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IMPACT OF SULPHUR DIOXIDE (SO_2) STRESS ON GROWTH AND PHYSIOLOGICAL RESPONSES OF *Calendula officinalis* L.

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ABSTRACT

Sustainable agriculture is need of era to maintain productivity and environment quality for farmers and society. The increased industrialization and urbanization are mainly responsible for increased air pollution. Pollutants can already lead to serious reduction of crop growth and yield. Sulphur compounds are the most harmful pollutants; the prime one is Sulphur dioxide (SO_2). At pre-flowering stages, the growth responses of field-grown marigold (*Calendula officinalis* L.) plants to SO_2 (320g/m3, 667g/m3, 1334g/m3) stress were investigated. Significant decrease in root and shoot length, leaf area, leaf number and chlorophyll content was observed with increased SO_2 concentration. Compared with control, fumigation of SO_2 at lower concentration also showed significant changes in plant attributes. SO_2 fumigation reduces plant growth and affects negatively various morphological and physiological attributes. The results clearly showed that increasing day to day pollution causes the adverse effect on human food and economic viability of farm operations.

Keywords: Fumigation; SO_2; pollution; *Calendula officinalis* L.

INTRODUCTION

The anthropogenic activity has augmented the concentration of harmful chemicals in the present environment. Pollution is mainly a problem of affluent countries. Man has converted the life-supporting systems and has vastly changed the natural ecological relationships. Air pollution has become a concern to plant existence in today's world [1]. The increased industrialization and urbanization are mainly responsible for increased air pollution.

Plants are the most important component of any ecosystem, so paying attention to their sensitivity to air pollution is more important than meeting any air pollution limits. The introduction of chemicals, particulate matter or biological materials that cause harm or discomfort to living being in air is known as air pollution.The alterations in ecosystems are due to addition of toxic substances in air. Serious degradation and depletion have caused through mismanagement to meet the needs and satisfying greed. Air pollution acts as an additional stress on plants because they respond to atmospheric pollution in the same manner as to other external stresses. The injurious effects of pollutants on plants may be visible or invisible. Many studies have evaluated the toxic effects of gases on morphological, biochemical and physiological conditions of plants [2,3].

Air pollutants have a significant impact on the natural and built environments, as well as the ecosystem. Nitrogen oxides (NOx), carbon monoxide (CO), sulphur dioxide (SO_2), troposphere ozone (O_3), suspended particulate matter, and heavy metals are the most diffuse and hazardous pollutants in the atmosphere. Many contaminants in the air have been recognised as phytotoxic agents. Nonetheless, sulphur dioxide (SO_2) phytotoxicity has been known for almost a century, acid rain for roughly 20 years, and the effects of ozone (O_3) for about 30 years.In last decade, other pollutant likewise nitrogen compounds, nitrogen oxides (NO_X), ammonia (NH_3), peroxyacetyl nitrate (PAN), fluorides and heavy metals also severely affect the environment.

A wide range of hazardous compounds, such as gases and particles as air pollutants, either directly show toxic effects on plants morphology or alter their physiology which reflects in the growth and yield of almost all crop plants and natural ecosystem structures. Herbaceous and juvenile woody plants often results in reduction of dry matter accumulation when exposed to gaseous air pollution with more effect on root growth than shoot [4]. Air pollutants cause stress conditions due to which net photosynthesis is curbed and growth rate is reduced resulting in acute visible damage [5,6]. On the basis of morphological and physiological point of view plants show various changes like colour, shapes, leaf number, area, root and shoot length, even after this plants survive in such areas [7]. The injury may either be acute or chronic as per the level of SO_2 absorbed by plants [8,9].

Sulphur compounds are the most harmful pollutants; the prime one is sulphur dioxide (SO_2) which is considered as inevitable and prominent abiotic stress parameter. Some earlier worker also recognized SO_2 as major atmospheric pollutants in urban and industrial areas in India [10]. In India, it has increased by over 50% in the last decade, and it has also surpassed the United States to become the second highest SO_2 generating country in 2014. Understanding area-specific pollution has been aided by pollution data and weather characteristics [11]. The sources of atmospheric sulphur are combustion of fossil fuels, organic matter and sulphur content of the ores of copper, iron, zinc, lead, nickel. It is primarily a by-product of electronic utility fuel usage, industrialization, and vehicle smoke. Several studies have indicated direct toxic effects of atmospheric pollutants on foliar loss and yield reduction in various crops [12]. These air pollutants, in particular, have a negative impact on the health of humans, animals, and vegetation, including many therapeutic plants. These pollutants affect directly via leaves or indirectly via roots through soil acidification. High SO_2concentration also affects leaf size and shape [13,14].

SO_2 is a byproduct of electronic utility fuel usage, industrialization, fossil fuel use, and vehicle emissions. The continual surge of SO_2 in the atmosphere has lately been measured and analysed, and it was discovered that it has increased by almost 50% in India over the last decade. In 2014, India overtook the United States as the world's second-largest emitter of SO_2.The phytotoxicity of SO_2 is due to various reasons. Through stomata SO_2 get accumulated in mesophyll tissue and form phytotoxic sulphite and bisulphite ions [15] which causes various physiological characters changes like foliar injury, stomatal behavior, transpiration, photosynthesis and reduce final yield [16]. The increased concentration of SO_2 results into decrease in stomata abundance and aborted stomata before the appearance of visible injuries [17-19].

The physiological responses of various plants to atmospheric pollution may vary widely and these variations depends on many factors, likewise differences in pollutant concentrations, distribution, physiological activity, phenological stage, the genetic origin and nutritional status of plants as well as various environmental stress factors. In particular, the easiest way of monitoring air pollution in field conditions is detection of physiological changes in plants and visible symptoms of injury by air pollution damage but sometimes alter physiological character of plants appear before visible, morphological damage takes place. Therefore, these pollutants absorbed by the plants leads to changes the chlorophylls and carotenoids contents of plants that also affecting plant production. However, the biochemical parameters such as chlorophyll content, soluble sugars, proline and carotenoids can give the valuable information about physiological status of plants. Reduced plant vigour and yield also reported when plant are exposure under pollutant stress condition.

Calendula officinalis which has a considerable medicinal value like antimicrobial [12], wound healing [20,21], immunomodulatory [22], antiviral and anti-tumoral [23] and also has increasing demand of good quality cut flowers availability.

The effect of low concentration of SO_2 on growth and development of *Calendula officinalis* has not been fully examined. Therefore, this study was designed with the aim of investigating the effect of low concentration of SO_2 on various morphological and physiological aspects of *Calendula officinalis*. As a result, extensive studies of the impact of this unavoidable air pollution on crops and other plants must be conducted in order to assess the level of harm it poses and to modify appropriate plant protection methods and methodologies in order to avoid severe pollutant damage.

MATERIALS AND METHODS

Site and Treatment

Calendula officinalis (pot marigold), family Asteraceae, a short lived aromatic, herbaceous annual with sparsely branched stem, leaves oblong lanceolate, inflorescence yellow capitulum disc florets (tubular and hermaphrodite) and ray florets (female tridentate), fruit is thorny curved achene. Various preparations from this plant are used as oil, antiseptic, diaphoretic, stimulant antispasmodic agents [24]. This plant can be grown in pots and field or in green houses.

The experiment was conducted in department of botany, Pt. C.L.S Govt. College, Karnal located in 29-32^{o}N latitude and 76-59^o E longitude under normal temperatures 32-34°C (day) to 17°-27°C (night). Seedlings were grown in polythene bags 10 cm. in diameter containing garden soil. Plants were exposed to SO_2 in continuous SO_2 flow chambers 60x60x60 cm. dimensions, 2 hours daily for 25 consecutive days. SO_2 was produced by passing air current in an aqueous solution of sodium metabisulphite [25] which under compressed air get decomposed producing SO_2. The sulphur dioxide is used in three different concentrations; 320µg/m^3, 667µg/m^3, 1334µg/m^3 and observations were recorded after 30 days, 45 days, 60 days growth on different morphological and biochemical parameters. The experiment had 4 treatment combinations: control (G_0), 320 µg/m^3 (G_1), 667 µg/m^3 (G_2), 1334 µg/m^3 (G_3) used for plant. Throughout the season, all additional agricultural techniques such as irrigation, weeding, and so on were carried out. Root length, number of leaves, shoot length, leaf area, chlorophyll content, ascorbic acid, and sulphur content were all measured. From the coteledonary node to the growing tip, root and shoot lengths were measured. Each plant's total number of leaves arising from the main stem was counted and reported as the number of leaves per plant. According to the manufacturer's instructions, all of the leaves were separated from the stem and their area per plant (sq. cm) was measured using a portable leaf area metre (Systronics 211, Ahmadabad, India). In biochemical analysis parameters likewise chlorophyll content, ascorbic acid and sulphur content were calculated. Leaf chlorophyll content was measured as described by Arnon [26]. Total amount of ascorbic acid mg/g fresh leaf was determined by using the formula of Keller Schwager [27]. Sulphur in the leaves was determined by turbidity method described by Patterson [28].

Statistical Analysis

The data obtained during the experiment as means of 2 consecutive years were subjected to statistical analysis using one-way analysis of variance and the differences were computed using Duncan's multiple range test at $p = 0.05$. All statistical analyses were performed using the SPSS software (version 11.5).

RESULTS

The results obtained in this study showed that exogenous application of SO_2 significantly influenced the agronomic and economic attributes of *Calendula* sp. crop. Data of our experiment shows that even lower concentrations of SO_2 effect on shoot length, root length, leaf number and leaf area attributes and cause considerable decrease in all the growth parameters. Higher decrement in these parameters was observed by applying G_3 (1334µg/m^3) treatment which shows its negative effect on plant growth attributes. There is no significant decrease in growth parameters with use of control. The decrease in plant growth might be attributed to decreased cell division and cell elongation induced by the application of SO_2 because of their pollutant effect. The results are in agreement with the findings of other researchers also working on air pollutant effect on physiological and morphological character of plants likewise Assadi et al. [29]. It was reported by earlier workers that pollutant in air decreased the plant growth, nutrient uptake and plant yield as well as quality of plant [4,7,14].

The treated plants exhibited retarded growth by decreased root length and shoot length despite augmenting pollutant dose root and shoot length continued to increase but with increasing SO_2 concentration maximum reduction was seen in 60 days old plants treated with 1334µg/m^3 of SO_2 and least reduction in 320µg/m^3 SO_2 treated plants as shown in Figs. 1 and 2. Prolonged exposure to SO_2 gases is observed to have a negative effect upon plant growth where root and shoot length were seen to be stunted after increased SO_2 concentration. With increased SO_2 concentration, the number of leaves decreases due to accumulation of sulphur compounds. Maximum reduction was seen in 45 days old plants treated with 1334µg/m^3 SO_2 and minimum in 60 days old plants treated with 320µg/m^3 (Fig. 3). Foliar injury symptoms appeared after 60days when light yellow chlorotic patches appeared in interveinal region. Symptoms were sporadic and appear earlier on 667ug/m^3 and 1334ug SO_2 treated plants. The leaf area injured increases with increased SO_2 concentration.

The results indicate that maximum increase in root and shoot length is observed in control by 9.42 cm. and 13.5 cm.

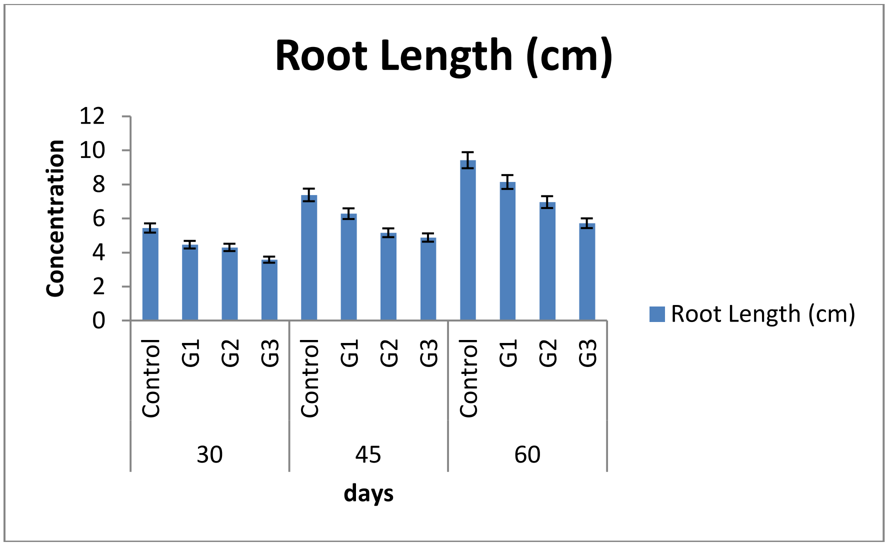

Fig. 1. Effect of SO_2 on root length. Values represent means ± SE (n = 3 per treatments)

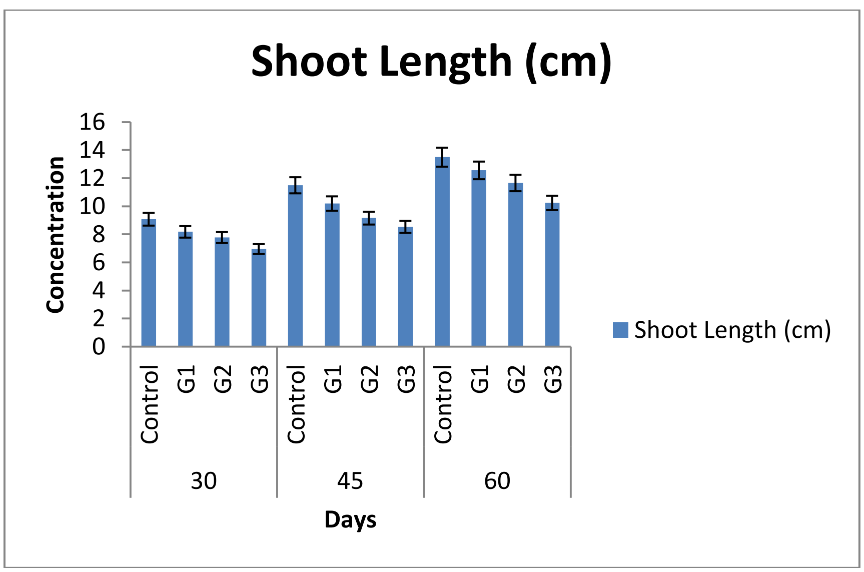

Fig. 2. Effects of SO_2 supply on Calendula shoot growth.SO_2 was supplied at 320µg/m^3, 667µg/m^3, 1334 µg/m^3. Data are means ± SE (n = 3 per treatment)

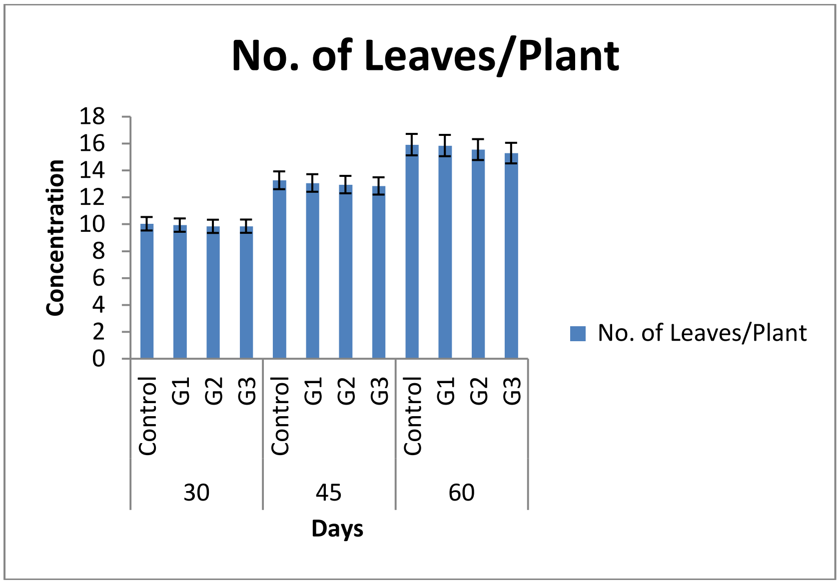

Fig. 3. Effect of SO_2 on number of leaves. The number of leaves was counted visually. One plant per plot was examined. Data are means ± SE (n = 3 per treatments)

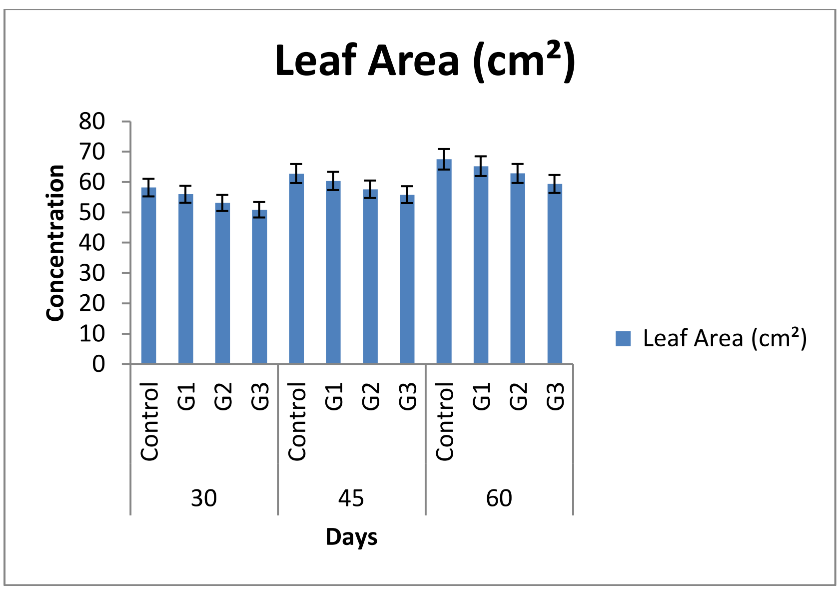

Fig. 4. Leaf area of *Calendula officinalis* as influenced by application of SO_2 at 30, 45 and 60 days. Values represent means ± SE (n = 3 per treatments) showing significant difference using Duncan's multiple range test at 5% probability level

The length of leaf is considered important characteristics, which reflect the capability of plant to protect itself from any kind of stress. As depicted in Fig. 4, the results of our experiment showed that the long-lasting impact of different air pollutants like SO_2 leads to causes a decrement the leaf size and growth that ultimately affect leaf surface area. Similarly increase in number of leaves and leaf area also occurs in control i.e. 15.91 and 67.49 cm. respectively. The maximum increase in all attributes occur in control is due to least pollution as there is no treatment with SO_2. The comparative data (Fig. 5) showed significant reduction in the morphological characteristics such as length of leaves and simultaneously its leaf area from 58.17(control) to 55.97 cm^2 (G_1), 53.1cm^2 (G_2), 50.86 cm^2 (G_3) treatments.

The biochemical parameters also show variations on treatment with SO_2. Various plant species shows different responses against same stress. The total chlorophyll content (Fig. 8) decrease in the treated plants but chlorophyll –b showed hardly any reduction in 30 days old plant in 320µg/m^3 of SO_2 concentration. With increased in age and concentration the amount of reduction was more in chlorophyll a in comparison to chlorophyll b as represented in Figs. 6 and 7.

45 days old plants treated with 1334µg/m^3 SO_2 shows maximum reduction while 30 days old plants treated with 320µg/m^3 shows minimum reduction in ascorbic acid content (Fig. 9). Marked increase in sulphur was observed with increase in sulphur concentration (Fig. 10). Maximum increase in 60 days old plants treated with 1334µg/m^3 was observed i.e. 0.52mg/g. The result of comparative analysis indicates with increase of SO_2 there is a decrease in biochemical parameters (Fig. 11).

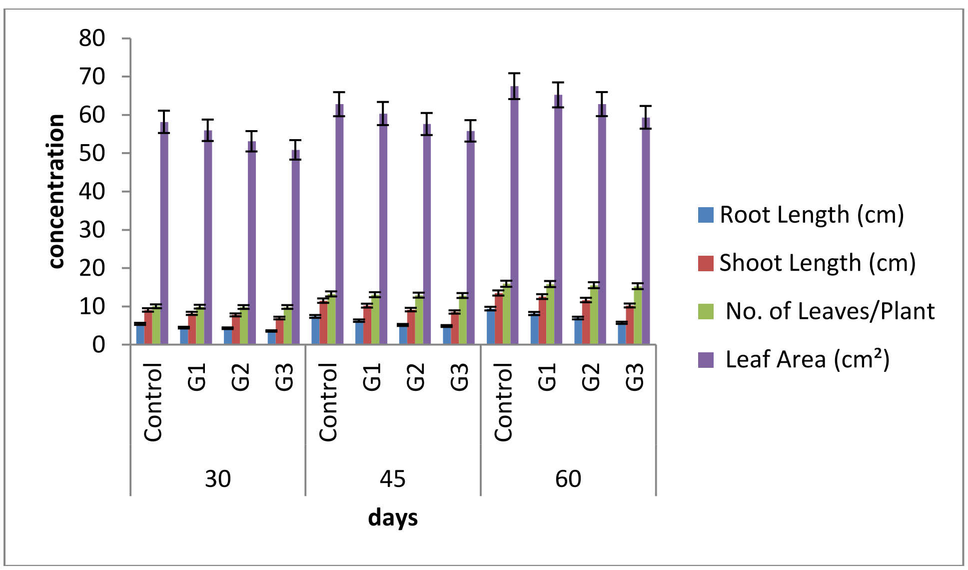

Fig. 5. Comparision of different growth attributes of *C. officinalis* as influenced by various SO_2 solutions. Different letter in figure represent values significantly different at 0.5% level, using Duncan multiple range test (DMRT)

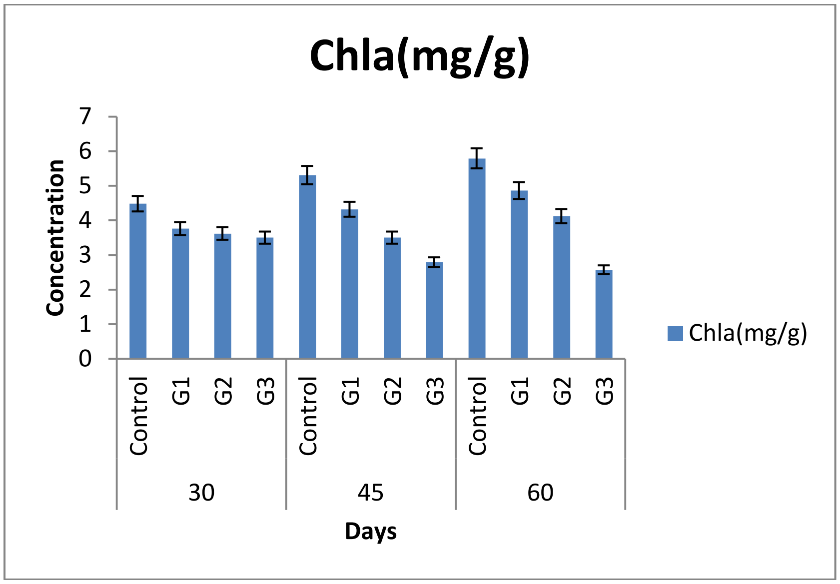

Fig. 6. Chlorophyll a content in leaves of *C. officinalis* treated with various SO_2 concentrations. Different letter in figure represent values significantly different at 0.5% level, using Duncan multiple range test (DMRT)

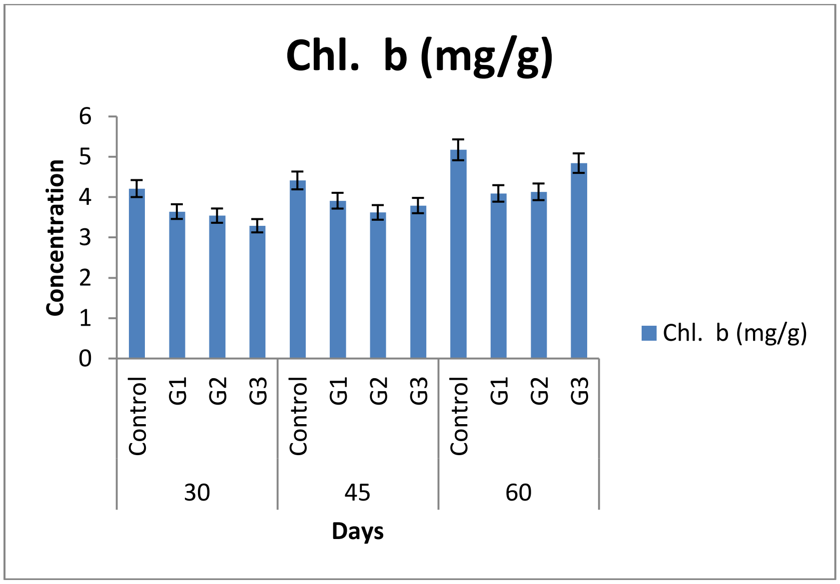

Fig. 7. Impact of SO_2 on Chlorophyll b content. Data are means ± SE (n = 3 per treatments)

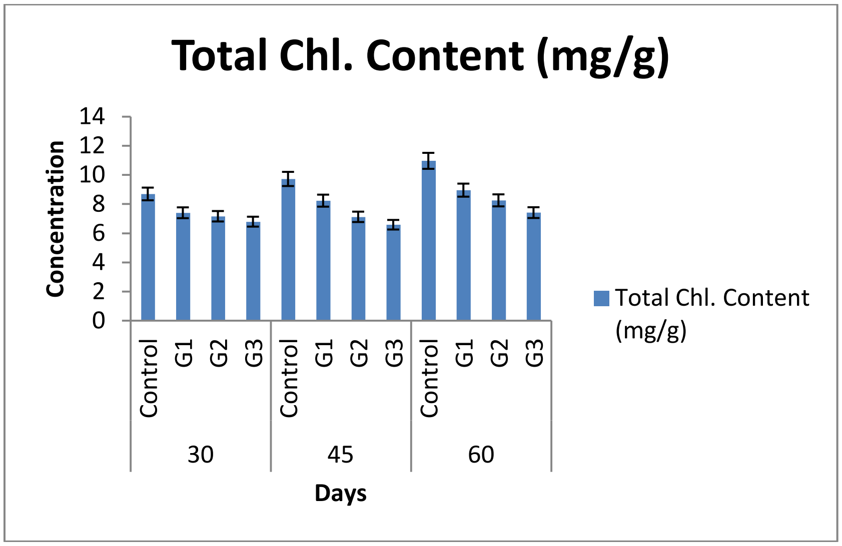

Fig. 8. Total chlorophyll content of *Calendula officinalis* as influenced by application of SO_2 at320µg/m^3, 667µg/m^3, 1334 µg/m^3. Data are means ± SE (n = 3 per treatments)showing significant difference using Duncan's multiple range test at 5% probability level

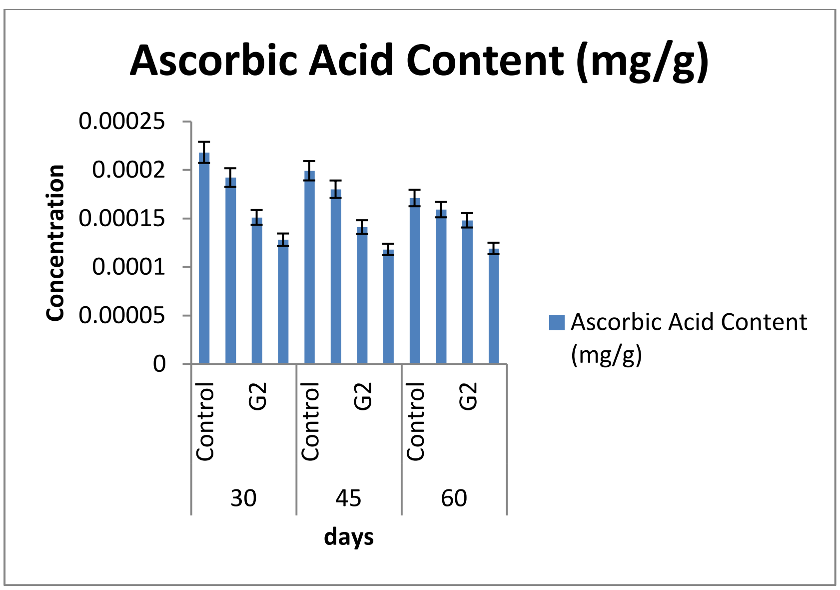

Fig. 9. Ascorbic acid of *Calendula officinalis* as influenced by application of SO_2 at 30, 45 and 60 days. Values represent means ± SE (n = 3 per treatments) showing significant difference using Duncan's multiple range test at 5% probability level

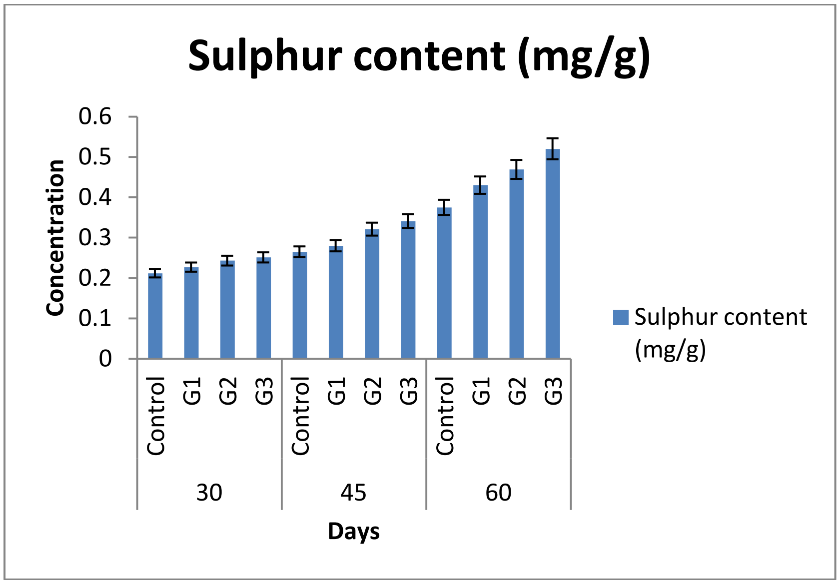

Fig. 10. Impact of SO_2 on sulphur content of *C. officinalis* leaves. Data are means ± SE (n = 3 per treatments)

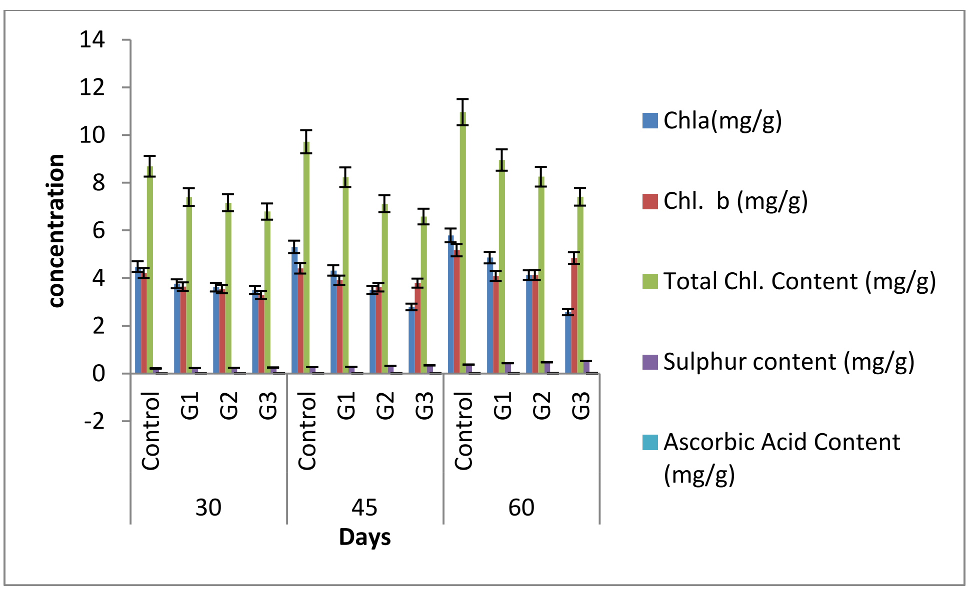

Fig. 11. Comparision of different biochemical parameters of *C. officinalis* as influenced by various SO_2 solutions. Different letter in figure represent values significantly different at 0.5% level, using Duncan multiple range test (DMRT)

DISCUSSION

Sulphur is a required micronutrient for the formation of amino acids (Cysteine and Methionine), hormones (Ethylene, polyamines), vitamins (Biotine), electron transport, photosynthetic oxygen generation, and a variety of other defense-related enzymes and chemicals in plants.

The application of SO_2 has proved to be marked effects on plant growth. Data from various experiments shows that sulphite radical are more toxic and reactive, mainly responsible for the primary damage symptoms like leaf yellowing, chlorotic spots, necrosis and finally leaf drying. While in later stages this symptoms become more severe leads to production of the reactive oxygen species (ROS) by damaging photosystem. By this, the cellular integrity damage and ultimately results in extensive tissue necrosis. SO_2 exposure also shows secondary negative impact by stomatal closure and cellular acidification.

The level of sulphur measured in leaves and soil differed significantly. As the amount of sulphur increases in the leaves, chlorophyll and nitrogen content decreases thereby confirming unfavorable effect of SO_2 on plant metabolism. Prakash et al. [30] observed reduced plant height, shoot and root length, number of leaves and fresh weight in SO_2 treated plants of barley. In plant cells so_2 is oxidized to sulphate (so_4-^{2}) through intermediate sulphite (so_3-^{2}) and the excess of sulphite may completely inhibit RUBP-Carboxylase. It drops electron flow within the chloroplast that leads to reduction in plant growth [31].

The leaf surface area of numerous plant species growing near significant air pollution was shown to be reduced. This data suggests that a hidden injury or physiological disruption may have occurred, resulting in negative effects on plant species' anatomical and morphological characteristics [32]. The current findings are consistent with Swain and Padhi's [33] findings, which showed a decrease in the number of leaves and their leaf area with higher SO2 exposure. The sulphur dioxide accumulates at faster rate in intercellular spaces aside from its oxidation and assimilation in tissues of plant that causes necrosis and cell injury, taking the form of reduced leaf growth and increased senescence. The phloem loading mechanism becomes a critical limiting step in restricting the translocation rate when exposed to SO_2. Majstrik [34] found that fumigating *Nicotiana tabaccum* with 0.02 ppm SO_2 for four weeks reduced the fresh weight of green leaves, shoots, and roots, as well as the root/shoot ratio, leaf area, and dry weight percentage. Hogsett et al. [35] observed growth responses to sequential and simultaneous exposure of NO_2 and SO_2 in radish (*Raphanous sativus cv cherry belle*) and spinach (*spinacea oleracea*) and found significant reduction in relative growth rate of total plants and roots. Rao and Dubey [36] found that different tropical trees responded differently to SO_2, with low conduction, decreased protein content, and increased sulphate content, superoxide dismutase, and peroxidase activities.

Fumigation of plants with SO_2 caused characteristics foliar injury symptoms. The phytotoxicity of SO_2 is due to various reasons likewise acute injury is caused by accumulation of sulphites in mesophyll tissues of leaves which are responsible for metabolic changes such as destruction of chloroplast and diminishing the enzymes activity. The acute and chronic effects cause the visible damage while the metabolic disturbances in plant system cause invisible damage. Acute injury may be due to rapid absorption of high concentration of pollutants and chronic injury due to low concentration of pollutants for longer duration.

In green plants, the chlorophyll and carotenoids is the main core of energy production and their amounts in leaves are significantly affected by environmental factor which reflected in their plant metabolism. As we know that Sulphur or nitrogen dioxides (SO_2, NO_2) are the main pollutants in the polluted area and react with O_2 and water vapour to form acid rain. By the entry of this pollutant materials in the plants via acid rain cause reformation of chlorophyll to pheophytin, because H^+ ion can substitute Mg+ ion in the chlorophyll molecule. Air pollution affects not only the chlorophyll and carotenoid content of the leaf, but also the shape and direction of the thylakoids [37].

Due to more SO_2 concentration, cells are first inactivated with or without plasmolysis and then killed showing a characteristic pattern of injury [38]. SO_2 alters the translocation pattern, causing more photosynthates to be held in the shoots and less to be delivered to the roots, which has a significant impact on root growth and physiology [39]. Suwannapinunt and Kozlowaski [40] found significant reduction in chlorophyll content in seedlings of Robinia when exposed to 2 ppm SO_2 for 1-4 hours. Exposure to sublethal concentration of SO_2 causes yellowing of leaves. SO_2 enters leaf through stomata mainly. The size of stomata in SO_2 treated plant is larger, rate and time of opening of stomata is more, it also affects potassium efflux, membrane permeability, enzymes respiration and other metabolic process. Roger [41] observed that photosynthesis and stomatal resistance decrease with increasing SO_2 concentration in *Glycine max*. There was a correlation between pore width and stomatal conductance before exposure to SO_2 which continued until visible irreversible changes occurred henceforth it disappeared [42].

In addition to acting as a potent reductant, ascorbic acid also appears to facilitate the conversion of sulphite to hydrogen sulphide, which lessens the toxicity of SO2. The amount of ascorbic acid in plants probably has little bearing on how resilient they are to sulphur dioxide pollution. Boraikar and Chaphekar [43] studied foliar injury to *Trigonellafoenum–graecum* due to SO_2 and observed that leaf area injury and chlorophyll loss enhanced at increasing SO_2 concentration. The leaf area injury was greater in younger than old leaves.

CONCLUSION

The plants under the prolonged exposure to SO_2 gases impart negative effect upon growth of plants and its physiology however the physiological and morphological characteristics of plants show drastic damage in chronic SO_2 application. On a comparative account, data shown that reduction up to 50% marked observed in plant treated with SO_2 and higher reduction found in plant growth rate where SO_2 amount was higher than permissible limit. From the grower's perspective, fumigation of so_2 constitutively harmful even at low concentration, because it acts as pollutant to reduce plant growth and were found to be increased gradually with high concentrations that affect negatively various morphological and physiological aspects. The morphological characters like root length, shoot length, number of leaves and leaf area showed decrease with increased concentration of SO_2. Root length, shoot length and leaf area showed maximum decrease in 60 days while number of leaves showed maximum decrease in 45 days.

Similarly the biochemical characters like chlorophyll content and ascorbic acid also showed decrease with increased SO_2 concentration in 45 days old plant. Sulphur content increased with increased days of experimentation. Lowest dose also showed significant changes in plant attributes. Thus, the result suggests that increasing day to day pollution causes the adverse effect on crop canopy as a whole.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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