

Plant responses to SO₂ pollution and its amelioration

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SUMMARY

Sulphur dioxide is a major air pollutant and it enters in to plants through stomata. Sulphur dioxide affects a number of plant processes in a variety of ways. Sulphur dioxide gas dissolves in extra cellular fluid of plants and reacts with plant materials to produce ionic species and free radicals, which are generally more reactive than sulphur dioxide. This dissolved sulphur dioxide is potentially capable of behaving as an oxidant and reductant depending upon redox potential of the system. As a result of reaction of these ionic species with lipid and proteins in cell walls and membranes, chain reactions are initiated and giving rise to more free radicals such as superoxides, single oxygen, hydroxyl ion (OH⁻) etc. So, the level of ascorbic acid, β-carotene and phenolic compounds increase which provide protection against sulphur dioxide phytotoxicity by removing these free radicals. Exogenous application of antioxidants as ascorbate and benzoate and nutrient supplementation may inverse/ mitigate the effect of sulphur dioxide pollution.

Key Words : Sulphur dioxide pollution, Effects on plants, Amelioration

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Sulphur is a very important plant nutrient and is essential for plant growth. Sulphur content of healthy leaves usually ranges from 500 to 14000 ppm on dry weight basis (0.5 – 14 mg/g dry weight) (Varshney and Garg, 1979). Plants usually absorb sulphur from soil in the form of sulphate ions. Sulphur can also be absorbed through leaves in the form of sulphur dioxide (SO₂) and sulphur trioxide (SO₃) from the atmosphere. Beyond a certain critical level, sulphur assimilation adversely affects photosynthesis, respiration and other plant processes. On the basis of experimental exposures, a concentration of about 429 µg sulphur dioxide per m³ (0.15ppm) has been widely accepted as a threshold level below which a range of higher plants would not be injured even during prolonged exposure (Zahn, 1963). High levels of sulphur dioxide in the atmosphere can increase the sulphur content in the leaves beyond the critical level so that prolonged exposure may cause irreversible injury and ultimately death of the plant.

Plant responses to sulphur dioxide:

Exposure of plants to SO₂ led to increase in seedling growth *i.e.* plant height, root and shoot lengths, fresh and dry weights of root and shoot (Awanish and Kumar, 2010). Initial SO₂ induced stimulation of growth has also been reported by Kumar and Singh (1986) and Prasad and Rao (1981). Faller (1971) reported that even under the condition of normal sulphur nutrition, very low level of sulphur dioxide can stimulate above ground growth. Thus, sulphur dioxide serves as a source of nutrient 'S' at low level and this may explain stimulation in root and shoot length and plant height.

The long term exposure of *Triticum aestivum* L. *Raphanus sativus* L. plants to 1306 µgm⁻³ SO₂, caused significant biochemical changes leading to reductions in growth of these plants as compared to control (Awanish and Kumar, 2010 and Awanish *et al.*, 2011).

The SO₂ gas is absorbed into mesophyll of leaves through the stomata, but it also gets deposited at significant rates to wet surfaces where it may dissociate to form sulphite or bisulphate and reacts with cuticular waxes. This can affect the cuticle to such an extent that a certain amount of SO₂ can enter via damaged cuticle (Wellburn, 1994). Toxicity of SO₂ is largely due to reducing properties of gas. SO₂ gas

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combines with water in intercellular spaces to form sulphurous acid (H_2SO_3), which dissociates into H^+ and HSO_3^- ions. Thus, the foliar injury in sulphur dioxide treated plants is caused by accumulation of sulphites in the mesophyll tissues of leaves and inside the leaf the sulphur dioxide or its breakdown products react with cellular components, mainly cellular membranes causing injury or death to tissues (Richard, 1965) and eventually leads to interveinal necrosis (Thomos, 1961 and Rao *et al.*, 1985).

Mature leaves are more susceptible to sulphur dioxide injury (Awanish and Kumar, 2010). This may be due to increased intercellular spaces in mature leaves which facilitate rapid gas flow (Evans and Ting, 1974; Kumar and Singh, 1986). The younger leaves being in synthetic phase (anabolic phase) can synthesize the metabolites against the pollutant stress and hence, resistant while mature leaves being in degrading phase (catabolic phase) and the degradation is accelerated by SO_2 pollution.

Awanish and Kumar (2010) observed that after 10-15 days of fumigation, whitish yellow chlorotic patches appeared in interveinal areas and on prolonged exposures, these patches became dark brown bifacial necrotic lesions. This may be due to the fact that when the limiting concentration of gas is exceeded; the cells are first inactivated with or without plasmolysis, then killed. When extensive areas are killed, the tissue collapse and dry up, leaving a characteristic pattern of interveinal and marginal acute injury. If only a few cells in an area are injured, this area may become chlorotic or brownish red in colour, owing to chronic injury (Thomas, 1961). When prolonged exposure occurs, these areas become necrotic due to death of large amount of cells in that area. A slow oxidation of sulphites to sulphate occurs in the cells and this reduces the toxicity of sulphites. The sulphate toxicity is in the form of chronic injury manifested by white or brownish red turgid areas on leaves caused by rupture of some cells or chloroplasts within the cells (Solberg and Adams, 1956; Thomos and Hendricks, 1956). Injury by sulphur dioxide is local and no systemic effects have been observed, while the injured areas of leaf never recover, the uninjured areas quickly and fully regain their functions and new leaves develop normally.

Sulphur content is high in plants exposed to sulphur dioxide (Murray *et al.*, 1992; Dwivedi *et al.*, 2008; Awanish and Kumar, 2010). It is observed that the increase in foliar injury of SO_2 exposed T_1 plants could be correlated with the decrease in leaf-extracts pH and not to increase in the sulphur content (Awanish and Kumar, 2010). Because with increase in the age of plants the sulphur content was found to be decreased in leaves. It is possible that at later stages of growth, the sulphur was translocated from leaves to other plant parts (Garsed and Read, 1977; Rao *et al.*, 1985). Perhaps this is the mechanism through which plants avoid foliar injury caused by S-accumulation.

It is already known that subsequent to absorption and

oxidation of SO_2 into sulphite and sulphate ions within the plant cells, H^+ and other active oxygen radicals are generated, which not only reduce the buffering capacity of the cells but also affect various cellular components (Jeyakumar *et al.*, 2003).

The leaf extract pH value of plants declined significantly due to SO_2 (Awanish and Kumar, 2010). Theoretical and experimental studies have pointed to H^+ exchange as primary reason for cation leaching from the leaf surface (Rao *et al.*, 1985; Pfanz *et al.*, 1987). Bytnerowicz *et al.* (1986) reported that H^+ concentration in plant tissue increases when buffering systems are weakened and changes of pH in leaf tissue can be correlated to leaf injury. Similar result was also observed by Wang *et al.* (2005). Pfanz *et al.* (1987) suggested that in SO_2 exposed plants, protoplasts are damaged by acidification rather than by accumulation of toxic anions. Cells are capable of regulating internal pH. Acidification by imported acid will threaten survival only if pH stabilizing mechanism of the cytoplasm can not cope with acidification.

Exposure of plants to sulphur dioxide stress leads to oxidative stress. Sulphur dioxide gas dissolves in extra cellular fluid of plants and is potentially capable of behaving as an oxidant and reductant depending upon redox potential of the system. In the cell, SO_2 is converted into sulphite and/or bisulphite ions (HSO_3^- and SO_3^{2-}), which react with lipid and proteins in cell walls and membranes, chain reactions are initiated giving rise to more reactive oxygen species (ROS=free radicals) such as O_2^- (superoxide), OH^\cdot (hydroxyl radical), O^\cdot (single oxygen) and H_2O_2 , which are generally more reactive than sulphur dioxide (Hoffman and Jacob, 1984; Halliwell and Gutteridge, 1999). So, the level of ascorbic acid, β -carotene, phenolic compounds, superoxide dismutase (SOD), catalase, ABA etc. increases, which provide protection against sulphur dioxide phytotoxicity by removing free radicals (Takahama, 1992; Mandal and Mukharji, 1998; Jeyakumar *et al.*, 2003; Surowka *et al.*, 2007).

Sulphur dioxide can easily penetrate, especially in the light, into chloroplasts, which are the main place of the action of sulphite ions (Pfanz *et al.*, 1987 and Surowka, *et al.*, 2007). Chloroplast exposed to sulphur dioxide shows disorganization and significant changes at ultrastructural level, for example, the swelling of thylakoid discs and disintegration of other intrachloroplast membranes resulting in the formation of small vesicles. The photosynthetic apparatus appears to be adversely affected due to the changes resulting at subcellular level in chloroplast fumigated with sulphur dioxide. Because of the destruction of chloroplasts leaves become chlorotic (Wellburn *et al.*, 1972).

It is observed by Awanish and Kumar (2010) that chlorophyll a, chlorophyll b and total chlorophyll contents in SO_2 exposed T_1 plants decreased maximally as compared to control. The loss of chlorophyll a was greater than that of

chlorophyll b.

Sulphur dioxide can react with chlorophyll in three distinct ways: bleaching (*i.e.* loss of colour), pheophytinization (*i.e.* degradation of chlorophyll molecules to photosynthetically inactive pigment pheophytin) and process responsible for blue shift in pigment spectrum (Nieboer *et al.*, 1976). In pheophytinization, the degradation of chlorophyll occurs by displacement of Mg⁺² ions by free H⁺ ion from tetrapyrrol ring of chlorophyll molecules and thus, converting them into pheophytin, a photosynthetically inactive brown pigment. Rao and Leblanc (1966) have shown experimentally that the breakdown of chlorophyll to pheophytin in higher acidity is accompanied by the removal of Mg⁺² ions from chlorophyll molecules. The enzyme chlorophyllase is responsible for degradation by removal of phytol or reversible reaction. Sulphur dioxide pollution increases the chlorophyllase activity and thus, decrease chlorophyll contents (Jeyakumar *et al.*, 2003).

Decrease in carotenoids in SO₂-exposed plants was observed and carotenoids were highly sensitive to SO₂ pollution. Sosnovschi *et al.* (2003) noticed 93.01 per cent reduction in carotenoid content under SO₂ contamination.

Stomatal size, stomatal frequency, stomatal index and stomatal coverage area are adversely affected in SO₂ exposed plants. The stomatal response to environmental changes is important in controlling the absorption of pollutants by plants. A decrease in the stomatal aperture or induced stomatal closure or decrease in stomatal frequency may be an avoidance mechanism against the inhibitory effect of a pollutant on physiological activities such as photosynthesis. The reduced stomatal aperture resists the entry of pollutants, thus, preventing their adverse effects on plants (Awanish and Kumar, 2010).

Several mechanisms have been proposed to explain the effects of SO₂ on stomatal movement (Heath, 1980). Stomatal movements are caused by changes in guard cell turgor arising from the movement of K⁺ and H⁺ with electroneutrality being maintained by movement of Cl⁻ or internal production of malate. Activation of plasma membrane-localized anion channels results in guard cell depolarization, potassium efflux and loss of guard cell turgor and volume, which causes stomatal closure (Blatt, 2000). Another possible controlling mechanism may be increased ABA accumulation in the plants. When ABA has been applied to plants, there has been an increase in stomatal closure in plants exposed to pollutants (Kondo and Sugahara, 1978).

Reactive oxygen species (ROS) serve an important signaling function, providing information about changes in the external environment. H₂O₂ has been implicated to play a signaling role in guard cells that permit gas exchange. ABA causes an increase in H₂O₂ production, which serves as a signaling intermediate to promote stomatal closure (Zhang *et al.*, 2001), although a study has suggested that H₂O₂ may

also function in a divergent pathway that controls stomatal movement (Kohler *et al.*, 2003). H₂O₂-induced stomatal closure was reversed by exogenous application of free radical scavengers such as ascorbate and benzoate (Zhang *et al.*, 2001). Consequently, plants with increased ascorbate and benzoate might be predicted to exhibit reduced responsiveness to ABA or H₂O₂ signaling (Chen and Gallie, 2004).

Jeyakumar *et al.* (2003) observed that stomatal frequency and stomatal index were not affected by sulphur dioxide. However, the size of stomata was significantly reduced.

The established effects of SO₂ on stomatal function, chlorophyll content, carotenoid content and transpiration rate are important factors that are responsible for reducing photosynthetic ability. This reduction in photosynthetic activity of chlorophyll molecules leads to overall reduction in growth, productivity and yield.

Another important factor that impairs photosynthetic activities is RuBP carboxylase, an enzyme which has a key role in photosynthesis. Since in a polluted atmosphere, SO₂ competes with CO₂ for reaction site on this enzyme (Mansfield and Jones, 1984), the activity of RuBP carboxylase is affected, which affects the photosynthetic rate and hence, cause growth reduction.

On assessment of growth performance, Awanish and Kumar (2010) observed that SO₂ treatments cause considerable reduction in various growth parameters such as lengths of root and shoot, number of leaves, number of roots, number of primary branches and number of nodules in pea, dry weight fractions and net primary productivity (NPP) in T₁ plants as compared to control. Ca(OH)₂ as spray and as nutrient, sodium benzoate as spray and potassium ascorbate as spray improve the growth of SO₂ exposed T₂, T₃, T₄ and T₅ plants. The shoot growth of SO₂ exposed plants were affected mostly due to the retarded development of leaves (Agrawal *et al.*, 1985; Rao *et al.*, 1985). Reduced leaf area has been found to reduce photosynthetic capacity and dry matter accumulation of plants (Byers *et al.*, 1992).

Since biomass accumulation is an integrated result of all biochemical, physiological and metabolic activities in plants (Agrawal *et al.*, 1985; Rajput and Agrawal, 1994), its significant reduction further confirms that SO₂ may directly interfere with these functional processes resulting in biomass reductions as well as growth retardation.

As the growth of underground plant parts is dependent on photosynthate translocated to them from the above ground parts, direct effects of SO₂ on the photosynthetic leaves are reflected in poor development of the root system and number of nodules in pea (Awanish *et al.*, 2011). In this study, reductions in root weight fractions are higher than those of the shoot weight. The reduction in root biomass is due to low translocation as a result of reduced photosynthetic

activity and inhibition of phloem loading system in polluted environment (Teh and Swanson, 1982).

Kumar and Singh (1985), Kumar and Singh (1986) and Awanish and Kumar (2010) have reported advance flowering in *Cicer arietinum*, *Pisum sativum* and *Vigna sinensis*, *Triticum aestivum*, respectively as a result of SO₂ pollution. This may be due to the fact that under stress conditions plants are in hurry to complete their life cycles.

Significant reduction in yield and yield contributing factors are observed in SO₂ treated plants as compared to control (Kumar and Singh, 1985; Kumar and Singh, 1986; Rajput and Agrawal, 2005; Awanish *et al.*, 2011). The decrease in seed yield was mostly attributed to a decrease in number of fruits and this decrease in number of fruit per plant may result from either a decrease in flower pollination and fertilization, a decrease in fruit retention or an inadequate development of young fruits. Inhibition of pollen germination and pollen tube growth have been observed by Varshney and Varshney (1981).

Photosynthesis is crucial to the discussion of any of the effects of pollutants on plants. SO₂ has been reported to considerably reduce the photosynthetic rate. This reduction in photosynthesis leads to decrease in weight of seeds, seeds per fruit and number of fruits per plant and hence, reduction in total yield. Kumar and Singh (1986), Rajput and Agrawal (2005) observed that the carbohydrate contents of stem, leaves and seeds are less in SO₂ exposed plants. The decreases in total carbohydrate content probably correspond with the photosynthetic inhibition or stimulation of respiration rate.

Significant reductions are observed in total protein content in mature plants due to exposure of SO₂ (Singh *et al.*, 2003 and Awanish and Kumar, 2010). It has been suggested that SO₂ interferes with enzymes regulating amino acid synthesis (Pierre and Queiroz, 1982) leading to qualitative and quantitative changes in amino acids. Such changes may reduce the protein content of SO₂ exposed plants.

Jeyakumar *et al.* (2003) observed that contents of total phenolics, anthocyanin and proline in leaves of plants are increased significantly in SO₂ exposed.

Phenolics are diverse secondary metabolites (flavonoids, tannins, hydroxycinnamate esters and lignin), abundant in plant tissues. Polyphenols possess ideal structural chemistry for free radical scavenging activity and they have been shown to be more effective antioxidants *in vitro*. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors and from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and also from their ability to chelate transition metal ions (Rice-Evans *et al.*, 1997). Another mechanism underlying the antioxidative properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of the membranes (Arora *et al.*, 2000). These changes can hinder the diffusion of free

radicals and restrict peroxidative reactions. Phenolic compounds also involved in the hydrogen peroxide scavenging cascade in plant cells (Takahama and Oniki, 1997).

Anthocyanins, a glycosylated form of anthocyanidins, are a group of flavonoids mostly responsible for the colour of fruits from red through purple to blue. Anthocyanins have been suggested to act as potent antioxidants. The potent antioxidant activities of anthocyanins are related to their unique structures. The O⁺ (oxonium ion) in the C-ring and their capacities to facilitate stable radical products after interrupting chain reactions (Van Acker *et al.*, 1996). Anthocyanins are also thought to act as important biological chelators able to indirectly reduce the oxidation of other biological antioxidants such as ascorbate. By forming complexes with transition metals, anthocyanins have been demonstrated to prevent the conversion of H₂O₂ and O₂ to destructive OH radicals through Haber-Weiss-Fenton reactions (Van Acker *et al.*, 1996).

Proline takes participation in a lot of reactions of plant metabolism such as activation of respiration, regulation of acceptance of O₂, contributes to synthesis of chlorophyll and supplies amino groups for the synthesis for some amino acids. It is well known that proline accumulates in plants during adaptation to various types of environmental stress, such as draught, salinity, nutrient deficiency, high temperature and exposure to different types of pollutants (Oncel *et al.*, 2000). There are three possible causes of the free proline accumulation under stress: first, stimulation of proline synthesis from glutamic acid (Bogges *et al.*, 1976), which has been found to be dependent on the abscissic acid concentration (Stewart, 1980); second, inhibition of proline oxidation to other soluble compounds and third, inhibition of protein synthesis (Stewart, 1973).

In contrast to its metabolism, the physiological significance of proline accumulation has not yet been completely clarified. Researchers have ascribed to proline a positive role associated with some sort of adaptive response. According to Stewart and Lee (1974), proline is a substance inducing osmotic adjustment. Other researchers have suggested that proline is a source of energy, carbon and nitrogen for the recovering tissues (Blum and Ebercon, 1976). Kurkdjian and Guern (1989) suggested that proline may be involved in alleviating cytosolic acids associated with several stresses. The removal of excess H⁺ occurring as a result of proline synthesis may have a positive effect on reduction of SO₂ induced damage. From the results obtained, it is suggested that proline can protect cells and tissues against damage induced by SO₂.

Amelioration of SO₂ phytotoxicity:

Cowling and Lockyer (1978) studied that how nutrient supply influences the effect of SO₂ on perennial ryegrass. They exposed plants for 85 days, in chambers, to a daily average of

55 µg m⁻³ SO₂ or filtered air. Plants were grown with or without added soil sulphur and two rates of added nitrogen (N: low and high). Plants grown with high N and no added soil S developed S deficiency symptoms. The S deficiency symptoms were reduced in the high N treatment in the presence of SO₂ while shoot dry weight was more than doubled and root dry weight was almost doubled. When high N plants were supplied with soil sulphate, the addition of SO₂ to the atmosphere did not alter root or shoot dry weight. The number of tillers increased with increased N, with added soil sulphate and when exposed to SO₂. There was little difference in shoot or root dry weights and number of tillers in the low N treatments in the presence or absence of SO₂. Total shoot sulphur and sulphate-S generally increased with the addition of soil sulphate and exposure to SO₂; although, high N treatments had lower levels of both, which was attributed to dilution, as the high N plants were larger.

SO₂ gas mainly diffuses in leaves through stomata. So, antitranspirants may help against air pollution by covering the stomatal apertures. It has been suggested that the foliar application of chemicals such as OED (oxyethylenedecosanol) green may be used to protect plants near stationary source of SO₂ emission. Perhaps OED green and other antitranspirants form a thin film over the leaves and may reduce injury caused by sulphur dioxide pollution, however, the efficacy of antitranspirants in preventing sulphur dioxide damage is uncertain, since they are also likely to affect the normal diffusion of carbon dioxide. Moreover, application of antitranspirants on large scale to protect plant from pollution may create its own problem (Varshney and Garg, 1979).

Shimazaki *et al.* (1980) observed that chlorophyll breakdown in SO₂ exposed plants can be checked by using various free radical scavengers. Winterhalden (1981) reported that Ca⁺⁺ as mineral nutrient provides protection to SO₂ exposed plants. Nandi *et al.* (1981) reported that potassium ascorbate acts as antidote to SO₂ phytotoxicity. Nandi *et al.* (1984) observed calcium hydroxide spray checks chlorophyll degradation by neutralizing the acidity of SO₂ in plants.

Rao *et al.* (1985) sprayed aqueous solution of lime water and sodium benzoate on SO₂-exposed *Vigna sinensis* plants and observed lower degradation of photosynthetic pigments and better growth than SO₂-exposed plants. Singh and Rao (1985) reported amelioration of SO₂-induced injury through foliar spray of urea.

Krishnaya and Date (1996) studied the impact of SO₂ and SO₂ + ascorbic acid on growth and partitioning of dry matter in *Trigonella foenum-graecum* L., two-week-old plants were exposed to SO₂ for 2 h daily over a 42 day period. One of the exposed sets was treated with ascorbic acid. Although ascorbic acid treatment could mitigate the effect of SO₂, the differences were not found to be statistically significant. Significant changes were seen in fruit yield, suggesting that the effect of ascorbic acid is cumulative.

Agrawal and Verma (1997) concluded that both nutrient deficiency and SO₂ caused the observed reductions of measured parameters but that the addition of NPK in different combinations was able to ameliorate the adverse effects of SO₂.

Bhagya Lakshmi and Raza (2000) report the nitrogen and sulphur levels of 11 tropical trees growing under industrial establishments and experimental results on ameliorating SO₂ in tree sampling by nitrogen fertilization. They analyzed percentage injury due to SO₂ and its percentage recovery due to nitrogen fertilization at weekly intervals and observed that nitrogen fertilization is quite useful in reducing the SO₂ toxicity.

Han (2001) observed the relation between tree stomatal infiltration and SO₂ injury and the protection effect of ABA. The effects of SO₂ smoking to the selected trees were observed and the results show that stomatal infiltration was a comparably constant index for certain tree species. The index is also positively correlated with K⁺ efflux in leaf (r = 0.92, alpha < 0.01). In the experiment of SO₂ smoking, the effect on infiltration of same species under different SO₂ concentration was little, less than one grade, while K⁺ efflux increased with the increment of SO₂ amount absorbed by the leaves. When the leaves were sprayed with ABA solution, the higher the ABA solution concentration was, the lower the K⁺ efflux was. He concluded that the ABA solution on leaves has remarkable effect of protection of SO₂ injury.

Tomato (*Lycopersicum esculentum*) fruits contain many antioxidants such as lycopene, vitamin C (ascorbate), vitamin E (tocopherol), polyphenols such as kemferol, querceten and huge levels of total phenolics typically has high oxygen radical absorbance capacity (ORAC) value, which helps in amelioration of SO₂ phytotoxicity (Singh *et al.*, 2004).

Singh *et al.* (2005) observed that increased fertilizer application over recommended dose of N, P and K resulted in positive response by reducing losses in photosynthetic pigments and total biomass by ambient air pollution of Allahabad city.

Awanish and Kumar (2010) and Awanish *et al.* (2011) observed better growth when SO₂ exposed plants were sprayed with lime water (as spray and as nutrient), sodium benzoate and potassium ascorbate and they concluded potassium as better ameliorating agent.

Conclusion:

In the last it is concluded that industrial development is necessary and sulphur dioxide pollution is side effect of this industrial development. It causes loss of productivity in crop plants. Although the complete check of sulphur dioxide can't be possible but its effects can be mitigated with the help of ameliorating agents such as antioxidants / free radical scavenging agents and nutrient supplementation.

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