form two molecules of 3-phosphoglycerate or it can catalyse reduction with oxygen to give one molecule of 2-phosphoglycolate and one molecule of 3-phosphoglycerate. Lebera, Ziegler and Ziegler (1975) demonstrated that, with isolated chloroplasts and concentrations of sulphite greater than 1

mM, fixation of $14CO_2$ declined rapidly and at 5 mM it was reduced to 20%. The relative amounts of radioactivity in phosphoglycerate and sugar phosphate were decreased whereas those in asperate and malate were increased. This indicated a possible shift towards the C-4 dicarboxylic type of fixation and may indicate a higher sensitivity of RuBP carboxylase than that of PEP carboxylase towards sulphite.

Hersham and Wellburn (1975) exposed Pisum sativum var. Faltham in air polluted with known amount of SO_2 and/or NO_2 for six days under constant conditions of temperature, light and relative humidity. At the end of this period RuBP carboxylase was extracted and assayed, whilst little change was observed at lower concentrations. At concentrations in excess of 1.5 to 2.0 ppm SO_2, RuBP carboxylase activity was reduced NO_2 was found stimulate at concentrations greater than 1.0 ppm. Stimulation has also been observed with tomato plants exposed to 0.4 to 0.5 ppm or NO_2.

Miszalski and Ziegler (1980) have investigated the use of RuBP carboxylase in plant material as a measure of toxicity to SO_2 exposure. These experiments had the advantage of using an assay pattern for RuBP carboxylase which ensured full activation and optimum catalytic rates. Miszalski and Ziegler (1980) confirmed that RuBP carboxylase activity was decreased at higher concentration of SO_2. Additional proof is provided by Hallgren and Gezelins (1982) who showed that fumigation with 'low' SO_2 concentration (400 ug SO_2 m^{-3} 0.15 ppm) decreased RuBP

carboxylase activity when expressed on a dry weight basis. However, no significant difference was observed between fumigated and control plants when the enzyme activity was calculated on protein basis. This indicates a decrease in the amount of active enzyme present rather than in its specific activity, but the reason for the decrease is not known. Any inhibition of the enzyme activity by SO_2 would probably be lost during extraction procedure.

Direct effects may be best observed in the third type of investigation in which the effect of pollutants on the catalytic activity of isolated enzymes in vitro has been studied. In view of the similar size and structure of the molecules SO_2 and CO_2 the influence of SO_2 on RuBP carboxylase has been extensively studied. Since SO_2 is largely thought to be active in the form of SO_3^{-2}, the effect of dissolved SO_3^{-2} has been investigated on RuBP carboxylase. Ziegler (1972) found SO_3^{-2} inhibited RuBP carboxylase competitively with respect to bicarbonate, presumably SO_3^{-2} replaces HCO_3^- by reacting at the same enzyme site. SO_3^{-2} showed a non-competitive inhibition with respect to RuBP and Mg^{2+}. The non-competitive type of inhibition suggests that SO_3^{-2} does not react with the keto group of RuBP. Since SO_2 binds to the enzyme in the same way as CO_2, the degree of inhibition by SO_3^{-2} will be independent of the RuBP and Mg^{2+} concentrations but highly independent on the concentration of CO_2 at the reaction site. If this is the case then it follows that in plants with the C-4 type of photosynthesis and an increased concentration of CO_2 in the bundle sheath cells, SO_2 should be a less powerful

inhibitor. Gezelius and Hallgren (1980) demonstrated an inhibitory effect on 10 mM SO_3^{-2} of approximately of the same order as for 10 mM SO_3^{-2}. Paulsen and Lane (1966) found that ammonium sulphate inhibited RuBP carboxylase competitively with respect to bicarbonate; presumably SO_3^{-2} replaces HCO_3 by reacting at the same enzyme site. SO_3^{-2} showed a non-competitive inhibition with respect to RuBP and Mg^{2+}. The non-competitive type of inhibition suggests that SO_3^{-2} does not react with the keto group of RuBP. Since SO_2 binds to the enzyme inthe same way as CO_2, the degree of inhibition by SO_3^{-2} will be independent of the RuBP and Mg^{2+} concentrations but highly dependent on the concentration of CO_2 at the reaction site. If this lis the case then it follows that in plants with the C-4 type of photosynthesis and an increased concentration of CO_2 in the bundle sheath cells, SO_2 should be a less powerful inhibitor. Gezelius and Hallgren (1980) demonstrated an inhibitory effect on 10 mM SO_4^{-2} of approximately of the same order as for 10 mM SO_3^{-2}. Paulsen and Lane (1966) found that ammonium sulphate inhibited RuBP carboxylase competitively with respect to RuBP and suggested that there was competition between the phosphategroup of the RuBP and the SO_4^{-2}ion. These results have recently been confirmed by Parry and Gutteridge (1983) who found a Ki of 1mM SO_4^{-2} suggesting that SO_4^{-2} is an inhibitor of RuBP binding. A mixed pattern of inhibition with respect to HCO_3^- was observed. Gazelius and Hallgren (1980) also examined crude extracts of spinach (Spinacea oleracea)using the same assay conditions as Ziegler (1972) and found that SO_3^{-2} was a less potent inhibitor than claimed previously. They observed Ki values with respect to

HCO_3^- between 9 and 13 mM compared with 3 mM found by Ziegler and found in addition that the pattern of inhibition was non competitive. Part of the confusion between these results may well be due to the conditions under which the enzyme is assayed. It has only recently been shown by Lorimer, Badger and Andrews (1976) that the enzyme must be pre incubated with Mg^{2+} and CO_2 to be in a fully activated stage and before the true affinity with respect to the bicarbonate can be observed. In recent experiments (Parry and Gutteridge, 1983), special attention has been given to ensure that the enzyme was fully activated. They found using enzyml purified from wheat and spinach that the inhibition of catalytic activity was complex. In the presence of SO_3^{-2} the time course for the reaction was biphasic so that over the first 30S carboxylation occured rapdily with little inhibition, but this rate declined over the next 2 min. to a much lower constant value. With higher concentrations of SO_3^{-2} the inactivation was more marked. The biphasic curves showed changes both in ;the apparent patterns of inhibition by SO_3^{-2} and in the kinetic constants with time. Thus, the inhibition pattern for SO_3^{-2} versus RuBP was mixed over the first period of the assay but became non-competitive over longer periods. The Ki increased from 2.5 mM SO_3^{-2} at 15s to 9mM at 4 min. The inhibition pattern for SO_3^{-2+} versus HCO_3^- was mixed throughout the assay period but the Ki decreasedfrom 8 to 1.2 mM during the assay. It is clearly important to follow the progress of the enzyme reaction in the presence of the inhibitor as a function of time, rather than attempt to deduce rates after a set reaction

period. Further studies to explain the nature of SO_3^{-2} inhibition indicated that preincubation of the substracte or enzyme with SO_3^{-2} prior to initiating the reaction did not alter the biphasic form of the reaction curves, moreover, no potent inhibitor was accumulated during the course of the reaction, since a further addition of the enzyme produced a two-phase curve almost identical to the first. The results of Parry and Gutteridge (1983) suggest that the chemistry of the catalytic reactions are so affected that some form of the enzyme common to both the carboxylase and oxygenase rections becomes modified in such a way that further substrate turn over proceeds at a much reduced rate. The effect of the progressive inactivation of the enzyme even by low concentrations (1mM SO_3^{-2}) suggests that the potential effects of SO_2 on this enzyme may have been underestimated. Certainly the complex changes noted here provide the basis for reaction.

Effects of SO_2 on RuBisco activity is Indian plants have not been done studied so far. Recently (1988) have started to examine the effect of various pollutants on Ribulose bisphosphate carboxylase activity on different types of plants including Ponac tree species. Varshney (1988) have reported that in Lycopersicon esculentum RuBisco activity decreased to 2.85 to 4.62 percent and 6.33 to 17.1 percent fumigated with 0.1 and 0.2 ppm of sulphur dioxide (unpublished).

Ascorbic acid.

The water soluble vitamin C (ascorbic acid) is an enediol-lactone of an acid with a confirmation similar to that of the sugar, L-glucose. It occurs in two forms. P-forms are inactive while naturally occuring vitamin C is L-ascorbic acid. It remains present in all living cells. It has been reported that it is involved in cellular oxidation-reduction reactions.

Ascorbic acid has been reported to scavenge certain toxic oxygen species. It is known to be very powerful reductant responsible for the photochlorophillide (Rudalph and Bukasch, 1966). It acts as an election donor for the reduction of sulphur dioxide (Fig.1) and its capacity to do so is enhanced under illuminated conditions (Mapson, 1958;Rudolph and Bukatsch, 1966; Keller and Schwager, 1977). The reduction of SO_2 may lead to the formation of H_2S (Silvius et al. 1976, Fig.1), which has been shown to be given by plants, fumigated with SO_2 (De-cormis, 1968). Super oxide radical formation in the plant due to SO_2, as known earlier, also oxidizes ascorbic acid to dehydroxy ascorbic acid (Eestner and Kramer, 1973).

Conditions affecting ascorbic acid synthesis adversaly such as shadding, oxidizing gaseous pollutants create stress situation which in turn renders plants relatively more susceptible to air pollutants Ref. Indegenous levels of ascorbic acid are however, one of the important factors influencing plant resistance to gaseous pollutants.

The detoxification of SO_3^{-2} in the plant cell generally takes place by the oxidation of sulphite to sulphite and also by the reduction of SO_2. In SO_2 reduction ascorbic acid has been found to play an important role. Keller and Schwager (1977) reported that in plants ascorbic acid may influence dloxification of SO_2, Continued SO_2 fumigation decreases ascorbic acid content long before visible role of ascorbic acid as an electron donor in SO_2 reduction (Silvins, et al. 1976) injury symptoms appear. (Freebairn, 1960; Freebairn and Taylor, 1960); Rao et al., 1981) reported that the spray of ascorbic acid in the form of potassium ascorbate over SO_2 fumigated Vicia faba reduced the SO_2 toxicity and suggested that potassium ascorbate acts as an antidote to SO_2 pollution.

Low levels of SO_2 affects ascorbic acid content in plants. Varshney and Varshney (1982) while working on Bpaseolus radiatus observed 10.8, 15.6 and 21.17 percent reduction in ascorbic acid by fumigating 3, 5 and 10 pphm SO_2 for six weeks. They also observed reduction in the content of ascorbic acid in Brassica nigra and Zea mays respectively. Prasad (1980) exposed wheat (Triticum aestivum) plants between 20 and 100 day ages to 1 ppm SO_2, 1 ppm NO_2 and 1 ppm SO_2 + NO_2 in polythene chambers. He observed reduction in ascorbic acid content. Ascorbic acid reduction has been reported in (caesalpinino phvechermna), Bauhinia variegata, Tabernaemontana coronaria, Ficus bengalensis and Polyalthia longifolia. Putrangiva roxburghii plants exposed to air pollutants (Sahare, 1984).

As antitoxicant ascorbic acid and its salts have been used with same success (Freebairn and Taylor, 1960; Siegel, 1952). Nandi et al. (1981) used potassium ascorbate (0.02 M) ($C_6H_7O_6K$) as an antidote to SO_2 phytotoxicity. Root feeding of Ca and K salts of ascorbic acid is also known to reduce air pollution injury. (Freebairn, 1963). Agarwal (1982) used potassium ascorbate ($C_6H_7O_6K$) as an antioxicant for O_3, SO_2 and O_3 + SO_2 pollutans to which Vicia faba plants were exposed. She sprayed 250 ml of 0.02 M $C_6H_7O_6K$ solution at 45, 60, 75, 90 and 105 days old plants, exposed to 0.5 ppm SO_2 0.08 ppm O_3 and 0.25 + 0.04 ppm SO_2 + O_3 pollutants for 1.5 hr. daily between 40 and 100 days of their life cycle. Foliar injury was seen in plants exposed to pollutants but the degree of injury in sprayed plants was much lower as compared to unsprayed ones.

CHAPTER - 2

MATERIALS AND METHODS

MATERIALS AND METHODS

Plant material :

Twelve month old saplings of the six plant species, viz., Bauhinia variegata, Linn Ficus bengalensis, Linn Ficus infectoria, willd. Sensu Roxb. Ficus religiosa, Linn Pongamia pinnata pierre and Psidium guava Linn. were selected for sulphur dioxide fumigation experiments.

Twelve uniform plants of individual species were selected from JNU ecological garden and private nursuries. They were planted in earthern pots (height 15 cm) containing equal amount of organic manure and soil.

Fumigation Chamber :

A dynamic fumigation chamber made of glass having 1 m^3 capacity was used in this study. The chamber was air tight with an inlet at the base and an outlet at the top on opposite sides. A small electric fan of 9" size was fixed in a corner of the chamber to ensure uniform mixing. The flow of air-gas mixture into the chamber was monitored with the help of a rotameter and kept constant at 1.55 l min^{-1}.

Sulphur dioxide generation :

Sulphur dioxide was generated by bubbling air at a constant rate of 1.55 l min^{-1} in an impinger containing a 100 ml of the aqueous solution of sodium metasulphite of desired strength. The sequence of reactions leading to sulphur dioxide evolution are as follows :

$$NaHSO_3 \quad - \quad Na^+ + HSO_3^- \qquad (1)$$

$$HSO_3^- + H^+ \quad - \quad H_2SO_3 \qquad (2)$$

$$H_2SO_3 \quad - \quad H_2O + SO_2 \qquad (3)$$

The sulphur dioxide was introduced into the fumigation chamber through an inlet. The SO_2 concentration was in the fumigation chamber was determined by drawing gas samples from the exist port of the fumigation chamber through Q 10 ml 0.4 percent aqueous solution of potassium tetrachloromercurate (TCM) for ten minutes. The dichloro-sulphitomercurate complex is made to react with pararosaline and formaldehyde making a complex pararosaline methyl sulphonic acid of pink colour. The intensity of the colour was measured spectrophotometrically at 548 nm and optical density was measured and converted in sulphur dioxide concentration was calculated using the formula described by West and Gaeke (1956) as :

$$\text{ug } SO_2\ m^{-3} = \frac{(A - Ao)\ X\ (10^3)\ X\ BS}{Vr} \times D$$

where,

A = sampler absorbance

Ao = reagent blank absorbance

10^3 = conversion of litres to cubic metres

Vr = the sample volume corrected to 25°C and 760 mm Hg litres

Bs = caliberation factor, ug/absorbance unit

D = dilution factor

The values obtained in ug SO_2^{m-3} were multiplied with 3.82×10^{-4} for converting the SO_3^{-2} concentration in ppm.

Complete scrubbing of sulpher dioxide from the air stream was achieved by passing the gas current through two bubblers connected in series containing TCM solution. The concentration of sulphur dioxide in the gas stream is dependent upon the strength of sodium metabisulphite solution at a given rate of air-flow.

Three sets of plants were subjected to fumigation with 0.2, 0.5 and 0.7 ppm of sulpher dioxide for 2 hr. daily for 65 days and one set of each species was kept without treatment to serve as control. Pots were regularly irrigated during the experimental period. At the end of 65 days of fumigation observations were made on morphological, physiological and biochemical parameters in addition to biomass measurements.

Morphological parameters :

Visible folioar injury

The plants particularly leaves were carefully examined to detect visible injury symptoms in fumigated plants. Presence of Chlorotic necrotic spots on leaves recorded and plants subjected to different concentration of SO_2 fumigation were compared .

Plant height

Plant height of control and fumigated plants was measured. The measurements of were made before starting the fumigation treatment during the fumigation period at regular intervals and the last measurement was made after the fumigation treament was over.

Leaf dynamics

Leaf dynamics was examined interms total number of new leaves produced and the number of leaves abscised and dropped during the fumigation period. The number of leaves per plant was counted at a regular interval and last counting was carried out after 65 days of exposure . Leaf counting was done after 15, 30, 45 and 65 days of fumigation. Dried leaves at the base was also counted, to note down the leaf fall.

Shoot biomass (leaf biomass + stem biomass) and root biomass:

Shoot and root biomass of control and SO_2 fumigated plants was measured after drying for 24 hours at 80^{oC} in an electric over.

Physiological and biochemical parameters were evaluated after 65 days at the end of fumigation experiment.

Chlorophyll estimation

Fresh leaves weighing 0.5 g were homogenized in 20 ml of 80 percent acetone (acetone = water v/v) in a mortar. The homogenate was filtered through a double layered muslin cloth. The filterate was centrifuged at 3,000 g for 15 minutes. The supernatant was made upto 100 ml with 80% acetone and the optical density of extract was measured at 645 and 663 nm wavelength using a spectronic 20 Bausch and Lamb Spectrophotometer (USA). The chlorophyll a and chlorophyll b were determined by using the formula described by MacLachlan and Zalic (1963). The values of

chl a and chl b were added to get total chlorophyll.

Chlorophyll a (mg g^{-1} fresh leaves)

$$= \frac{12.3\ D\ 663 - 0.86\ D\ 645}{dx\ 1000\ x\ w} \times V$$

chlorophyll b (mg g^{-1} fresh leaves)

$$= \frac{19.3\ D\ 645 - 3.6\ D\ 663}{d\ x\ 1000\ x\ w} \times V$$

where,

d = length of light path in the cell (usually 1 cm)

w = fresh weight of the leaves (g)

v = volume of the chlorophyll extract in acetone (ml)

Carotenoids estimation - Two gram freshly harvested leaf was homogenized with 20 ml of acetone. The extract was filtered through a Buchner funnel using Whatman No. 42 filter paper. Pooled the filtrates and partitioned with equal quantity of peroxide free ether thrice using a separatory funnel. The either phase contained carotenoids. Evaporated the combined ether extracts under reduced pressure at 35°C on a hot water bath. Dissolved the residue in minimum quantity of ethanol, and added 60% KOH. This removed chlorophylls and interfering lipids and also cleaved the esterified carotenoids. Kept the mixture in the dark and left it overnight at room temperature. Added equal amount of water and partitioned twice with peroxide-free ether. Evaporated the ether under reduced pressure and dissolved the residence in a minimum volume of ethanol. After this measured

the absorbance of this solution at 450 nm in a calorimeter, and calculated the carotenoid content .

$$C = \frac{D \times V \times f \times 10}{2500}$$

where,

C = total amount of carotenoid in mg

D = absorbance at 450 nm in a 1.0 cm cell

V = volume of the extract in ml

f = dilution factor, and

2500 = average extinction coefficient of the pigments

Ascorbic acid :

The ascorbic acid content of leaf tissue was estimated according to the method given by Tillman et al. (1932). In oxidized form 2.6 - dichlorophenolindophenol (DCPIP) is purplish blue' in neutral or alkaline medium and pink in an acid solution. When reduced it is colourless. The following reagents were required :

a) Oxalic acid solution (0.5 percent): 5 g of oxalic acid was dissolved in 1 litre of distilled water to obtain a strength of 0.5 percent oxalic acid.

b) Ascorbic acid standard solution: 100 mg of ascorbic acid was dissolved on 0.5 percent oxalic acid and made upto 500 ml. The solution is unstable so the dye was standardized immediately.

c) 2,6 - dichlorophenol indophenol solution (DCPIP): 50 mg sodium salt of DCPIP was taken was dissolved in 150 ml of distilled water. The DCPIP solution was placed in an oven which was set at 80°C for 5 minutes, and it was stirred with a magnetic stirrer. To it 42 mg $NaHCO_3$ was added. When latter dissolved, it was decanted into a 200 ml volumetric flask. After cooling and filtering, the volume was made with distilled water. The dye was stored in a dark bottle. The bottle was kept inside a refrigerator where it remain stable for one week.

For standardization, 5 ml ascorbic acid standard solution was taken in a small beaker and placed over a sheet of white paper. The DCPIP which was taken in burette and titrated with the ascorbic acid solution until a pink end point was reached and which lasts at least for 15 seconds. As 5 ml of the standard ascorbic acid solution contains 1 mg of vitamin C, the burette reading is the amount of dye required to oxidize 1 mg ascorbic acid. The amount of ascorbic acid oxidized by 1 ml of the dye was then calculated.

A 0.5 g of fresh leaf tissue was homogenized in a pestle and mortar using 10 ml of 0.5 percent oxalic acid (extracting) solution. The slurry was decanted into a volumetric flash and made upto 20 ml with extracting solution. The homogenate was centrifuged at 1800 g for 15 minutes. A 10 ml extract was titrated with dye till the pink colour persists for at least 15 seconds.

The number of mg ascorbic acid per g sample was calculated

as :

$$\frac{V \times T}{W} = \text{mg ascorbic acid 1 g sample}$$

where,

V = ml dye used for titration of extract of diluted sample

T = AA equivalent of dye solution expressed as per ml of dye

W = g of sample in extract titrated

Net photosynthesis :

Net photosynthesis was measured with the help of a portable photosynthesis system, LI-COR 6000, Lincoln, Nebrasaka, USA. For the measurement of net photosynthesis a fully sunlit heathy leaf near perpendicular to the sun was chosen. The leaf chamber of LI-6000 was installed after slightly (one litre size) elevating the CO_2 concentration in the leaf chamber CO_2. Logging was started with a time step appropriate for a CO_2 draw-down of about 30 ppm. All precautions mentioned in the mannual was carefully observed.

Ribulose bisphosphate carboxylase (RuBisCO) :

RuBP carboxylase determination was carried out following the method described by Marco and Tricoli (1983). It was determined by an enzymic estimation method in which D-3-pGA formed.

The reagents in this method, as used were Ribulose-1-S-bisphosphate (RuBP), bicine, mercapto ethanol, phosphocreatine,

creatine phosphokinase, glyceraldehyde 3-phosphate dehydrogenase (GADPH), phosphoglycerate kinase (PGK) etc. All reagents were obtained from Sigma Chemical Company, USA.

One gram leaves were homogenized in a mortar with glass beads in 10 ml per gram of 100 mM bicine (pH 8.2), 10 mM $MgCl_2$, 5 mM $NaHCO_3$ and 5 mM mercaptoethanol. The extract after filteration through cheese cloth was centrifuged in K-24 centrifuge at 16000 x rpm for 50 min at $0^{\circ}C$. 0.1 ml of crude plant extract was incubated for 5 minutes. After 5 min of incubation in the reaction mixture, reaction was started by adding 0.5 u mol of RuBP in 50 ul of reaction buffer minus $NaHCO_3$. The reaction was stopped after 2 min by adding 100 ul of 1 M HCL.

The spectrophotometric assay was effected by performing the carboxylation reaction and then to the reaction mixture adding 100 ul of 1 M NaOH with 1.3 ml of 100 mM bicine pH 8.2, containing 5 mM mercaptoethanol. This reaction mixture was transferred to 3 ml quartz cuvette. To this mixture was added to 100 ul of 5 mM NaDH, 100 ul of 100 mM ATP. 50 ul of 200 mM phosphocreatine and 5 units of creatine phosphokinase to give a final volume of 2.4 ml. After recording the absorbance of this solution against a blank containing the same amount of NaDH in bicine on Beckman DU-20 spectrophotometer 5 units of phosphoglycerate kinase (PGK) and 5 units of glyceraldehyde phosphate dehydrogenase (GADPH) as a suspension in ammonium sulphate solution (10 ul) were added. The reduction of D-3-PGA went to completion in about 5 min at $28^{\circ}C$.

Following precautions were observed :

1. Homogenization of leaves should be carried out in chilled mortar and pastle so that heat caused by friction may not denaturate the enzyme RuBisCO.

2. pH of the reaction should be around 8.2

3. After centrifugation, the supernatant should be crystal-clear, as crude plant-extract is used for determining enzymic activity.

4. RuBP, NaDH, ATP, phosphocreatine are to be freshly prepared i.e. approximately half an hour before the enzymic assay has to be carried out.

5. Mercaptoethanol is to be added to buffer just before making use of the buffer.

6. Ribulose 1-5 bisphosphate dissolves in slightly acidic solution i.e. pH 4.5 - 5.9.

Homogenize 1 gm of leaves in 10 ml of 10 mM Bicine

+ 100 mM $Mgcl_2$

+ 5 mM $NaHCO_3$

+ 5 mM Mercaptoethanol

Filter the extract through muslin cloth centrifuge for 50 minutes on a K-24 refrigerated centrifuge

To 3 ml cuvette added 1.3 ml of 100 mM Bicine

+ 5 mM Mercapto ethanol (pH 8.2)

+ 200 ul of plant extract

Incubate for 5 minutes

Added 50 ul of RuBP (1.02 mg of RuBPin 150 ul of

+ 100 mM Bicine

+ 10 mM $MgCl_2$

+ 5 mM $NaHCO_3$

Stop the carboxylation reaction after two minutes by adding 100 ul of 1 M HC1

Neutralize, by adding 100 ul of 1M NaOH

Add 100 ul of 100 mM ATP

+ 50 ul of 200 mM Phosphocreatine

$\pm$ 5 units of creatine phosphokinase

+ 500 ul of NaOH

Read O.D. at 340 nm

5 units of phoglycerate kinase (PGK)

+ 5 units of glyceraldehyde phosphate dehydrogenase (GADPH) 10 ul.

Read change in O.D. for 5 minutes at 340

Fig. : RUBP carboxylase determination by enzymic estimation of D-3-PGA formed.

Statistical Analysis :

The variability or dispersion of the data was subjected to statistical analysis and standard deviation was calculated. The sum of squares of the deviations from the mean divided by number of observations. The square root of the resultant represents the value of standard deviation :

$$6 = \frac{(X - \bar{X})^2}{N}$$

where,

6 = standard deviation

= sign of algebric sum

X = observed value

$\bar{X}$ = mean of observed values

N = number of observations.

RESULTS

The effect of sulfur dioxide pollution on twelve month old tree splings of B. variegata, F. bengalensis, F. infectoria, F. religiosa, P. pinnata and P. guava was studied. Plants were daily fumigated for 2 hrs. with o.2, 0.5 and 0.7 ppm of SO_2 for 65 days, The SO_2 effects were evaluated in terms of visual injury, plant height, leaf dynamics, biomass, chlorophyll content, carotenoids content, ascorbic acid content, net photosynthesis and RuBisco activity.

Visible foliar injury

B. variegata, F. bengalensis and P. guava exhibited foliar injury while F. infectoria, F. religiosa and P. pinnata did not show any injury symptoms.

The maximum leaf injury was observed in B. variegata. Chlorotic and necrotic patches in the interveinal regions were observed after 0.2, 0.5 and 0.7 ppm of sulfur dioxide treatment. The plants subjected to 0.5 and 0.7 ppm of SO_2 showed maximum foliar injury,necrosis, bronzing and pre mature defoliation. Production of new leaves was also slowed down in fumigated plants.

PLATE NO.1

B. variegata saplings exposed to 0.5, 0.7 ppm of SO_2. Reduced growth, chlorosis and necrosis in the leaves of treated saplings are clearly visible.

PLATE NO.2

F. infactoria saplings exposed to 0.2, 0.5 and 0.7 ppm of SO_2. Reduced growth in 0.7 ppm treated saplings are clearly visible.

PLATE - 1

PLATE - 2

PLATE NO.3

F. bengalensis saplings exposed to 0.2, 0.5 and 0.7 ppm of SO_2 . Stunted growth and chlorosis in the leaves of treated saplings are clearly visible.

PLATE NO.4

P. guava saplings exposed to 0.2 and 0.7 ppm of SO_2. Reduced growth and chlorosis in the leaves of treated saplings are clearly visible.

PLATE-3

PLATE-4

PLATE NO. 5

F. raligiosa saplings exposed to 0.2, 0.5 and 0.7 ppm of SO_2. Reduced growth in treated saplings is clearly visible.

PLATE NO.6

P. pinnata saplings exposed to 0.2, 0.5 and 0.7 ppm of SO_2. Reduced growth in treated saplings is clearly visible.

PLATE-5

PLATE-6

In the case of F. bengalensis injury symptoms were somewhat different like plants fumigated with 0.2 ppm of SO_2 were free fromvisual injury while plants treated with 0.5 and 0.7 ppm showed maximum injury. The injured leaves were dull patchy and appeared somewhat like variegated type due to significant loss of chlorphyll.

In P. guava, no visual injury was observed in plants exposed to 0.2 ppm, but clear chlorotic spots were obsered in 0.5 and 0.7 ppm sulfur dioxide treated plants. The most severely injured leaves were observed in plants exposed to 0.7 ppm of SO_2 F. infectoria, F. religiosa and P. pinnata did not show any visible injury symptoms.

Leaf Dynamics

The criterion of the extent of injury due to SO_2 exposure was also observed in terms of number of leaves produced, damaged and dropped during the fumigation period , Data recorded in table - 3 show that SO_2 Fumigation has induced severe leaf damage and subsequently leaf abcission..

In the case of B. variegata the average number of leaves for control plants were 36.5± 1.00, 42.55±.2.5, 46.85±0.54, 49.48± 0.65, and 52.35± 0.75 at the beginning of the experiment and after 15, 30 , 45 and 65 days respectively. The average leaves produced during this period were 6.05, 10.35, 12.88 and 19.85 after 15, 30, 45 and 65 days respectively. No leaf fall was observed in control plants. In the 0.2, 0.5 and 0.7 ppm of

TABLE NO. - 3

Effect of SO_2 fumigation (0.2, 0.5 and 0.7 ppm) 2 hr. daily on leaf dynamics in six local tree species after 65 days of treatment.

Plant Species	Fumigation (ppm)	Initial No. of leaves at beginn. of exp.	No. of leaves after 15 days	Changes in number of leaves after 15 days	No. of leaves after 30 days	Changes in number of leaves after 30 days	No. of leaves after 45 days	Changes in number of leaves after 45 days	No. of leaves after 65 days	Changes in number of leaves after 65 days
B. variegata	0.0	36.5±1.00	42.55±2.5	+ 6.05	46.85±0.5	+10.35	49.48±0.65	+12.98	52.35±0.75	+15.85
	0.2	38.25±1.01	39.35±1.5	+ 1.10	37.66±0.40	- 0.59	36.42±2.5	+ 1.83	32.45±3.5	- 5.8
	0.5	48.25±2.0	48.56±1.0	+ 0.31	48.45±2.5	+ 0.2	45.52±1.0	+ 2.73	45.55±1.4	- 2.7
	0.7	39.5±1.5	40.25±1.5	+ 0.25	42.48±1.0	+ 2.09	37.46±1.40	+ 2.04	35.25±1.25	- 4.25
F. bengalensis	0.0	8.5±2.0	8.5±2.0	0.0	8.5±2.0	0.0	9.0±1.5	0.0	10±2.0	+ 1.5
	0.2	8.5±1.5	8.25±1.5	- 0.25	8.0±1.4	- 0.5	7.0±0.5	- 1.5	7±0.5	- 1.50
	0.5	9.5±1.0	9.5±1.0	0.0	8.0±1.0	- 1.5	8.0±1.0	- 1.5	5±1.5	- 4.50
	0.7	9.25±1.5	9.5±1.6	0.0	7.0±1.0	- 2.25	7.0±1.0	- 2.25	5±1.0	- 4.25
F. infectoria	0.0	47±4.0	23±3.0	-24.0	12.56±3.2	-34.44	0.0	0.0	30±2.0	+ 3.0
	0.2	48±3.5	29±4.0	-19.0	9.25±2.2	-38.75	0.0	0.0	34±4.0	+ 34
	0.5	48±2.0	21±1.0	-27.0	11.54±1.4	-36.36	0.0	0.0	22±1.0	+ 22
	0.7	47±2.0	26±1.5	-21.0	10.26±2.6	-36.74	0.0	0.0	22±2.5	+ 22
F. religiosa	0.0	8.5±0.5	8.5±0.5	0.0	6.5±1.0	- 2.0	0.0	0.0	6.0±4.0	+ 6.0
	0.2	9.0±0.5	9.0±0.5	0.0	4.0±0.0	- 5.0	0.0	0.0	52.0±6.0	+52.0
	0.5	9.0±1.0	9.0±1.0	0.0	4.0±0.5	- 5.0	0.0	0.0	-	-
	0.7	8.5±1.5	8.5±1.5	0.0	3.0±0.5	- 5.5	0.0	0.0	12.0±2.5	+12.0
P. pinnata	0.0	12.0±2.0	12±2.0	0.0	14±1.0	+ 2.0	14±2.0	+ 2.0	12.0±2.0	- 2.0
	0.2	12±2.0	13±2.0	+ 1.0	11±2.0	- 2.0	8.5±1.5	- 3.5	7.0±0.0	- 5.0
	0.5	13±1.0	13±1.0	0.0	13±1.0	0.0	12±1.0	- 1.0	10.5±1.5	- 2.5
	0.7	14±1.0	12±1.0	- 2.0	12±1.0	- 2.0	12.25±1.4	- 1.75	9.25±1.5	- 4.75
P. guava	0.0	52.5±4.5	74.5±6.0	-22.0	87.88±4.65	+25.38	92.56±4.5	+40.06	104.45±8.5	+51.95
	0.2	48.72±2.5	48.7±5.5	0.0	56.5±2.85	+ 7.80	62.5±6.4	+13.80	67.25±6.0	+18.55
	0.5	58.5±4.0	63.5±4.0	+ 5.0	69±4.0	+10.5	70.5±2.4	+12.0	74.48±2.4	+15.98
	0.7	55.5±3.0	55.5±4.0	0.0	63.25±2.3	+ 7.75	64.5±2.0	+ 9.0	67.68±5.6	+12.18

treated plants the average number of leaves were 38.25± 1.01, 39.35±1.5, 37.66± 0.40, 36.42±2.5 and 32.45± 3.5; 48.25±2.0, 48.56±1.0, 48.45±2.5, 45.52± 1.0 and 45.55±1.4, 39.5±1.5, 40.25±1.5, 42.48±1.0, 37.46±1.40 and 35.25±1.25 at the beginning and after 15, 30, 45 and 65 days respectively. In 0.2 ppm treated plants leaves were produced only upto 15 days, after that in 65 days treated plants on an average 5.8 leaves were dropped. In 0.2 and 0.7 ppm treated plants the leaf fall was also observed after one month. Thus SO_2 induced defoliatron, while no leaf fall was observed in control plants. No new leaves were produced after 30 days of fumigation. Thus SO_2 also inhibted production of leaves.

In case of F. bengalensis the average number of leaves for control plants were 8.5±2.0, 8.5±2.0, 8.5±2.0, 9.0±1.5 and 10±2.0 at the beginning and after 15, 30, 45 and 65 days respectively. The average number of leaves produced during this period was 1.5 after 45 days. No leaf fall was observed in control plants. In the 0.2, 0.5 and 0.7 ppm treated plants the average number of leaves were 8.5±1.5, 8.25±1.5, 8.0±1.4, 7.0±0.5 and 7±0.5; 9.5±1.0, 9.5±1.0, 8.0±1.0, 8.0±1.0 and 5±1.5; 9.25±1.5; 9.5±1.6,7.0±1.0, 7.0±1.0 and 5±1.0 at the beginning and after 15, 30, 45 and 65 days respectively. New leaves were not produced in the SO_2 treated plants. It clearly shows SO_2 inhibits leaf production. In 0.2, 0.5 and 0.7 ppm treated plants 1.54.5 and 4.25 leaves were dropped respectively. Data clearly show the effect of SO_2 in promoting premature leaf abcission..

In case of F. infectoria the average number of leaves for control plants were 47±4.0, 23±3.0, 12.56±3.2,. and 30±2.0 at the beginning and after 15, 30 and 65 days respectively. The leaf fall took place due to seasonal changes in control plants. After 45 days the number of new leaves produced was 30. In the same way in 0.2,0.5 and 0.7 ppm treated plants leaf fall took place, but the rate of fall was faster than control. After 45 days the new leaves were produced in all the plants, but the number of leaves in 0.5 and 0.7 ppm treated plants was very less. Only 22 leaves were produced, incomparison to 30 in control and32 in 0.2 ppm treat plants. The results show that in SO_2 treated plants leaf fall is acceierated and production of new leaves is some what supressed.

In case of F. religiosa the average number of leaves in control plants were 8.5±0.5, 8.5±0.5, 6.5±0.5, 6.0±4.0 at the beginning and after 15, 30 and 65 days respectively. The leaf fall took place in the control plants due to seasonal changes. After 45 days the new leaves produced were 6.0±4.0. In o.2, and 0.7 ppm treated plants. Leaf fall also took place and the number of new leaves produced were 52 and 12 respectively. But interestingly the new leaves were very small though they were many more number in comparision to control, plants.

In P. pinnata the average number of leaves for control plants were 12±2.0, 12±2.0, 14±1.0, 14±2.0, and 12.0±2.0 at the beginning of the experiment and after 15, 30 45 and 65 days respectively. New leaves were produced in control plants. In

0.2,0.5 and 0.7 ppm treated plants the average number of leaves were 12±2.0, 13±2.0, 11±2.0 8.5±1.5 and 7.0±0.0; 13±1.0, 13±1.0, 13 ±1.0, 12±1.0 and 10.5±1.5; 14±1.0, 12±1.0 12±1.0, 12.25 ±1.4 and 9.25±1.5 at beginning and after 15, 30, 45 and 65 days respectively,. The new leaves were produced only after 15 days in 0.2 ppm treated plants, other wise leaf fall took place in the fumigated plants, viz., 3.5, 1.0 and 1.75 in 0.2,0.5 and 0.7 ppm treatment respectively.

In P. guava the average number of leaves for control plants were 2.5±4.5, 74.5±6.0, 87.88±4.65, 95.86±4.5, and 104.45±8.5 at the beginning and after 15,30, 45 and 65 days respectively. In 0.2,0.5 and 0.7 ppm treated plants the average number of leaves were 48.7±2.5, 48.72±.55 56.5±2.85, 62.5±6.4, and 67.25±6.0; 58.5±4.00, 63.5±4.0, 69±4.0,70.5±2.5,74.48±2.4; 55.5±3.0, 55.5±4.00, 63.25±2.3, 64.5±2.00, 67.66±5.6 at the beginning and after 15, 30 and 65 days of fumigation respectively. There was no leaf fall in case of P. guava after SO_2 treatment, but the production of new leaves was reduced greatly. While 40.06 the number of leaves produced in control were 40.06, while 13.80, 12.0 and I leaves were produced in 0.2,0.5 and 0.7 ppm treated plants.

Plant height

The height of plants fumigated with 0.2,0.5 and 0.7 ppm of SO_2 was measured after 15, 30, 45 and 65 days. The data recorded in Table - 4 clearly shows that maximum reduction in plant heightwas observed in F. bengalensis while minimum change was seean in F.infectoria.

TABLE NO - 4

Effect of SO_2 fumigation (0.2, 0.5 and 0.7 ppm) for 65 days, 2 hr daily on Plant height in six local tree species.

Plant Species	Fumigation (ppm)	Plant Height at beginning (cm)	Plant Height after 15 days	Plant Height after 30 dyas	Plant Height after 45 days	Plant Height after 65 days	% Red'n over control after 15 days	% Red'n over control after 30 days	% Red'n over control after 45 days	% Red'n over control after 65 days
B. Variegata	0.0	94±2.5	98.5±0.5	98.85±2.3	106.26±3.2	112.75±1.6	-	-	-	-
	0.2	92±1.5	93.48±1.8	94.67±1.8	98.25±1.25	99.65±1.5	3.18	2.12	6.26	11.63
	0.5	93±0.5	94.15±0.8	94.95±1.5	95.46±0.86	96.68±1.24	3.55	3.06	10.75	15.99
	0.7	97±3.5	98.05±1.4	98.51±3.5	99.23±2.4	100.96±1.8	3.7	3.6	10.47	16.79
F. bengalensis	0.0	21.50±0.85	24.50±2.2	26.25±0.58	28.50±2.4	32.35±1.82	-	-	-	-
	0.2	22.50±1.4	23.20±1.5	23.90±1.2	26.60±1.35	28.82±2.6	10.84	15,87	16.70	22.38
	0.5	22.75±0.5	23.15±0.86	23.25±0.72	24.80±0.75	26.66±0.48	12.20	19.90	25.91	33.28
	0.7	21.61±1.62	21.90±1.5	22.45±1.24	23.54±1.26	25.24±1.34	12.61	18.21	30.58	33.67
F. infectoria	0.0	83±3.5	85.38±1.5	86.86±2.4	88.85±3.5	90.66±2.4	-	-	-	-
	0.2	80±1.5	82.15±4.5	82.95±1.7	85.32±1.0	83.65±1.8	0.18	0.97	0.39	4.66
	0.5	85±2.0	85.85±2.5	86.28±2.5	87.66±2.4	88.68±1.5	1.86	3.15	3.92	4.90
	0.7	80±2.0	80.78±1.6	81.25±1.5	82.47±2.6	83.48±1.7	1.88	3.09	3.96	4.87
F. religiosa	0.0	18±3.6	19.85±2.5	19.38±2.4	19.88±1.8	21.68±1.65	-	-	-	-
	0.2	21±1.5	21.78±1.65	21.97±1.75	22.25±2.6	23.27±1.0	1.01	3.52	4.49	9.60
	0.5	22±2.65	23.05±1.28	23.08±1.6	23.28±3.2	-	0.05	2.76	4.63	-
	0.7	22.75±1.0	23.05±1.58	23.87±2.0	23.98±2.4	24.85±0.77	0.77	2.69	5.04	11.21
P. pinnata	0.0	25.75±1.25	26.45±1.34	27.85±2.23	29.88±1.86	30.88±1.35	-	-	-	-
	0.2	21.65±3.2	22.15±2.65	22.94±1.44	23.14±1.24	24.15±2.24	0.41	6.08	9.15	10.32
	0.5	20.75±2.6	20.95±2.52	21.05±0.88	21.15±1.36	22.21±1.24	1.75	10.58	14.96	14.82
	0.7	23±1.58	23.14±1.38	23.22±1.62	23.38±1.76	24.34±1.82	2.11	11.07	14.37	16.034
P. guava	0.0	48.50±2.6	52.45±1.84	52.85±1.57	54.75±1.6	57.18±1.15	-	-	-	-
	0.2	58.50±1.3	59.45±0.85	59.46±1.48	60.85±1.2	62.87±0.78	6.52	6.47	8.87	10.42
	0.5	54.50±0.68	55.8±1.35	55.25±0.84	56.85±2.34	58.78±1.22	5.73	5.75	8.94	9.33
	0.7	51±1.24	52.15±1.4	52.59±1.28	53±1.65	55.25±0.82	5.89	5.85	8.96	9.46

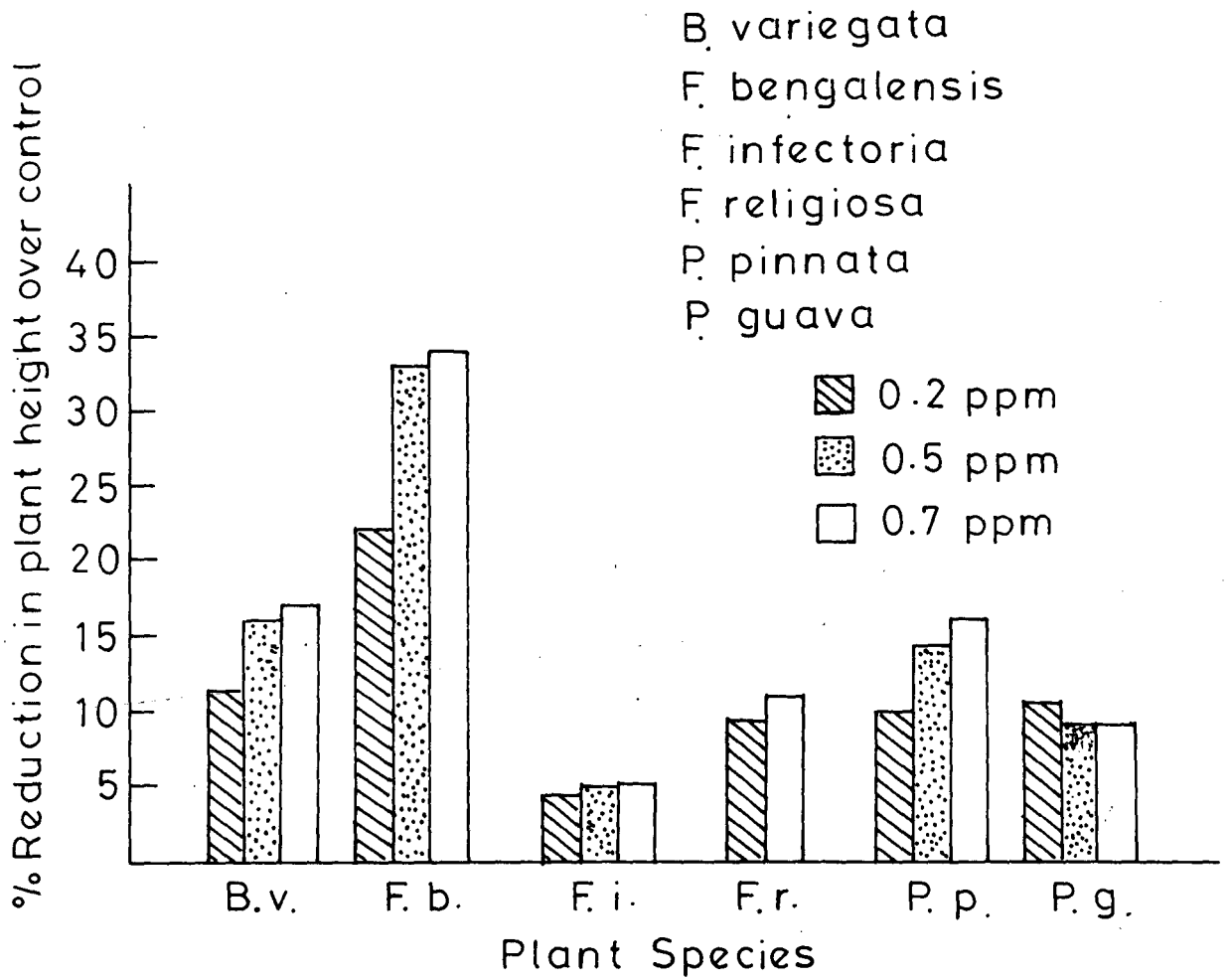

Figure - 1 **Effect of 65 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on plant height.**

In the case of B. variegata reduction in plant height was observed due to sulphur dioxide fumigation. The maximum reduction was observed in plants fumigated with 0.7 ppm of SO_2 for 65 days. The height of 15 days fumigated plants was reduced by 3.18, 3.55 and 3.7 percent over control in 0.2,0.5 and 0.7 ppm of SO_2 treatments respectively. After 30 days of fumigation the plant height was reduced by 2.12, 3.06 and 3.6 percent in 0.2,0.5 and 0.7 ppm of SO_2 treatments respectively. The percentage reduction in plant height was 6.26, 10.75 and 10.47 after 45 days on 0.2, 0.5 and 0.7 ppm of SO_2 treatments respectively. Ater 65 days the plant height was reduced by 11.63, 15.99 and 16.79 percent over control in 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively.

F. bengalensis was affected seriously due to SO_2 fumigation. After 15 days of fumigation the difference in plant height was 10.84, 12.20 and 12.61 percent over control in 0.2, 0.5 and 0.7 ppm of SO_2 trea respectively. After 30 days the plant height was reduced by 15.87, 19.90 and 18.21 perecent over control on 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively. The percentage reduction in plant height was 16.70, 25.91 and 30.58 after 45 days in 0.2,0.5 and 0.7 ppm SO_2 treatments respectively. After 65 dayw the height plant was reduced by 22.38, 33.28 and 33.67 percent over control on 0.2 0.5 and 0.7 ppm of SO_2 treatment respectively.

F. infectoria showed minimum reduction in growth in plant height due to SO_2 fumigation. After 15 days of fumigation the difference in plant height was 0.18, 1.86 and 1.88 percent in 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively. After 30 days the reduction in plant height was 0.97, 3.15 and 3.09 percent over control in 0.2, 0.5, and 0.7 ppm of SO_2 treatment respectively. After 45 days the reduction in plant height was 0.39, 3.92 and 3.96 percent over control in 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively. After 65 days the plant height was reduced by 4.66, 4.90 and 4.87 percent over control in 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively.

In the case of F. religiosa, after 15 days the percentage reduction in plant height was 1.01, 0.05 and 0.77 over control in 0.2,0.5 and 0.7 ppm of SO_2 treatments respectively. After 30 days the plant height was reduced by 3.52, 2.76 and 2.69 percent over control in 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively. The percentage reduction in plant height was 4.49, 4.63 and 5.04 over control after 45 days on 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively. After 65 days the plant height was reduced by 9.60 and 11.21 percent over control on 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively.

The reduction in plant height was also observed in P. pinnata due to SO_2 fumigation. After 15 days of fumigation the plant height was reduced by 0.41, 1.75 and 2.11 percent over control on 0.2,0.5 and 0.7 ppm of SO_2 treatments respectively.

After 30 days the percentage reduction in plant height was 6.08

10.58 and 11.07 percent over control in 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively. After 45 days the plant height was reduced by 9.15, 14.96 and 14.37 percent over control in 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively . After 65 days the percentage reduction in plant height was 10.32, 14.82 and 16.034 over control in 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively.

In the case of P. guava, after 15 days of fumigation the percentage reduction in plant height was 6.52, 5.73 and 5.89 over control in 0.2,0.5 and 0.7 ppm of SO_2 treatments respectively. After 30 days, the plant height was reduced by 6.47, 5.75 and 5.85 percent over control in 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively. After 45 days the plant height reduced by 8.87, 8.94 and 8.96 percent over control in 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively. After 65 days, the reduction in plant height was 10.42, 9.33 and 9.46 percent over control in 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively.

Biomass and root/shoot ratio

Shoot, root and total plant biomass were measured at the end of the experiment and root/shoot ratios were calculated, for B. variegata, F.bengalensis, F. infectoria, F. religiosa, P. guava and P. pinnata the data are recorded in Table 5. The maximum reduction in total biomass was observed in P. pinnata followed by B. variegata, F. infectoria and F. religiosa. (Figure - 2).

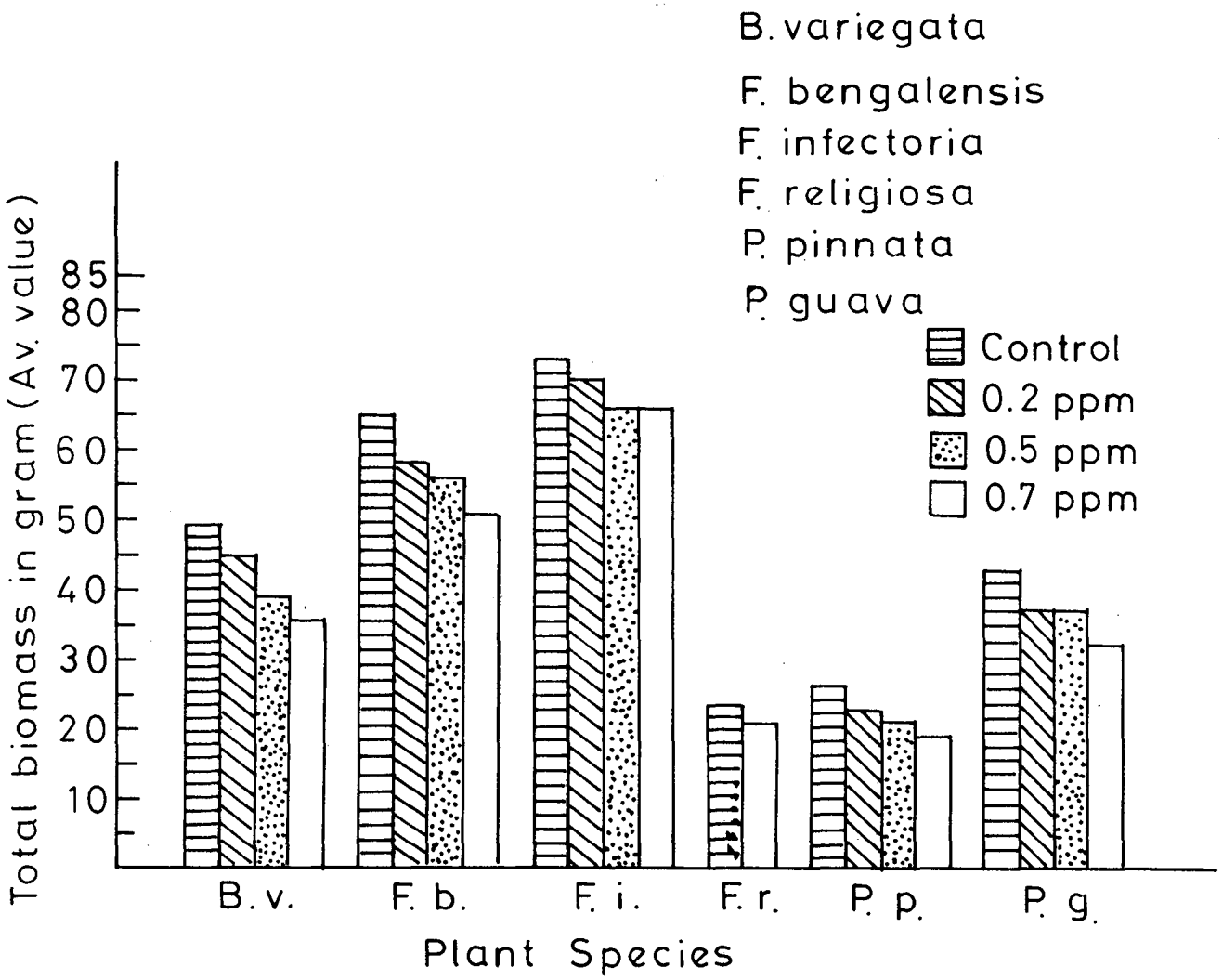

Figure - 2 **Effect of 65 days SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on plant biomass.**

In the case of B. variegata the average value for root, shoot and total biomass was 20.5± 0.81, 28.5± 1.5 and 49.0 g for control plants. The plants treated with 0.2 ppm of SO_2, their average value for root, shoot and total biomass were 18.5 ±0.5, 26.5±2.0 and 45.0 g. In 0.5 and 0.7 ppm of SO_2 fumigation, the average value of shoot, root and total biomass were 15.5±1.0, 24±1.5, 39.5 and 14±1.0 22.5±0.40 and 36.5g respectively. The reduction in total biomass over control was 8.16, 19.38 and 25.51 for 0.2,0.5 and 0.7 ppm of SO_2 treatment respectively.

In F. bengalensis the average value for root, shoot and total biomass was 25.5±, 40±0.5 and 65.50 for control plants, 20.5±0.5, 37.5±0.4 and 58.0 for 0.2 ppm for treated plants; 19.5±1.5, 36.5±0.5 and 56.0 for 0.5 ppm treated plants and 16.5±1.0, 34±1.5 and 51.0 for 0.7 ppm treated plants. The percentage reduction in total biomass over control values was 11.45, 14.50 and 22.13 on 0.2,0.5 and 0.7 ppm of SO_2 fumigation respectively.

In F. infectoria the average value for root, shoot and total biomass wee 18±0.28, 55±1.0, 73.00 for control plants; 17.5±0.4, 52.5±0.81, 70.0 for 0.2 ppm treated plants; 16±0.5, 50.±0.5, 66.0 for 0.5 ppm treated plants and 15.5± 2.0, 50.5±1.5 and 66.0 for 0.7 ppm exposed plants. The percentage reduction in total biomass over contrrol value were 4.10, 9.58 and 9.85 for 0.2, 0.5 and 0.7 ppm of SO_2 treated plants respectively.

TABLE NO - 5

Effect of SO_2 fumigation (0.2, 0.5 and 0.7 ppm) for 65 days, 2 hr. daily on the biomass and Root/Shoot ratio in six local tree species.

Plant Species	Fumigation in ppm	Root biomass (gm)	Shoot biomass (gm)	Total biomass (gm)	R/S ratio	% reduction over control
B. variegata	0.0	20.5±0.81	28.5±1.5	49.0	0.71	-
	0.2	18.5±0.5	26.5±2.0	45.0	0.69	8.16
	0.5	15.5±1.0	24±1.5	39.5	0.62	19.38
	0.7	14±1.0	22.5±0.40	36.5	0.62	25.51
F. bengalensis	0.0	25.5±1.0	40±0.5	65.50	0.63	-
	0.2	20.5±0.5	37.5±0.4	58.00	0.54	11.45
	0.5	19.5±1.5	36.5±0.5	56.0	0.53	14.50
	0.7	16.5±1.0	34.5±1.5	51.0	0.47	22.13
F. infectoria	0.0	18±0.28	55±1.0	73.0	0.32	-
	0.2	17.5±0.4	52.5±0.81	70.00	0.33	4.10
	0.5	16±0.5	50±0.5	66.00	0.32	9.58
	0.7	15.5±2.0	50.5±1.5	66.00	0.306	9.58
F. religiosa	0.0	7.5±1.0	16±0.35	23.5	0.46	-
	0.2	-	-	-	-	-
	0.5	-	-	-	-	-
	0.7	6±0.5	14.5±1.0	20.5	0.41	12.76
P. pinnata	0.0	12±0.25	14.5±1.5	26.5	0.82	-
	0.2	10±0.5	12.5±0.8	22.5	0.80	15.09
	0.5	9.5±0.5	12±0.5	21.5	0.79	18.86
	0.7	8.5±0.3	11±0.5	19.5	0.77	26.41
P. guava	0.0	15±0.57	27.5±2.02	42.5	0.54	-
	0.2	12.5±0.35	24.5±0.5	37.00	0.51	12.94
	0.5	12.5±1.0	25±1.00	37.5	0.50	11.76
	0.7	10±1.54	22±0.5	32.0	0.45	24.70

In F. religiosa the average value for root shoot, and total biomass was 7.5±1.0, 16±0.35 and 23.5gm for untreated control plants; 6±0.5, 14.5±1.0, 20.5 for 0.7 ppm treated plants respectively. The percentage reductiion in total biomass over control was 12.76 in 0.7 ppm treated plants.

In P. pinnata the average value for root,shoot and total biomass were 12±0.25, 14.5±1.5, 26.5 gm for control plants; 10±0.5, 12.5±0.8, 22.5 for -0.2 ppm treated plants; 9.5±0.5, 12.0±0.5, 21.5 for 0.5 ppm treated plants and 8.5±0.3, 11±0.5 and 19.5 for 0.7 ppm exposed plants. The percentage reduction in total biomass over control value were 15.09, 18.86 and 26.41for 0.2, 0.5 and 0.7 ppm of SO_2 treated plants respectively.

In P. guava the aveage value for root, shoot and total biomass were 15±0.57, 27.5±2.02, 42.5 for control plants, 12.5 ±0.35, 24.5±0.5 and 37.0 for 0.2 ppm treated plants; 12.5±1.0, 37.0 for 0.2 ppm treated plants ; 12.5±1.0, 25±1.0, 37.5 for 0.5 ppm treated plants and 10±1.54, 22.0±0.5 and 32.0 for 0.7 ppm treated plants respectively. The percentage reduction in total biomass over control value were 12.94, 11.76 and 24.70 on 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively .

Root/shoot ratio

A change in root/shoot ratio was observed in the SO_2 fumigated saplings of B. variegata, F. bengalensis, F. infectoria, F. religiosa, P. pinnata and P. guava. The data are recorded in Table - 5.

In B. variegata the average root/shoot ratio value for control plants were 0.71 whereas for 0.2, 0.5 and 0.7 ppm of SO_2 treated plants the value was shifted and decreased to 0.69, 0.62 and 0.62 respectively showing reduction in root/shoot ratio.

In F. bengalensis the average root/shoot ratio for control plants was 0.63 where as for 0.2, 0.5 and 0.7 ppm treated plants the value decreased to 0.54, 0.53 and 0.47 respectively showing clear reduction in root/shoot ratio.

In F. infectoria the average root/shoot ratio for control plants was 0.32 whereas for 0.2, 0.5 and 0.7 ppm of SO_2 treated plants the value changed to 0.33, 0.32 and 0.306 respectively showing reduction in root/shoot ratio.

In F. religiosa the average root/shoot ratio for control plants was 0.46 where as for 0.7 ppm of SO_2 treated plants the value changed to 0.41 showing reduction in root/shoot ratio.

In P. pinnata the average root/shoot ratio for control plants was 0.82, whereas for 0.2, 0.5 and 0.7 ppm of SO_2 treated plants the value changed to 0.80, 0.79 and 0.77 respectively, showing reduction in root/shoot ratio.

In P. guava the average root/shoot ratio for control plants was 0.54 whereas for 0.2, 0.5 and 0.7 ppm of SO_2 treated plants the value changed to 0.51, 0.50 and 0.45 respectively showing reduction in root/shoot ratio.

Chlorophyll content

The chlorophyll content (mg/g fresh leaf weight) was found out after 65 days. Reduction in chlorophyll content in SO_2 fumigated plants over control was determined and it was observed that the maximum reduction was in B. variegata while minimum was in F. religiosa.(Table 6 ; Fig. 3).

In the case of B. variegata after 65 days the chl a, chl b and total chlorophyll content in control (untreated) plants was 4.52 ± 0.098 3.14 ± 0.014 and 7.66 ± 0.084 respectively. In the 0.2, ppm SO_2 treated plants, the chl a, chl b and total chlorophyll contents were 3.78 ± 0.028, 2.91 ± 0.042 and 6.69 ± 0.014 respectively. The amount of chl a, chl b and total chlorophyll in the 0.5 pmm SO_2 treated plants were 2.715 ± 0.049, 2.17 ± 0.042 and 4.88 ± 0.007 respectively. In 0.7 ppm treated plants, the chl a, chl b, and total chlorophyll contents were 2.42 ± 0.07,1.78 ± 0.049 and 4.20 ± 0.021 respectively. The chl a, chl b and total chlorophyll were reduced by 16.37, 7.32 and 12.66, 39.93, 30.89 and 36.29, 46.46, 43.31 and 45.16 percent in 0.2, 0.5 and 0.7 ppm treatement respectively.

In F. bengalensis after 65 days the chl a, chla b and total chlorophyll contents of untreated control plants were 3.8 ± 0.07, 3.38 ± 0.056 and 7.18 ± 0.014 respectively. In the 0.2 ppm So_2 treated plants the a b and total chlorophyll contents were 3.1 ± 0.14, 3.28 ± 0.042 and 6.38 ± 0.098 respectively. The amount of a, b and total chlorophyll in the 0.5 ppm treated plants were 2.8 ± 0.063, 2.16 ± 0.028, 4.96 ± 0.084 respectively. In 0.7 ppm

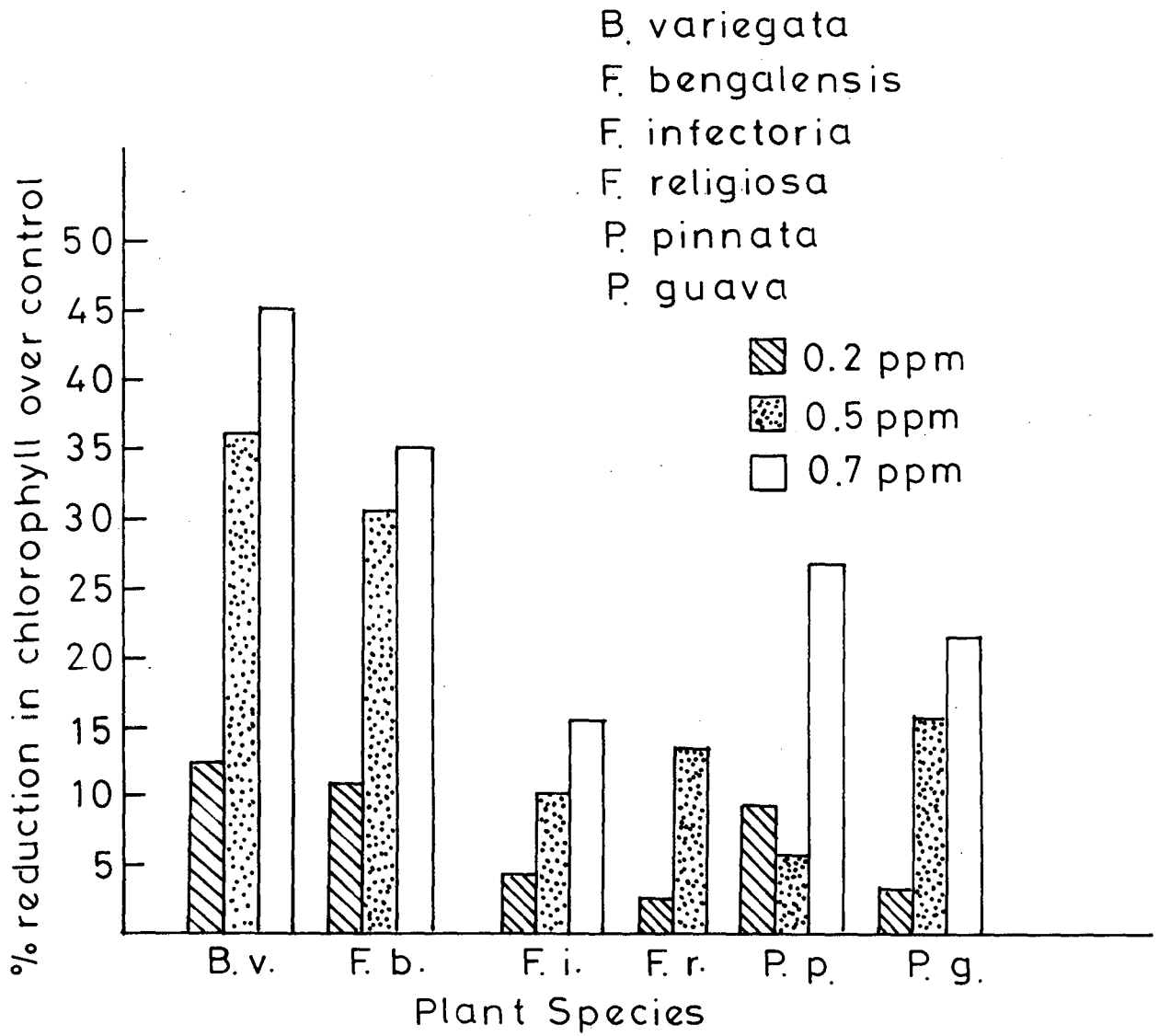

Figure - 3 **Effect of 65 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2hr. daily) on chlorophyll content.**

TABLE NO. - 6

Effect of SO_2 fumigation (0.2, 0.5 and 0.7 ppm) for 65 days, 2 hr daily on chlorophyll content (mg/g freshg leaf weight) in six local tree species.

Plant Species	Chlorophyll content of control plants	Chlorophyll content of 0.2 ppm exposed plants	% reduction over control	Chlorophyll content of 0.5 ppm exposed plants	% reduction over control	Chlorophyll content of 0.7 ppm explosed plants	% redution over control
B. variegata	a 4.52±0.098	3.78±0/028	16.37	2.715±0.040	39.93	2.42±0.07	46.46
	b 3.14±0.014	2.91±0.042	7.32	2.17±0.042	30.89	1.78±0.49	43.31
	T 7.66±0.084	6.69±0.014	12.66	4.88±0.007	36.29	4.20±0.021	45.16
F. bengalensis	a 3.8±0.07	3.1±0.14	18.42	2.8±0.063	26.51	2.72±0.021	28.42
	b 3.38±0.056	3.28±0.042	2.95	2.16±0.028	36.09	1.94±0.028	42.60
	T 7.18±0.014	6.38±0.098	11.14	4.96±0.084	30.91	4.66±0.007	35.00
F. infectoria	a 3.28±0.035	3.08±0.035	6.09	3.15±0.028	3.96	2.71±0.049	17.37
	b 2.5±0.028	2.44±0.014	2.4	2.04±0.014	18.4	2.16±0.028	13.6
	T 5.78±0.007	5.52±0.049	4.49	5.19±0.042	10.20	4.87±0.0212	15.74
F. religiosa	a 3.73±0.021	5.47±0.035	6.97	-	-	3.26±0.014	12.60
	b 2.89±0.042	2.97±0.007	+ 2.76	-	-	2.48±0.035	14.18
	T 6.62±0.063	6.45±0.028	2.56	-	-	5.74±0.021	13.29
P. pinnata	a 2.768±0.073	2.5±0.035	9.68	2.54±0.028	8.23	1.97±0.014	28.82
	b 1.94±0.028	1.77±0.056	8.76	1.88±0.077	3.09	1.49±0.007	23.19
	T 4.708±0.045	4.27±0.021	9.30	4.42±0.106	6.11	3.46±0.021	26.50
P. guava	a 3.28±0.007	3.11±0.07	5.18	2.72±0.049	17.07	2.42±0.07	26.21
	b 2.42±0.056	2.39±0.056	1.23	2.07±0.04	14.46	2.08±0.049	14.04
	T 5.7±0.048	5.5±0.014	3.50	4.79±0.02	15.96	4.5±0.05	21.05

a - chlorophyll a; b - chlorophyll b; T - total chlorophyll

treated plants the chl a, chl b and total chlorophyll contents were 2.72 ± 0.21, 1.94 ± 0.028 and 4.66 ± 0.007 respectively. The a, b and total chlorophyll were reduced by 18.42, 2.95 and 11.14; 26.51, 36.09 and 30.91; 28.42, 42.60 and 35.09 percent on 0.2, 0.5 and 0.7 ppm treatment respectively.

In F. infectoria after 65 days the chl a, vhl b and total chlorophyll contents of control plants were 3.28 ± 0.035, 2.5 ± 0.028 and 5.78 ± 0.007 respectively. In 0.2 ppm treated plants the a, b and total chlorophyll contents were 3.08 ± 0.035, 2.44 ± 0.014 and 5.52 ± 0.049 respectively. The amount of a, b and total chlorophyll in the 0.5 ppm trreated plants were 3.15 ± 0.028, 2.04 ±0.014 and 5.19 ± 0.042 respectively. In 0.7 ppm exposed plants the a, b and total chlorophyll contents were 2.71 ± 0.049, 2.16 ± 0.028 and 4.87 ± 0.0212 respectively. The a, b and total chlorophyll were reduced by 6.09, 2.4 and 4.49; 3.96, 18.4 and 10.20; 17.37, 13.6 and 15.74 percent on 0.2, 0.5 and 0.7 ppm of SO_2 exposure respectively.

In F. religiosa afterr 65 days the chl a, chl b and total chlorophyll content of control plants were 3.73 ± 0.021, 2.89 ± 0.042 and 6.62 ± 0.063 respectively. In 0.2 ppm treated plants, the a, b and total chlorophyll contents were 3.47 ± 0.035, 2.97 ± 0.007 and 6.45 ± 0.028 respectively. The amount of a, b and total chlorophyll in the 0.7 ppm treated plants was 3.26 ± 0.014, 2.48 ± 0.035 and 5.74 ± 0.021 respectively. The a, b and total chlorophyll were reduced by 6.97, + 2.76 and 2.56; 12.60 14.18 and 13.29 percent in 0.2 and 0.7 ppm of SO_2 fumigation respectively.

In P. pinnata after 65 days the chl a, b and total chlorophyll content of control plants was 2.768 ± 0.073 1.94 ± 0.028 and 4.708 ± 0.045 respectively. In 0.2 ppm treated plants the a, b and total chlorophyll contents were 2.5 ± 0.035, 1.77 ± 0.056 and 4.27 ± 0.021 respectively. The amount of a, b and total chlorophyll in the 0.5 ppm treated plants was 2.54 ± 0.028, 1.88 ± 0.077 and 4.42 ± 0.106 respectively. In 0.7 ppm treated plants the a, b and total chlorophyll contents were 1.97 ± 0.014, 1.49 ± 0.007 and 3.46 ± 0.021 respectively. The amount of a, b and total chlorophylls was reduced by 9.68, 8.76 and 9.30; 8.23, 3.09 and 6.11; 28.82, 23.19 and 26.50 percent in 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively.

In P. guava after 65 days chl a, chl b and total chlorophyll content of control plants was 3.28 ± 0.007, 2.42 ± 0.056 and 5.7 ±0.48 respectively. In 0.2 ppm treated plants the amount of a, b and total chlorophyll was 3.11 ± 0.007, 2.39 ± 0.056 and 5.5 ± 0.014 respectively. The amount of a, b and total chlorophyll in 0.5 ppm treated plants was 2.72 ± 0.049, 2.07 ± 0.04 and 4.79 ± 0.02 respectively. In 0.7 ppm treated plants the a, b and total chlorophyll content was 2.42 ± 0.07, 2.08 ± 0.049 and 4.5 ± 0.05 respectively. The a, b and total chlorophyll were reduced by 5.18, 1.23 and 3.50; 17.07, 14.46 and 15.96; 26.21, 14.04 and 21.05 percent in 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively.

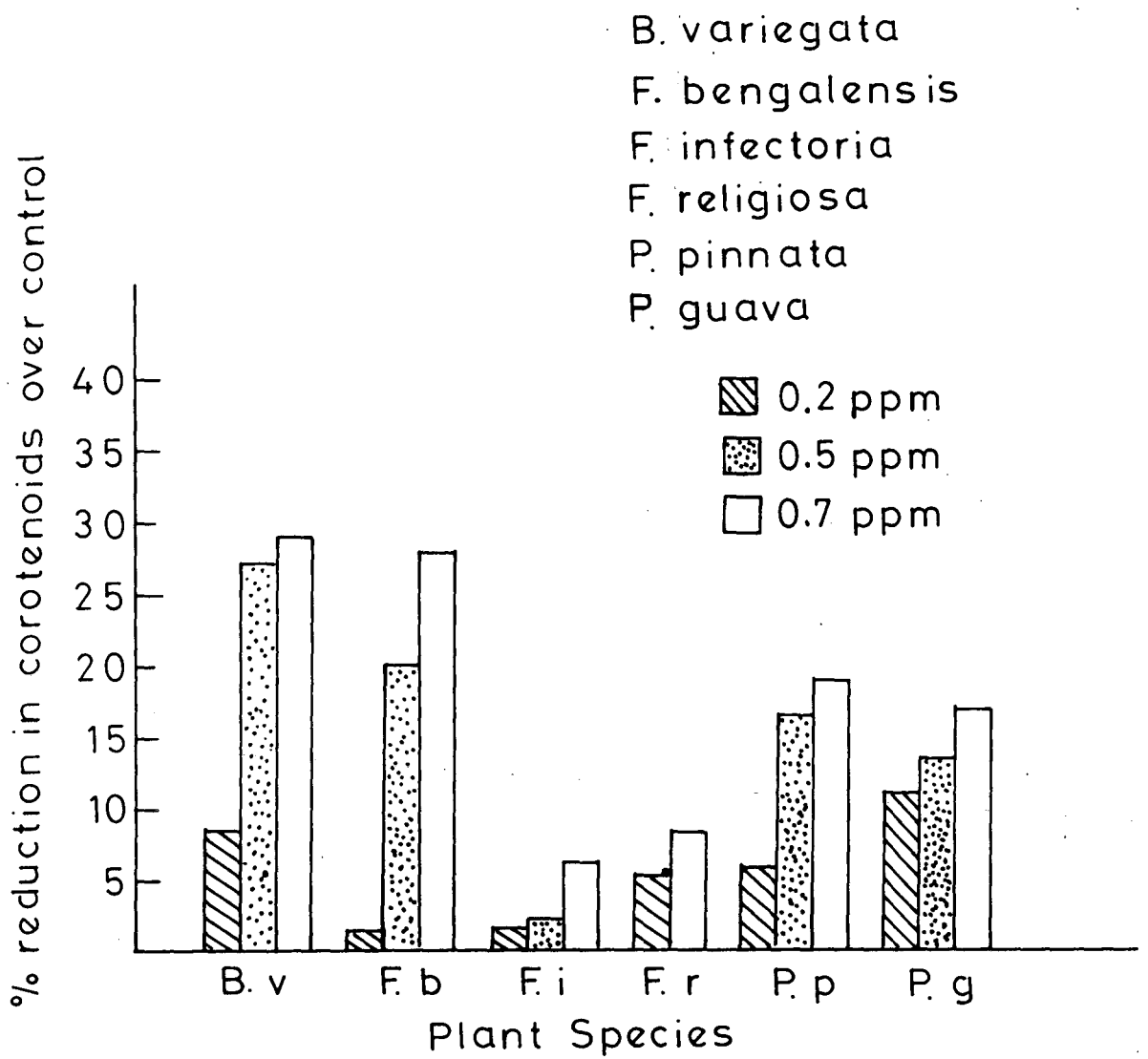

Figure - 4 Effect of 65 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on carotenoids content.

TABLE NO. - 7

Effect of SO_2 fumigation (0.2, 0.5 and 0.7 ppm) for 65 days, 2 hr daily on carotenoid content (mg/g fresh leaf weight) in six local tree species.

Plant Species	Carotenoids content of control plants	Carotenoids content of 0.2 ppm exposed plants	% reduction over control	Carotenoids content of 0.5 ppm exposed plants	% reduction over control	Carotenoids content of 0.7 ppm explosed plants	% redution over control
B. variegate	1.143±0.009	1.043±0.038	8.74	0.835±0.021	26.94	0.813±0.083	28.87
F bengalensis	1.435±0.0212	1.415±0.049	1.39	1.144±0.091	20.27	1.031±0.05	28.15
F. infectoria	1.048±0.045	1.031±0.009B	1.62	1.024±0.002	2.29	0.98±0.04	6.48
F. religiosa	1.125±0.176	1.065±0.035	5.33	-	-	1.032±0.009B	8.35
P. pinnata	0.95±0.042	0.893±0.072	6.00	0.793±0.0692	16.52	0.77±0.014	18.94
P. guava	1.32±0.056	1.17±0.014	11.36	1.143±0.009B	13.40	1.09±0.014	17.42

Carotenoids

The carotenoids content (mg/g fresh leaf weight) in tree saplings of *B. varegata*, *F. bengalensis*, *F. infectoria*, *F. religiosa*, *P. pinnata* and *P. guava* in control and fumigated plants was determined after 65 days of fumigation. Reduction in carotenoids content was observed in SO_2 fumigated plants. Maximum reduction was observed in *B. variegata* while minimum was in *F. infectoria*. (Table - 7 ; Fig. 4).

In *B. variegata* after 65 days the carotenoids content (mg/g) in control plants and SO_2 treated plants with 0.2, 0.5 and 0.7 ppm, were 1.143 $\pm$ 0.009, 1.043 $\pm$ 0.038, 0.835 $\pm$ 0.021 and 0.813 $\pm$ 0.083 respectively. The carotenoids content in 0.2, 0.5 and 0.7 ppm SO_2 treated plants were reduced by 8.74, 26.94 and 28.87 percent over control respectively.

In *F. bengalensis* after 65 days of fumigation, the carotenoids content of control and 0.2, 0.5 and 0.7 ppm treated plants was 1.435 $\pm$ 0.0212, 1.415 $\pm$ 0.049, 1.144 $\pm$ 0.091 and 1.031 $\pm$ 0.05 respectively. The caotenoids content in 0.2, 0.5 and 0.7 ppm SO_2 fumigated plants was reduced by 1.39, 20.27 and 28.15 percent respectively over control.

In *F. infectoria* after 65 days of fumigation the carotenoids content of control and fumigated plants with 0.2,0.5 and 0.7 ppm of SO_2 fumigated plants was 1.048 $\pm$ 0.45, 1.031 $\pm$ 0.0098, 1.024 $\pm$ 0.002 and 0.98 $\pm$ 0.04 respectively. The percentage reduction in carotenodis content of 0.2, 0.5 and 0.7 ppm SO_2 treated plants was 1.62, 2.29 and 6.48 perrcent respectively, over control.

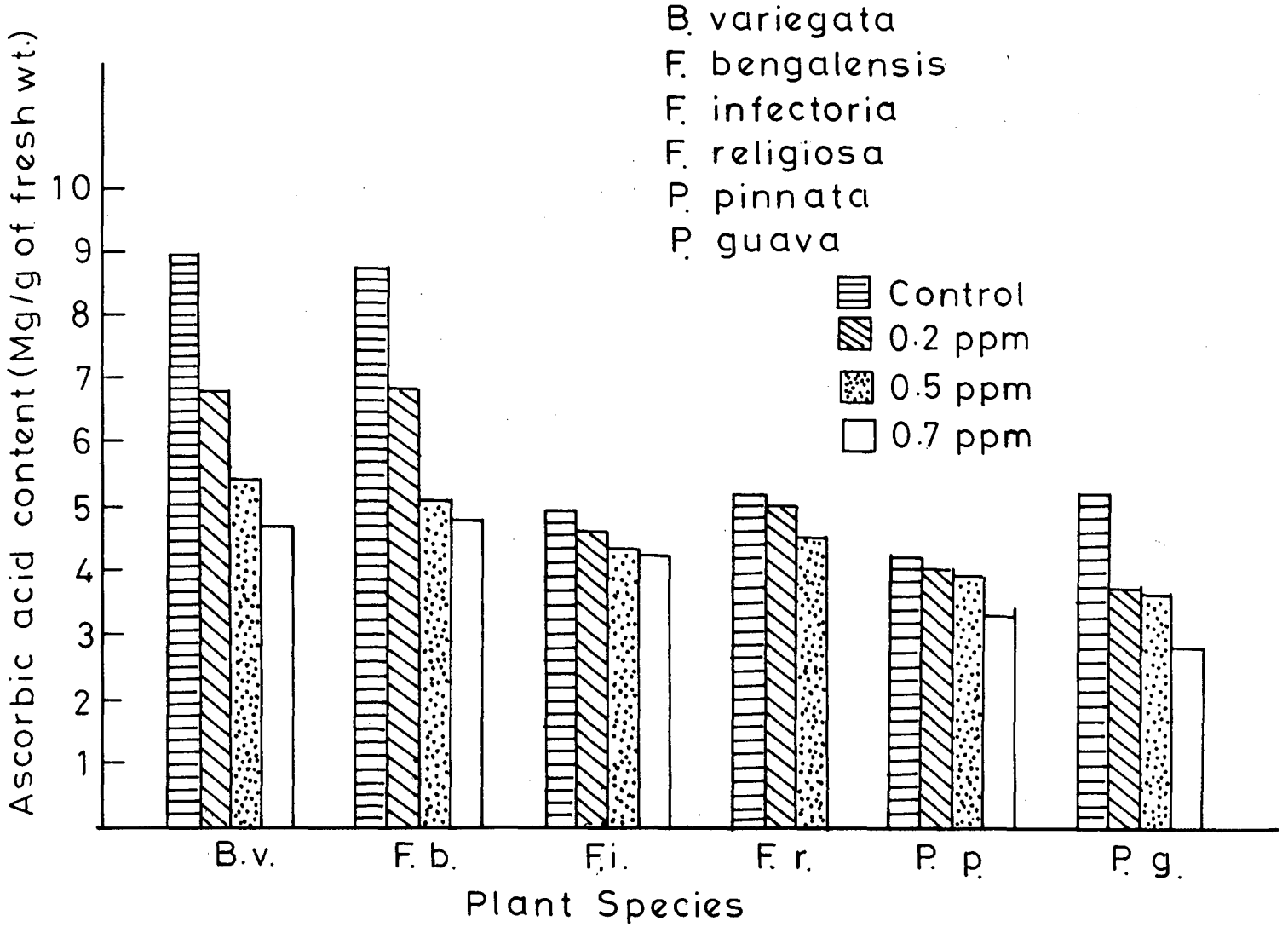

Figure – 5 **Effect of 65 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on ascorbic acid content.**

In F. religiosa after 65 days the carotenoids content of control and 0.2, 0.5 and 0.7 ppm fumigated plants was 1.125 ± 0.176, 1.065 ± 0.035 and 1.032 ± 0.0098 respectively. The carotenoids content in 0.2 and 0.7 ppm SO_2 fumigated plants was reduced by 5.33, and 8.33 percent over control respectively.

In P. pinnata after 65 days the carotenoids content of control plants and fumigated plants with 0.2, 0.5 and 0.7 ppm of SO_2 was 0.95 ± 0.042, 0.893 ± 0.072, 0.793 ± 0.0692 and 0.77 ± 0.014 respectively. The percentage reduction in carotenodis content of 0.2, 0.5 and 0.7 ppm SO_2 treated plants were 6.00, 16.52 and 18.94 percent respectively over control.

In P. guava after 65 days the carotenodis content of control and 0.2, 0.5 and 0.7 ppm treated plants was 1.32 ± 0.056, 1.17 ± 0.014,1.143 ± 0.0098 and 1.09 ± 0.14 respectively. The carotenodis content in 0.2, 0.5 and 0.7 ppm of SO_2 fumigated plants was reduced by 11.36, 13.40 and 17.42 percent respectively over control.

Ascorbic acid

The ascorbic acid content (mg/g fresh leaf weight) was determined in control and fumigated plants after 65 days. The reduction in ascorbic acid content was maximum in F. bengalensis and minimum in F. religiosa. (Table - 10; Fig. 5 and 6).

In B. variegata after 65 days the ascorbic acid contents (mg/g fresh leaves) of control and fumigated plants 0.2, 0.5 and 0.7 ppm oftreated plants was 8.95 ± 0.63, 6.8±0.14, 5.42±0.056 and 4.77±0.038 respectively. The percentage reduction over

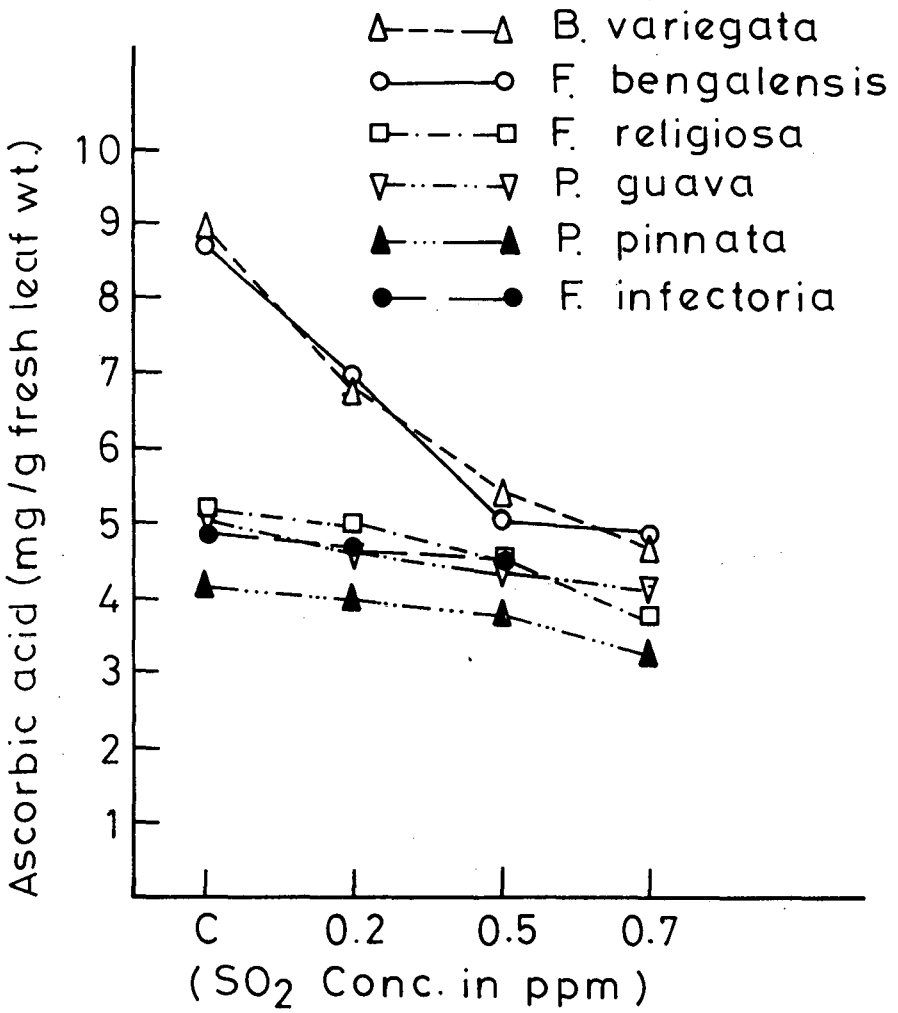

Figure – 6 **Effect of 65 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on ascorbic acid content.**

TABLE NO. - 10

Effect of SO_2 fumigation (0.2, 0.5 and 0.7 ppm) for 65 days, 2 hr. daily on ascorbic acid content (mg/g fresh leaf weight) in six local tree species.

Plant Species	Ascorbic acid in control plants	Ascorbic acid in 0.2 ppm fumigated plants	% change over control	Ascorbic acid in 0.5 ppm fumigated plants	% change over control	Ascorbic acid in 0.7 ppm fumigated plants	% change over control
B. variegata	8.95±0.63	6.8±0.14	24.02	5.42±0.056	39.44	4.77±0.38	46.70
F. begalensis	8.7±0.42	6.85±0.212	21.26	5.06±0.62	41.83	4.805±0.48	44.77
F. infectoria	4.95±0.71	4.68±0.088	5.45	4.32±0.113	12.72	4.28±0.159	13.53
F. religiosa	5.18±0.049	5.05±0.00	2.50	-	-	4.56±0.084	11.96
P. pinnata	4.205±0.049	4.0725±0.53	3.15	3.92±0.32	6.77	3.3±0.63	21.52
P. guava	5.145±0.13	4.68±0.081	9.03	4.61±0.056	10.39	3.82±0.1838	25.75

control was 24.02, 39.44 and 46.70 on 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively.

In F. bengalensis after 65 days the ascorbic acid content (mg/g fresh leaves) of control and 0.2, 0.5 and 0.7 ppm treated plants was 8.7±0.42, 6.85±0.212, 5.06±0.62 and 4.805±0.48 respectively. The percentage reduction over control was 21.26, 41.83 and 44.77 in 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively.

In F. infectoria after 65 days the ascorbic acid content (mg/g fresh leaves) of untreated control plants and fumigated plants for 0.2, 0.5 and 0.7 ppm of SO_2 were 4.95±0.71, 4.68±0.088, 4.32 and 0.113 and 4.28±0.159 respectively. The percentage reduction over control was 5.45, 12.72 and 13.53 in 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively.

In F. religiosa after 65 days the ascorbic acid content (mg/g fresh leaves) of untreated plants and 0.2, 0.5 and 0.7 ppm treated plants was 5.18±0.049, 5.05±0.00, ---, 4.56±0.084 respectively. The percentage reduction over control was 2.50 and 11.96 in 0.2 and 0.7 ppm of SO_2 treatment respectively.

In P. pinnata after 65 days the ascorbic acid content (mg/g fresh leaves) of control plants and 0.2, 0.5 and 0.7 ppm treated plants was 4.205±0.049, 4.072±0.53, 3.92±0.32 and 3.3±0.63 respectively. The percentage reduction over control was 3.15, 6.77, 21.52 in 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively.

In P. guava after 65 days the ascorbic acid content (mg/g

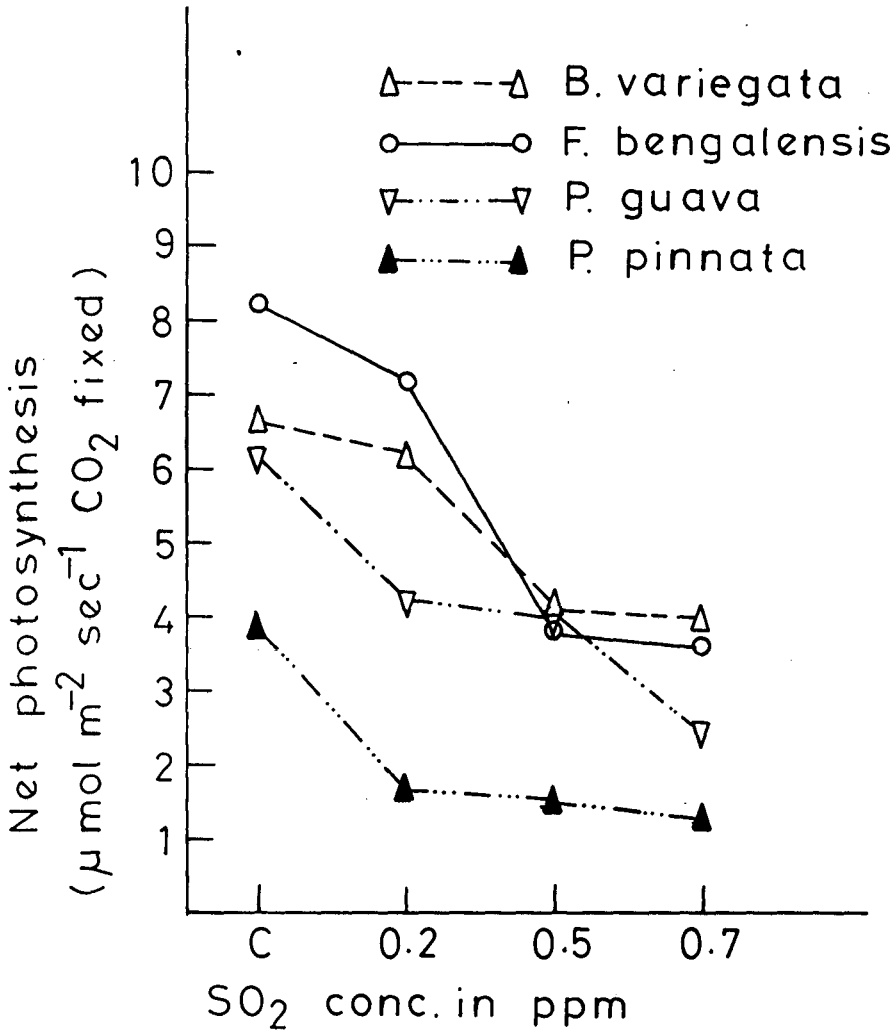

Figure 7B Effect of 45 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on net photosynthesis.

TABLE NO. - B

Effect of SO_2 fumigation (0.2, 0.5 and 0.7 ppm) for 45 days, 2 hr daily on net photosynthesis (u mol $m^{-2} sec^{-1}$ CO_2 fixed) in six local tree species.

Plant Species	Net photosynthesis in control plants	Net photosynthesis in 0.2 ppm exposed plants	% reduction over control	Net photosynthesis in 0.5 ppm exposed plants	% reduction over control	Net photosynthesis in 0.7 ppm explosed plants	% redution over control
B. variegata	6.739±0.095	6.275±0.1	6.88	4.198±0.06	37.70	4.038±0.047	40.08
F. bengalensis	3.939±0.12	1.534±0.025	61.05	1.506±0.022	61.76	1.363±0.019	65.39
P. pinnata	6.253±0.19	4.276±0.201	31.61	3.952±0.075	36.79	2.564±0.13	58.99
P. guava	8.148±0.027	7.273±0.015	10.73	3.854±0.023	52.70	3.640±0.107	55.32

fresh leaves) in untreated plants and 0.2, 0.5 and 0.7 ppm treated plants was 5.143±0.13, 4.68±0.081, 4.61±0.056 and 3.82±0.183 respectively. The percentage reduction over control was 9.03, 10.39 and 25.75 in 0.2, 0.5 and 0.7 ppm of SO_2 treatment respectively.

Net photosynthesis

The net photosynthesis was measured (u mol m^{-2} sec^{-1} CO_2 fixed) in tree saplings of B. variegata, F. bengalensis, P. guava and P. pinnata both in control and fumigated plants with SO_2 after 45 and 65 days of fumigation (Table 8 - 9). After 45 days the maximum reduction was in F. bengalensis and minimum in B. variegata, while after 65 days the maximum reduction was in P. pinnata and minimum in B. variegata. (Fig. 7 - 8).

Net photosynthesis after 45 days of SO_2 treatment :

In B. variegata after 45 days of fumigation, the average value of net photosynthesis was 6.739±0.095, 6.275±0.1, 4.198±0.06, 4.038±0.047 in control and with 0.2, 0.5 and 0.7 ppm treated plants respectively. The percentage reduction over control was 6.88, 37.70 and 40.08 on treatment of 0.2, 0.5 and 0.7 ppm of SO_2 respectively.

In F. bengalensis after 45 days of treatment, the average value of net photosynthesis was 3.939±0.12, 1.534±0.025, 1.506±0.022 and 1.363±0.019, in control and fumigated plants with 0.2, 0.5 and 0.7 ppm treated plants respectively. The percentage reduction over control were 61.05, 61.76 and 65.39 on fumigation

with 0.2, 0.5 and 0.7 ppm treated plants fespectively.

In P. guava after 45 days of fumigation the average value of net photosynthesis was 8.148±0.027, 7.273±0.015, 3.854±0.023 and 3.640±0.107 in control and in 0.2, 0.5 and 0.7 ppm treated plants respectively. The percetage reduction over control was 10.73, 52.70 and 55.32 in 0.2, 0.5 and 0.7 ppm treated plants respectively.

In P. pinnata after 45 days of fumigation the average value of net photosynthesis was 6.253±0.19, 4.276±0.201, 3.962±0.075 and 2.564±0.13 in control and treated plants with 0.2, 0.5 and 0.7 ppm treated plants respectively. The percentage reduction over control was 31.61, 36.79 and 58.99 respectively.

Net photosynthesis after 65 days of SO_2 treatment :

In B. variegata after 65 days of fumigation, the average value of net photosynthesis was 6.519±0.12, 5.491±0.081, 4.932±0.18, 4.437±0.28 in control and in 0.2, 0.5 and 0.7 ppm treated plants respectively. The percentage reduction over control was 15.65, 24.23 and 31.03 in 0.2, 0.5 and 0.7 ppm treated plants respectively.

In F. bengalensis after 65 days of fumigation the average value of net photosynthesis was 11.04±0.042, 10.16±0.16, 8.395±0.2, 7.045±0.05 in control and in 0.2, 0.5 and 0.7 ppm treated plants respectively. The percentage reduction over control was 7.97, 23.95 and 36.18 in 0.2, 0.5 and 0.7 ppm treated plants respectively.

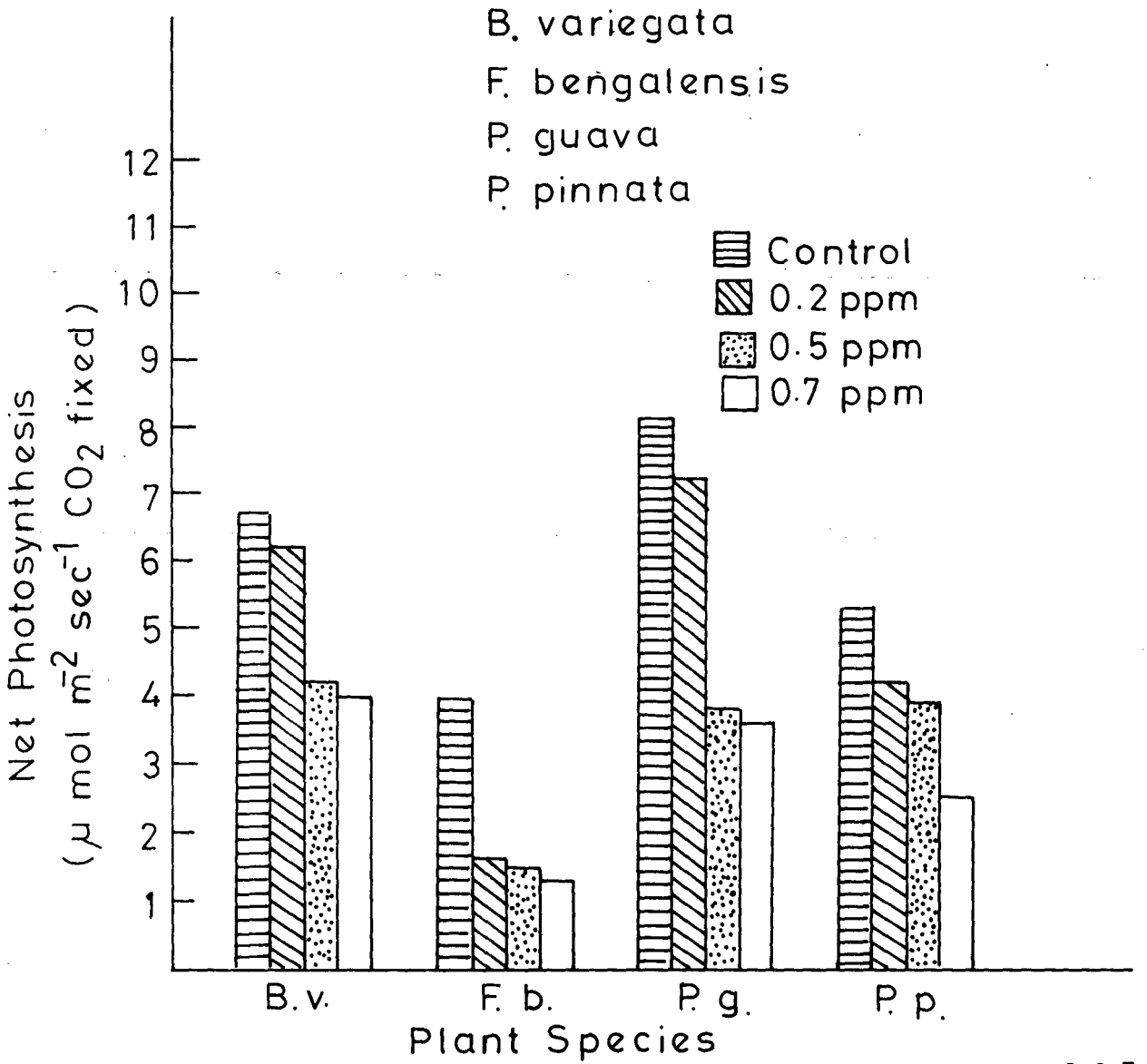

Figure - 7A Effect of 45 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on net photosynthesis.

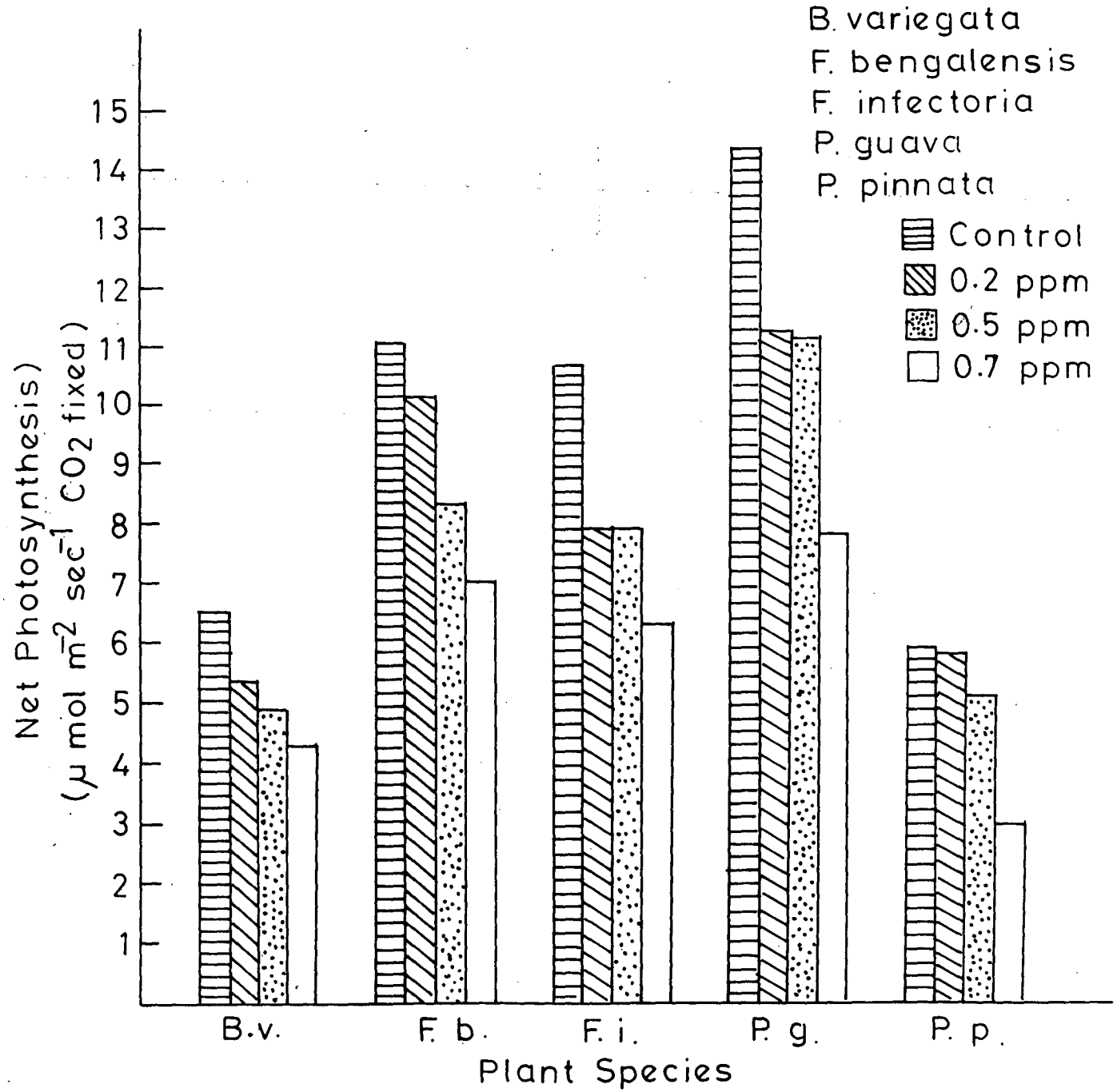

Figure - 8A Effect of 65 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on net photosynthesis.

TABLE NO. - 9

Effect of SO_2 sumigation (0.2, 0.5 and 0.7 ppm) for 65 days, 2 hr daily on net photosynthesis (u mol m^{-2} sec^{-1} CO_2 fixed) in six local tree species.

Plant Species	Net photosynthesis in control plants	Net photosynthesis in 0.2 ppm exposed plants	% reduction over control	Net photosynthesis in 0.5 ppm exposed plants	% reduction over control	Net photosynthesis in 0.7 ppm explosed plants	% redution over control
B. variegata	6.510±0.12	5.491±0.081	15.65	4.932±0.18	24.23	4.437±0.28	31.93
F. bengalenis	11.04±0.042	10.16±0.16	7.97	8.395±0.2	23.95	7.045±0.05	36.18
F. infectoria	10.59±0.21	7.888±0.19	25.59	7.888±0.12	25.56	6.396±0.17	39.60
P. pinnata	5.943±0.05	5.803±0.14	2.35	5.173±0.05	12.95	3.051±0.08	48.66
P. guava	14.32±0.22	11.21±0.59	21.71	11.14±0.00	22.20	7.897±0.11	44.85

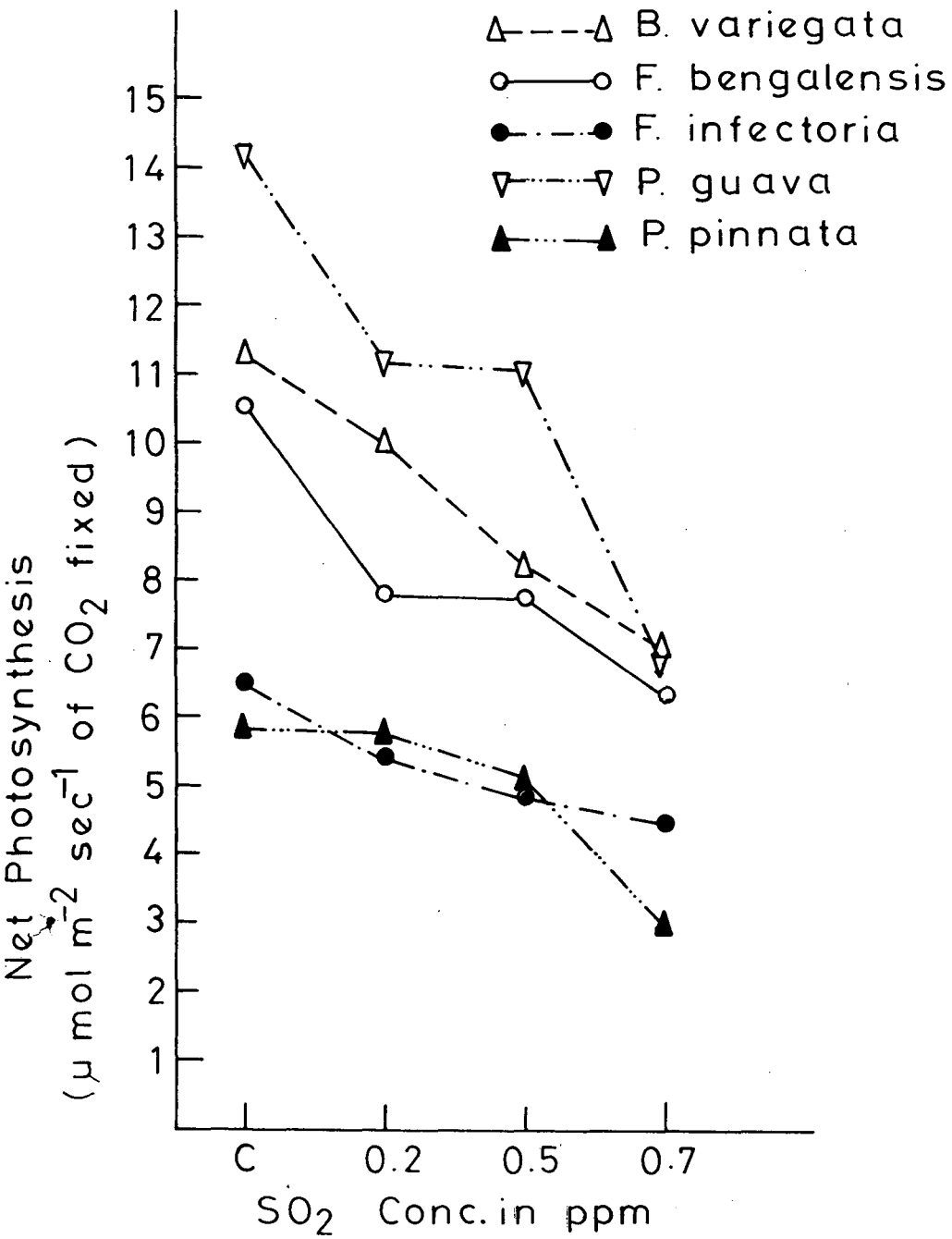

Figure - 8B Effect of 65 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on net photosynthesis.

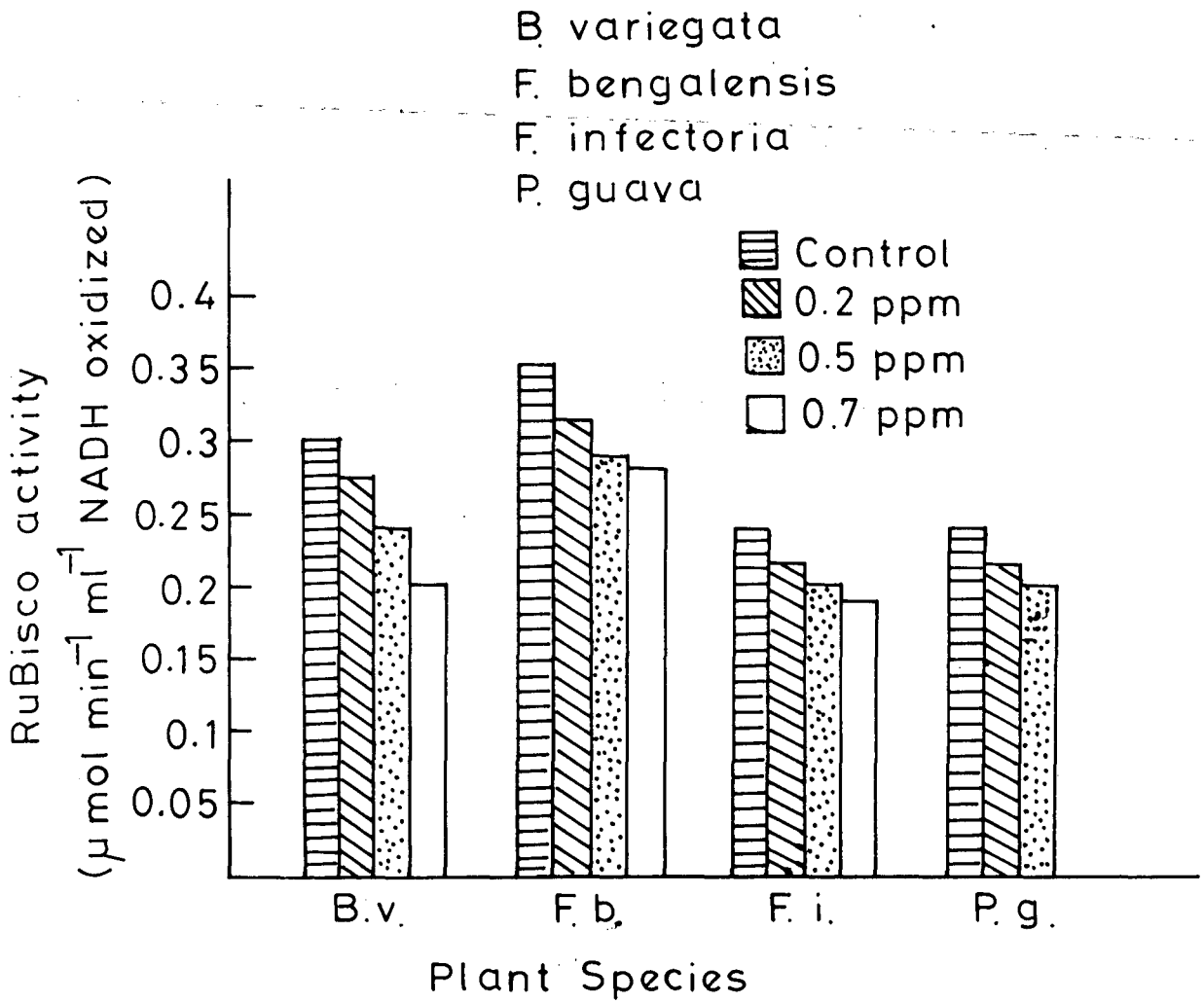

Figure - 9 **Effect of 65 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on RuBisco activity.**

In F. infectoria after 65 days of fumigation the average value of net photosynthesis was 10.59±0.21, 7.888±0.19, 7.880±0.12 and 6.396±0.17 of control and treated plants with 0.2, 0.5 and 0.7 ppm of SO_2 respectively. The percentage reduction over control was 25.59, 25.56 and 39.60 in 0.2, 0.5 and 0.7 ppm treated plants respectively.

In P. guava after 65 days of fumigation the average value of net photosynthesis was 14.32±0.22, 11.21±0.59, 11.14±0.00 and 7.897±0.11 in control and 0.2, 0.5 and 0.7 ppm treated plants respectively. The percentage redution over control was 21.71, 22.20 and 44.85 in 0.2, 0.5 and 0.7 ppm treated plants respectively.

In P. pinnata after 65 days of fumigation, the average value of net photosynthesis were 5.943±0.005, 5.803±0.14, 5.173±0.05 and 3.051±0.08 of control and treated plants with 0.2, 0.5 and 0.7 ppm of SO_2 respectively. The percentage reduction over control were 2.35, 12.95 and 48.66 on treatment of 0.2, 0.5 and 0.7 ppm of SO_2 respectively.

Ribulose bisphosphate carboxylase (RuBisco) activity:

RuBisco activity was determined by the method described by Marco and Tricoli (1983). It was determined by an enzymic estimation method in which D-3 PGA formed.

RuBisco activity was measured in B. variegata , F. bengalensis , F. infectoria and P. guava both in control and fumigated plants. The changes in RuBisco activity due to SO_2

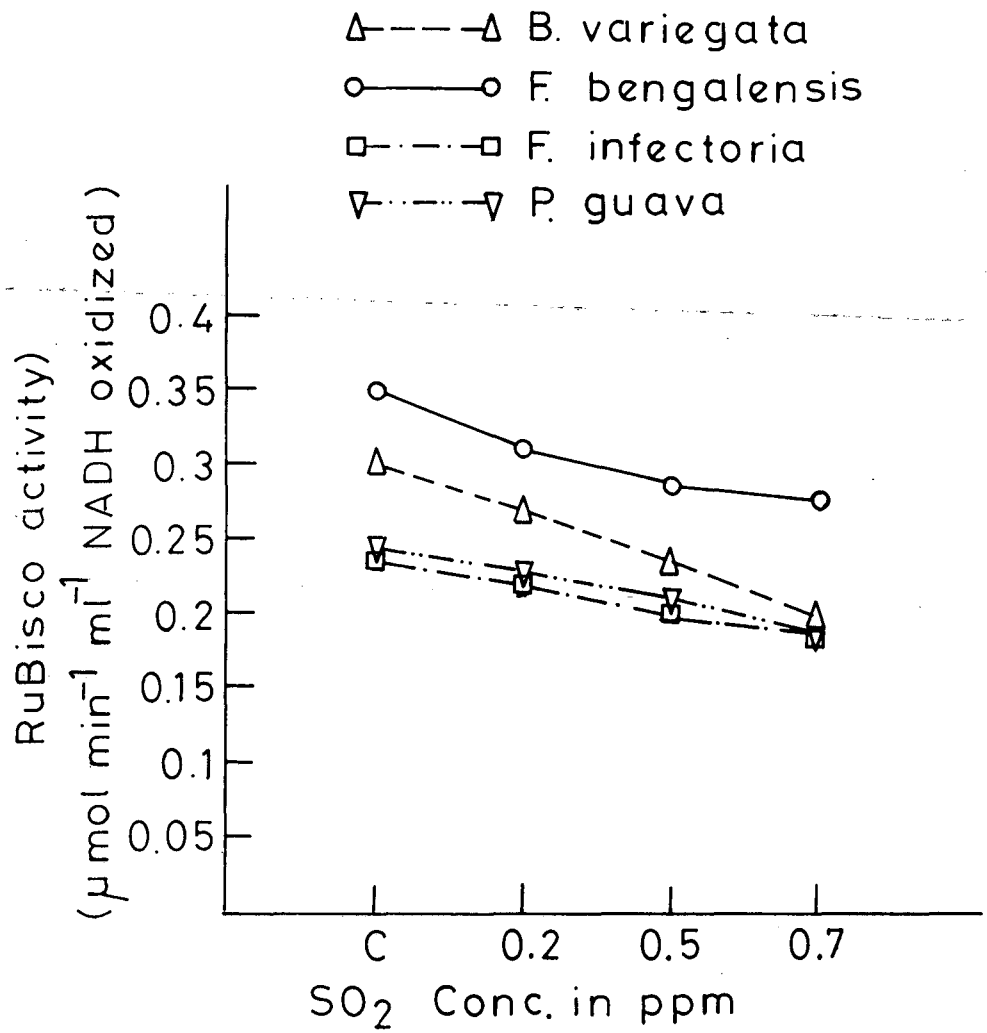

Figure - 10 Effect of 65 days of SO_2 fumigation (0.2, 0.5 and 0.7 ppm, 2 hr. daily) on RuBisco activity.

TABLE NO - 11

Effect of SO_2 fumigation (0.2, 0.5 and 0.7 ppm) for 65 days, 2 hr. daily on RuBISSO activity - (μ mol min^{-1} mil^{-1} of NADH Oxidized) in six local tree species.

Plant Species	RuBisCo activity in control plants	RuBisCo activity in 0.2 ppm exposed plants	% Change over control	RuBisCo activity in 0.5 ppm exposed plants	% Change over control	RuBisCo activity in 0.7 ppm explosed plants	% Change over control
B. variegata	0.305±0.0014	0.272±0.00088	10.81	0.245±0.0027	19.67	0.207±0.0036	32.13
F. bengalensis	0.355±0.0047	0.318±0.0044	10.42	0.28±0.00	21.12	0.273±0.0038	23.09
F. infectoria	0.253±0.0037	0.224±0.00B2	4.68	0.202±0.0018	14.04	0.198±0.0047	15.74
P. guava	0.247±0.005	-	-	0.217±0.002B	12.14	0.208±0.000B	15.7B

fumigation were maximum in B. variegata and minimum in F. infectoria. (Table 11; Fig. 9-10).

In B. variegata after 65 days the RuBisco activity (u mol min^{-1} ml^{-1} NADH oxidized) of control plants and 0.2, 0.5 and 0.7 ppm treated plants was 0.305±0.0014, 0.272±0.0083, 0.245±0.0027 and 0.207± 0.0036 respectively. The percentage reduction over control in 0.2, 0.5 and 0.7 ppm of SO_2 treatment were 10.81, 19.67 and 32.13 respectively.

In F. bengalensis after 65 days the RuBisco activity (u mol min^{-1} ml^{-1} NADH oxidized) in control and 0.2, 0.5 and 0.7 ppm fumigated plants of SO_2 was 0.355±0.0047, 0.318±0.0044, 0.28±0.00 and 0.273±0.0038 respectively. The percentage reduction over control on 0.2, 0.5 and 0.7 ppm of SO_2 treatment was 10.42, 21.12 and 23.09 respectively.

In F. infectoria after 65 days the RuBisco activity (u mol min^{-1} ml^{-1} NADH oxidized) of control and 0.2, 0.5 and 0.7 ppm treated plants was 0.235±.0037, 0.224±0.0082, 0.202±0.0018 and 0.198±0.0047 respectively. The percentage reduction over control in 0.2, 0.5 and 0.7 ppm treated plants was 4.68, 14.04 and 15.74 respectively.

In the case of P. guava after 65 days the RuBisco activity (u mol min^{-1} ml^{-1} NADH oxidized) in control and in 0.5 and 0.7 ppm treated plants was 0.247±.0005, 0.217±0.0028, 0.208±.008 respectively. The percentage reduction over control in 0.5 and 0.7 ppm treated plants was 12.14 and 15.78 respectively.

DISCUSSION

The low levels of SO_2 fumigation produced visible injury symptoms in *B. variegata*, *F. bengalensis* and *P. guava* saplings. The injury symptoms were similar to those described by Jacobson and Hill (1971). The sequence of effects can be shown as following *B. variegata*> *F. bengalensis*> *P. guava*.

In *B. variegata* fumigation with 0.2 ppm of SO_2 produced mild chlorotic spots only but plants fumigated with 0.5 and 0.7 ppm of SO_2 have shown necrotic patches in interveinal regions. The *F. bengalensis* plants treated with 0.2 ppm of SO_2 did not show any visual injury but in 0.5 and 0.7 ppm treated saplings the injured leaves were dull, patchy and some what variegated type. The chlorotic spots were seen in *P. guava* in 0.5 and 0.7 ppm of SO_2 treatment. Leaf sensitivity was found to be dependent on leaf age. Fully expended leaves were most sensitive, as the visible injury was mostly confined to such leaves. Young leaves at the apex and older leaves at the lower nodes, were free from any visible injury symptoms. The injury also depends on pollutant concentration and exposure period (Guderian 1970). Plants exposed to low concentration of pollutants over a long period develop injury symptoms similar to chronic injury (Norby and Kozlowasky, 1981). However, once a threshold has reached the chronic injury symptoms may transform into symptoms of acute damage.

It is interesting that the young leaves are more resistant to SO_2 than older leaves. The fully expanded and mature leaves were affected in most of the cases. In B. variegata mature leaves in the middle of the sapling were affected, where as the new developing leaves at the apex. and basal leaves did not show any injury symptoms. Same trend was seen in other species also.

Haut and Stratum (1970) have shown that younger leaves are generally more resistant than fully expanded leaves. Gudlrian (1970) also observed that in apple, pear, beet and broad bean during the long term exposure to low concentrations of SO_2 the older leaves were usually injured before the younger leaves. Bressan et. al. (1978) have suggested that relatively greater resistance of younger leaves is probably on account of same sort of developmentally controlled metabolic resistance to SO_2.

In some studies attempts have been made to correlate leaf damage with yield reduction. Chlorosis and necrosis have been shown to be related with the loss in plant yield (dry weight). (Bleasdale, 1952; Tingey, 1971; Heggested, 1972; Tangamn and Sawanka,1973 and Molhotra, 1977). In this study biomass reduction was observed in all the six species namely B. variegata F. bengalensis, F. infectoria, F. religiosa, P. pinnata and P. guava.

The tree saplings under SO_2 exposure develop visible foliar injury. Even low concentrations of SO_2 could produce foliar injury in some tree species. Fully expanded mature leaves suffer

more from visible injury.

The plant height and leaves per plant were found to be reduced in all the SO_2 fumigated plants (Table No. 3; 4) after 65 days of treatment. However F. religiosa was an exception as many small new leaves were produced in fumigated plants with 0.2 and 0.7 ppm of SO_2 treatment.

Rao et. al. (1981) have reported reduction in shoot length number of branches as well as in the number of leaves. Height of the pine seedlings has been shown to be reduced by SO_2 fumigation (Rading and Boyar, 1983). Number of leaves per plant were significantly reduced in Ulmus americana seedlings (Constantinidun and Kozlowsky, 1970).

Data given in tables (3; 4) show that the sensitivity of different species to SO_2, as judged from plant height and the number of leaves, differ from one species to another. B. variegata was found to be relatively sensitive whereas F. infectoria appeares relatively resistant.

Biomass of fumigated saplings of B. variegata, F. bengalensis, F. religiosa, F. infectoria, P. pinnata and P. guava was found to be reduced (Table - 5). The root/shoot ratio in 65 day SO_2 fumigated plants shifted from 0.71 to 0.62, 0.63 to 0.47, 0.32 to 0.306, 0.46 to 0.41, 0.82 to 0.77 and 0.54 to 0.45 in B. variegata, F. bengalensis, F. infectoria, F. religiosa, P. pinnata and P. guava respectively. The root/shoot ratio in F. bengalensis was changed from 0.63 to 0.47 and in B. variegata

o,71 to 0.62 showing maximum derivation. In general SO_2 fumigation influenced the root system more as compared to shoot. Biomass reduction of 25.91 percent was observed in P. pinnata which was maximum among the six species studied while in F. infectoria lowest biomass reduction amounting to 9.58 percent was observed.

Rao et. al. (1981) observed that dry weight of leaf, stem and root in fumigated wheat plants was reduced. Constantinidun and Kozlorowski (1979) also observed reduction in shoot and root biomass in Ulmus americana after 5 weeks of fumigation with 2 ppm of sulphur dioxide.

Dubey et. al. (1982) and Pandey (1982) have reported adverse effects of SO_2 pollution on plants growing around thermal power stations. Under artificial exposure of 140 ppb SO_2 plant yield was reduced (Hill and Thomas, 1933, Katz 1949). Reduced growth was observed by Farror et. al. (1977). Reduction in growth of plants exposed to high concentration of air pollutants (0.25 ppm SO_2) was observed by Pierra and Quiroz (1981).

Brinkmann et. al. (1971), Sij and Swanson (1974); Silvims et. al. (1976) observed that the reduction in phytomass can be correlated with the reduction in photosynmthetic leaf area.

Radish (Reinert and Gray, 1981; Reinert et. al., 1982), alfa alfa (Tingey and Reinert, 1975) blue grass (Poa pratensis), Whitemore and Mansfield, 1983), perennial rye grass (Lolium perenne), (Bell et. al., 1979), Scots pine (Pinus syvestris) and Sitka pruce (Picea sitchnensis) Garsed and Rutter, 1984), all

show greater suppression of root than of shoot biomass. In contrast root and shoot growth in hardwood tree species appear to be either unaffected or equally afected by SO_2 pollution (Garsed et. al., 1979; Roberts, 1975).

The effect of SO_2 pollution on the allocation priorities of photosynthate in plant leads to the reduction in the root : shoot (Norby and Kozlowshki (1981)). This may be due to SO_2 inhibiting the phloem loading system (Teh and Swanson, 1982). The import of an altered root : Shoot ratio lies in the possibility that the acquisition of carbon, energy, water and nutrient resources will be impaired. Thus plants growig in polluted areas may become more susceptible to environmental stress such as drought flooding etc. because proportionately less root is available to supply water to transpiring leaves (Lechowicz, 1987).

The pattern of biomass accumulation in plants is greatly altrered leading to strategic changes in the root/shoot ratios under air pollution strees.

The total chlorophyll content was greatly reduced in all the SO_2 fumigated plants (Table - 6). Reduction in chlorophyll in plants treated with 0.5 and 0.7 ppm of SO_2 was higher as compared with 0.2 ppm. In most of the cases it was found that chl a, was affected more as compared to chlorophyll b. The maximum chlorophyll reduction upto 46.46 percent was observed in *B. variegata* while minimum up to 13.29 percent was observed in *F. religiosa*. Same response of chlorophyll to SO_2 was observed by Rao and Le Blanc (1966, 1968), Malhotra (1977), Rabe and Kreeb

(1979), Lamenroth and Dodd (1981), Williams et. al., (1971), Kondo et. al.; (1980); Shimazaki et. al., (1980).

In F. bengalensis 0.2 and 0.5 ppm of SO_2 treatment the reduction in chllorophyll b, was more than chl a, in the same way in 0.5 ppm SO_2 treatment chllorphyll b, was reduced more than chllorophyll a, in F. infectoria. In contrast to these observations chllorophyll b was increased in F. religiosa fumigated with 0.2 ppm of SO_2, whereas chllorophll a, was reduced. Aggarwal, Nandi and Rao (1986) observed that in rice plants chlorophyll b was more sensitive to SO_2 damage than chlorophyll a. They attributed this to the increase in chlorophyll a activity (Malhotra, 1977) and/or inhibition of chlorophyll b synthesis (Arom\noff and Kwok, 1977), Castel franco (1983) observed similar effects.

It has been suggested that chlorophyll a is converted to phaeophytin following SO_2 fumigation. The breakdown of chlorophyll to phaeophytin resulted by replacing the Mg^{+2} with $2H^{+}$ formed due to increased cell acidity due to sulphur dioxide (Rao and Le Blanc, 1966). However, production of phaeophytin is not specific to SO_2 only, Arndt (1971) reported that hydro fluoric acid and hydrochloric acid also convert chlorophyll to phaeophytin as a result of increased acidity of the cell. Quantitative determination of various pigments by Malhotra (1977) suggests that chlorophyll a is converted into phaeophytin and chlorophyll b into chlorophyllide b following sulphur dioxide fumigation.

Rabe and Kreeb (1979) observed that the phaeophytrization of chlorophyll in spinach occurred around pH 4 *in vitro* conditions. However, Hill (1971) suggested that inactivation of chlorophyll was a secondary effect of SO_2. The activity caused by the low levels of SO_2 would not be strong enough to explain the inactivation of chlorophyll.

Peiser and Yang (1978) observed that free radicals from linoleic acid (LooH) decomposition by HSO^{-3} were responsible for chlorophyll destruction. Later they reported increased amounts of malandialdehyde (MDA) in leaves damaged by sulphur dioxide. The MDA formation decreases with the reduction of chlorophyll a by the addition of tririon (1.2, dihydroxy benzene - 3.5 disul phonate) a 5 scavenger of sulphur oxides (O_2^-) radical (Shimazaki *et. al.*, 1980). Now it has been suggested that the destruction of chlorophyll may be due to the formation of superoxide radical in plants fumigated with sulphur dioxide.

The effect of SO_2 on chlorophyll may be considered under two cellular conditions, i.e., at pH values below and above 3.5. At pH 2.2 to 3.5 the free H^+. ions in cell from the splitting of H_2SO_3 in SO_3^{2-} and H^+ displace Mg^{2+} from chlorophyll molecules to degrade them in to phaeophytin molecules (Rao and Le-Blanac, 1966). At pH above 3.5 SO_2 cause effects the thylakoid membrane of chloroplast by causing oxidation of carotenoids through generation of O_2^- (super oxide radicals) from HSO_3^- (Peiser and Yang, 1978). Once the carotenoid protection is lost, the

chlorophyll molecules get oxidixed and reduced quantitatively decreasing the photosynthetic ability of the plant.

The sulphur dioxide treatment of tree sapling of B. variegata, F. bengalensis, F. infectoria, F. religiosa, P. pinnata and P. guava with 0.2, 0.5 and 0.7 ppm for 2 hr daily for 65 days, has reduced the carotenoids content of the treated plants when compared with control (Table - 7). The maximum reduction was observed in B. variegata followed by F. bengalensis and minimum reduction was in F. infectoria followed by F. religiosa.

Carotenoids are important accessory pigments of chloroplasts. They may undergo several photochemical reactions when exposed to SO_2 treatment. These reduced and excited molecular species of oxygen are highly reactive and oxidize cell components. At pH above 3.5 these reactive species of oxygen may affect the thylakoid membrane of chloroplast by causing oxidation of carotenoids through generation of O_2^- (super oxide radicals) from H_2SO_3. Finally reducing the amount of carotenoids in cells. Tanaka and Sughara (1980) reported that the SO_2 damage is partly due to the toxicity of active oxygen.

The RuBisco activity was found to be decreased in tree saplings of B. variegata, F. bengalensis, F. infectoria and P. guava fumigated with 0.2, 0.5 and 0.7 ppm of sulphur dioxide. RuBisco activity was found to be suppressed in B. variegata followed by F. bengalensis while minimum change in the activity of this enzyme was observed in F. infectoiria followed by P.

RuBisco activity decreases after pretreatment with sulphur dioxide (Miszalski and Ziegler, 1981). It has been observed that sulphur dioxide treatment suppresses the activity of certain enzymes while the activity of other enzymes is relatively increased (Horsman and Wellburn, 1976; 1977; Pierre, 1977, Malhotra and Khan, 1980). Hallgren and Gezelims (1982) showed that fumigation with 'low' SO_2 concentration (400 ug SO_2^- 0.15 ppm) for 8 days, in Pinns sylvestris decreased RuBP carboxylase when expressed on dry weight basis.

Ziegler (1972) suggested that the mechanism by which sulphur dioxide interfered with photosynthesis was due to the potent and competitive inhibition of RuBisco with respect to HCO_3^- presumably SO_3^{-2} replaces HCO_3^- by reacting at the same enzyme site. SO_2^- showed a non-competitive inhibition with respect to RuBP and Mg^{2+}. However, Gezelims and Hallgren (1980), reported using similar preparations of RuBisco from Spinach and Pinus that SO_3^{-2} associated carboxylease activity to a lesser extent and was non-competitive with respect to HCO_3^-.

Hallgren and Gerelims (1982) proposed that a decrease in RuBisco activity in senescing plants has been associated with proteolytic enzyme activity (Peterson and Huffaker, 1975). Whether the SO_2 effect is asociated with a stimulation of hydrolytic enzymes or with an increased access of RuBisco is not known. Godzik and Linskens (1974) have suggested that the lower levels of RuBisco activity after SO fumigation should be

considered in relation to decrease in protein synthesis.

Net photosynthesis was affected in SO_2 treated plants of B. variegata, F. bengalensis, F. infectoria, P. pinnata and P. guava as compared to control plants (Table - 8; 9). The maximum reduction was observed in F. bengalensis upto 65.39 percent after 45 days of treatment, while in P. pinnata upto 48.66 percent after 65 days of treatment. The minimum reduction was observed in B. variegata upto 40.08 and 31.93 percent on 0.7 ppm treatment, after 45 and 65 days of fumigation respectively.

Libera, Ziegler and Ziegler (1973) demonstrated that exposure of isolated spinach chloroplasts to low concentrations of sulphite below > 1 mM produced a stimulation of carbon fixation. Higher levels of sulphite (upto 3 mM) stimulated photosynthetic electron transport but inhibited carbon fixation. Careson (1983), Bennet and Hill (1973), Black and Unsworth (1979) and Tayler (1965) have observed inhibition of photosynthesis in dicotyledenous crop species exposed to SO_2. Black and Unsworth (1979) and Bennet and Hill (1973) observed that the relationship between photosynthesis and exposure dose was curvilinear. They observed 10% inhibition of photosynthesis from 0.45 ppm of SO_2 in a single exposure.

Barton, Mcamghin and MC Conathy (1980) have reported that photosynthesis is more sensitive to SO_2 in low-vapour-pressure deficit regimes than in high vapour-pressure deficit regimes. They explained it on the basis of differences in pollutant

uptake into leaf interior (McLaughin and Taylor, 1981).

In light of above observations it is seen that the rate of photosynthesis is reduced due to SO_2 fumigation. The sequence of effects of SO_2 on net photosynthesis in different tree saplings is in the following sequence:

P. pinnata> P. guava> F. infectoria> F. bengalensis> B. variegata.

Reduction in ascorbic acid content was observed in SO_2 fumigated saplings (Table - 10). The plants exposed with 0.5 and 0.7 ppm of SO_2 have shown maximum reduction in ascorbic acid content as compared to 0.2 ppm treated ones. The maximum reduction in ascorbic acid content was observed in B. variegata followed by F. bengalensis and minimum reduction was observed in F. religiosa.

Varshney and Varshney (1982) while working on P. radiatus, Z. mays and B. nigra observed that, there was significant loss of ascorbic acid in B. nigra and P. radiatus while Z. mays did not show any appreciable change due to SO_2 exposure (3,5 and 10 ppm). Sahare (1984) working on local trees, found that plants growing in areas suffering from SO_2 pollution have less amount of ascorbic acid as compared to the ascorbic acid content in the trees of the same species growing at relatively pollution free sites.

Ascorbic acid has also been reported to scavenge certain toxic oxygen species. Ascorbic acid is known to be a powerful

reductant responsible for the photoreduction of chlorophyllide (Rodolph and Bukatsch, 1966). It acts as an electron doner for the reduction of SO_2 and its capacity to do so is enhanced under illuminated conditions (Manpson, 1988, Rudalph and Bukatsh, 1966, Keller and Schwager, 1977). The reduction of SO_2 may lead to the production of Hydrogen sulphide (H_2S) in plants subjected to SO_2 fumigation (Silvims et. al. 1976). In spinch H_2S has been shown to be given out by plants fumigated with SO_2 (De Cormis, 1968). Superoxide radical formation in plants grown in SO_2 stress oxidizes ascorbic acid to dehydroxy ascorbic acid (Elstner and Kramer, 1973).

Conditions affecting synthesis of ascorbic acid adversely such as shading, oxidizing gaseous pollutants reduce the ascorbic acid content in plants and thus render them relatively more susceptible to air pollutants. Indigenous levels of ascorbic acid appears to be one of the important factors in determing plant resistance to gaseous pollutants.

Fumigation of plants with SO_2 seriously affects ascorbic acid content in plants (Table - 10). Keller and Schwager (1977) observed ascorbic acid as one of the important factors which influences resistance to unfavourable enviromental conditions besides air pollutants.

SUMMARY

The effect of sulphur dioxide on six local tree species namely, B. variegata, F. bengalensis, F infectoria, F relegiosa, P. puinnnata and P. guava was studied under controled fumigation. The saplings of these species were fumigated with 0.2, 0.5, and 0.7 ppm of SO_2 for 2 hr. daily for 65 days. The SO_2 effects were examined at morphological, physiological and biochemical levels,

In specific terms the impact of SO_2 fumigation was evaluated in terms of plant height, visual foliar injury, leaf dynamics, biomass and root/shoot ratio, chlorophyll content, carotenenoids content, ascorbic acid, net photosynthesis and Ru Bisco activity.

Exposure of tree saplings to sulphur dioxide fumigation produced wide ranging effects in the plants studies. Foliar injury and foliar damage was observed in B. variegata, F. bengalensis and P guava. Maximum leaf injury e.g. chloretic and necrotic patches at interveinal region and finally the whole leaf dies in B varicgata. No leaf injury was observed in F. infectoria, F. religiosa and P. puinnata. However, there was appreciable reduction in other parameters such as leaf dynamics of fumigated plants.

Biomass of the six tree species exposed to 0.2, 0.5 and 0.7

ppm of SO_2 was reduced. The root-shoot ratio of the six tree species saplings exposed to 0.2, 0.5 and 0.7 ppm of SO_2 treatment was altered with increasing concentration of SO_2.

The chlorophyll content in plants exposed to sulphur dioxide was adversely effected. In most of the cases chlorophyll a was found to be more affected than chlorophyll b. In case of F. bangalensis and F.infectoria reduction in chlorophyll b was more than chlorophyll a in plants treated with 0.5 ppm of SO_2. Rreduction in chlorophyll b was more than chlorophyll a in plants treated with 0.5 ppm of SO_2. The chlorophyll contents of plants fumigated with 0.7 ppm of SO_2 were more affected than those subjected to 0.5 ppm of SO_2. Plants exposed to 0.2 ppm exhibited minimum change.

Corotenoides content in the plants exposed to SO_2 fumigation was also found to be adversely affected, Reduction was more in 0.7 ppm exposed plants as compared to 0.5 ppm and 0.2 ppm treated plants. The maximum effect was on B.variegata and minimum was on F. infectoria.

The rate of net photosynthesis was also reduced in all the SO_2 treated tree saplings. After 65 days of SO_2 treatment, the maximum reduction in net photosynthesis was observed in P pinnata and minimum was in B variegata.

The ascorbic acid content in plants exposed to SO_2 was reduced in all the species. The maximum reduction was observed in B. variegata.

The enzyme ribulose 1-biphosphate activity was found to be reduced in SO_2 treatment with the increase in the concentration of SO_2, the enzymic activity decreases steadily. Relatively maximum effect was observed in B. variegata and minimum effect in F. infectoria.

Although visible foliar injury was not prominent in most of the cases, but the SO_2 stress was quite marked in terms of plant height, leaf dynamics, biomass, chlorophyll content, carotenoids content, ascorbic acid, net photosynthesis and RuBisco activity. On the basis of the results obtained from this study it can be concluded that in terms of relative sensitivity the six tree species fumigated with SO_2 fall in the following sequence ; B.variegata > F.bengalensis> P.pinnata> P.guava> F.infectoria>F. religiosa.

The evaluation of relative performance of plant species to specific pollutants provides valuable information to access the impact of pollution stress and to develop a scientific basis for tree plantation in polluted areas.

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