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Effect of Elevated Ozone on Plant Nutrients, Chlorophyll Content and Antioxidant Enzymes in Cauliflower (*Brassica oleracea* var. botrytis L.)

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An experiment was conducted at woodhouse farm, Horticultural Research Station, Ooty, in the period of October 2017 to March 2018, to quantify the impact of tropospheric ozone and ozone protectants spray on chlorophyll content and antioxidant enzyme levels of cauliflower crop in a factorial completely randomized block design replicated thrice. During the study period, the plant exposed to ambient ozone level + 3% Panchagavya foliar spray had higher chlorophyll 'a', chlorophyll 'b' and total chlorophyll. The ambient ozone level + 3% Neem oil foliar spray had significantly higher total nitrogen, total phosphorous, total potassium. The elevated ozone exposure at 200 ppb significantly reduced the, total nitrogen, total phosphorous, total potassium, Chlorophyll 'a', Chlorophyll 'b', total chlorophyll and increased catalase and peroxidase enzyme activities in cauliflower plant. It is concluded that the tropospheric ozone had detrimental effect on chlorophyll contents and anti-oxidant enzymes in cauliflower crop. Further, the ozone protectants played a major role to nullify the tropospheric ozone effect on growth, physiology, development and yield of cauliflower and among the protectents, panchagavya performed well followed by neem oil and ascorbic acid.

Key words: Elevated ozone, Chlorophyll, Anti-oxidative enzymes, Cauliflower crop.

Air pollution, which is trans-boundary in nature, has detrimental effect on all living organisms in the world and ambient air pollution plays a vital role in atmospheric climate change, human health and ecosystem (Cohen *et al.,* 2017). They are classified as primary air pollutants such as methane, CO_2, NO_X, sulfur oxides are produced from natural and anthropogenic activities and secondary air pollutants *viz.*, PAN (Peroxyl acetyl nitrate), ozone (O_3) and smog are produced in the air by the interaction of primary air pollutant. Tropospheric ozone (O_3) is one of the prominent secondary air pollutant in the world. Surface ozone is not only a greenhouse gas, which is next to CO_2 and methane also, has a deleterious impact on growth and yield of both agricultural and horticultural crops with long lasting impact in most parts of the world (IPCC, 2001).

The anthropogenic emissions of O3 precursors such as nitrogen oxides and volatile organic compounds (VOC) across Asia also increased, in particular over the Indian and Chinese region. Formation of ozone occurs in the troposphere by photochemical oxidation of CO, CH4 and non-methane volatile organic compounds by the hydroxyl radical (OH) in the presence of reactive nitrogen oxides. In India, urban and rural agriculture is mainly affected by the ozone pollution (Bell *et al.,* 2011). In atmosphere, the tropical regions are mostly referred as active photochemical region for production of ozone. Higher level of ozone affects plants in different mechanisms such as premature leaf fall, reduction in photosynthetic activity and biomass loss and also physiological and growth changes (Tetteh *et al.,* 2015). Ambient ozone causes a wide range of negative impact on major crop species and their physiological functions such as weakening of plants, retarded growth, inferior crop quality, altered carbon metabolism and decreased yield.

Ozone injury is also reported in various crops *viz.,* potato (*Solanum tuberosum*) in India, radish (*Raphanus sativus*) and turnip (*Brassica rapa*) in Egypt. It is affirmed that the ozone concentration is increasing in the atmosphere and it has potential threat to agricultural production and sustainability. There are no sufficient information and studies on quantifying the effect of ozone on growth and yield of cauliflower. Hence, the purpose of the study is to identify the critical issues related to the understandings of the effects of ozone exposure on cauliflower crop with the objectives of studying the impact of elevated ozone level on chlorophyll contents and anti-oxidant enzymes of cauliflower and the impact of ozone protectants on chlorophyll contents and anti-oxidant enzymes of cauliflower.

Material and Methods

The Indian Space Research Organization (ISRO) Climate Change Observatory is located at Woodhouse house farm, Horticultural Research

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Station (TNAU) with a latitude 11.4°N, longitude 76.7°E and altitude of 2520 m above MSL in Western Ghats of Nilgiris Biosphere Reserve at Ooty town. The monthly mean values with average maximum temperatures ranging between 13 - 22°C and average minimum temperatures between approximately 5 -12°C. Maximum rainfall occurs in monsoon (June to November) and post monsoon period (80% of total rainfall). The average annual precipitation is 1250 mm. Relative humidity is observed from 40 to 80% with highest value (> 80%) in monsoon and post monsoon. Wind direction is north and north easterlies during summer and changing over to western lies during monsoon and post monsoon season. The experiment was conducted by Factorial completely randomized block design (FCRD) with twelve treatments and three replications. The treatments include T_1 – Ambient ozone level, T_2 – Elevated ozone exposure @ 150 ppb, T_3 – Elevated ozone exposure @ 200 ppb, T_4 - Elevated ozone exposure @ 150 ppb + foliar spray 0.1% Ascorbic Acid, T_5 - Elevated ozone exposure @ 150 ppb + foliar spray 3% Neem Oil, T_6 - Elevated ozone exposure @ 150 ppb + foliar spray 3% Panchagavya, T_7 - Elevated ozone exposure @ 200 ppb + foliar spray 0.1% Ascorbic Acid, T_8 - Elevated ozone exposure @ 200 ppb + foliar spray 3% Neem Oil, T_9 - Elevated ozone exposure @ 200 ppb + foliar spray 3% Panchagavya, T_{10} – Ambient ozone level + foliar spray 0.1% Ascorbic Acid, T_{11} – Ambient ozone level + foliar spray 3% Neem Oil, T_{12} – Ambient ozone level + foliar spray 3% Panchagavya

Estimation of plant nutrients, chlorophyll contents and biochemical properties

During the study experiment, plant nutrients such as nitrogen was analysed by micro-kjeldahl method, phosphorous by triple acid extraction (HNO_3: H_2SO_4: $HClO_4$ in 9: 2: 1 ratio) method and potassium by flame photometry method (Shang *et al.,* 2018). Biochemical properties such as catalase activity was determined by titration method, peroxidase activity was determined by UV spectrophotometry at 430 nm (Rai *et al.,* 2015). Chlorophyll content such as, Chlorophyll a, Chlorophyll b and Total chlorophyll were analyzed by spectrophotometry method (Tetteh *et al.,* 2015). All analyses were carried out in three replicates. Standard Errors (SE) were calculated for each data series. One-way analysis of variance (ANOVA) was used to quantify the impact of tropospheric ozone and ozone protectants spray on chlorophyll contents and anti-oxidant enzymes of cauliflower crop. The significance of the differences between the means was determined with Duncan's multiple range test with 5% error probability. All the experimental data were analyzed using SPSS version 16.

Results and Discussion

Plant nutrients

The experimental results showed that, the treatment T_{11} (Ambient ozone level + 3% Neem oil) had significantly higher amount of N, P, and K levels compared with all other treatments and lowest N, P, and K level was observed in treatment T_3 (Elevated ozone exposure @ 200 ppb). N, P, and K level showed a significance difference among the all treatments. Compared with elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure significantly decreases the N, P, and K level content in cauliflower plant (Table 1).

Previous studies reported that ozone exposure increased the N, P and K elements in plants (Zhang *et al.*, 2018). In the present study, elevated ozone exposure strongly decrease the N, P, and K, in cauliflower plant. The increasing concentration of phosphorus may be the enhanced amount of plant defense mechanism against ozone stress (Shang et al., 2018). Broberg et al. (2017) also reported that ozone exposure directly affects the plant nitrogen and potassium levels in cereal crops. Nitrogen is an important element in the chlorophyll biosynthesis and chlorophyll production in plants. Kou *et al.* (2017) also reported a strong correlation between the plant nutrients and chlorophyll contents in rice cultivars.

Table 1.Effect of ambient and elevated ozone on plant uptake of nutrients (N, P, K)

Treatment	N (%)	P (%)	K (%)
T_1	9.68 ± 0.387^{b}	2.06 ± 0.416^{cba}	20.91 ± 0.335^{b}
T_2	7.00 ± 0.416^{ed}	0.89 ± 0.057^{fe}	11.29 ± 0.127^{g}
T_3	5.870 ± 0.479^{e}	0.71 ± 0.051^{e}	11.03 ± 0.335^{g}
T_4	9.63 ± 0.549^{b}	1.43 ± 0.219^{fedc}	19.24 ± 0.208^{c}
T_5	9.06 ± 0.567^{cb}	1.93 ± 0.104^{dcba}	20.39 ± 0.237^{b}
T_6	9.00 ± 0.472^{cb}	1.51 ± 0.190^{edcb}	17.06 ± 0.358^{d}
T_7	7.93 ± 0.069^{dc}	1.25 ± 0.214^{fed}	12.58 ± 0.479^{f}
T_8	7.93 ± 0.260^{dc}	1.38 ± 0.069^{fedc}	15.06 ± 0.162^{e}
T_9	7.520 ± 0.283^{d}	1.40 ± 0.144^{fedc}	16.69 ± 0.174^{d}
T_{10}	9.99 ± 0.416^{b}	2.11 ± 0.560^{cba}	22.76 ± 0.191^{a}
T_{11}	11.33 ± 0.566^{a}	2.34 ± 0.132^{a}	23.05 ± 0.439^{a}
T_{12}	9.79 ± 0.433^{b}	2.25 ± 0.121^{ba}	22.72 ± 0.156^{a}
P Value	0.000	0.001	0.000

±: Standard Error, Values followed by same letters with in columns are not significantly different at $P \leq 0.05$

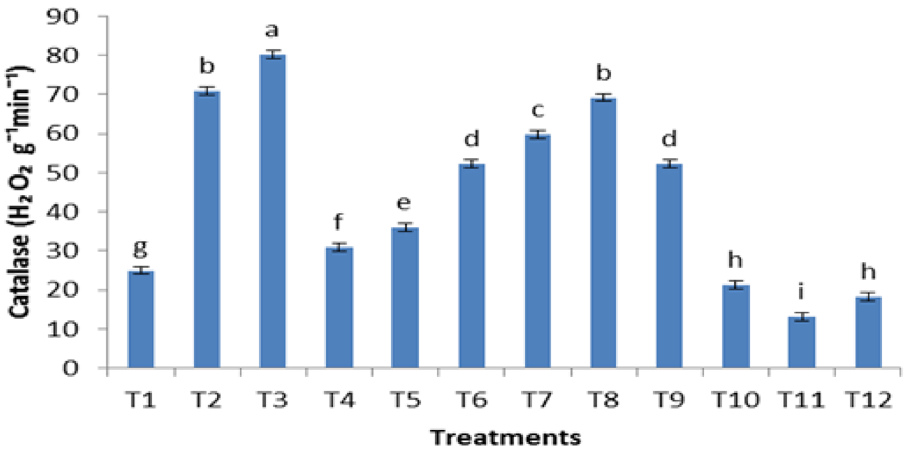
Elevated ozone decreases photosynthesis even at comparatively low concentrations followed by decreasing the nitrogen concentration in the leaves. The present findings also agreed that the elevated ozone directly affect the chlorophyll content, which may lead to decrease the N, P and K uptake in cauliflower.

Table 2. Effect of ambient and elevated ozone on chlorophyll contents

Treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
T_1	0.320 ± 0.006^{b}	0.310 ± 0.006^{d}	0.809 ± 0.017^{cb}
T_2	0.161 ± 0.003^{e}	0.140 ± 0.004^{h}	0.409 ± 0.006^{g}
T_3	0.160 ± 0.004^{e}	0.111 ± 0.012^{i}	0.378 ± 0.006^{g}
T_4	0.301 ± 0.046^{cb}	0.290 ± 0.005^{fed}	0.740 ± 0.006^{ed}
T_5	0.288 ± 0.014^{cb}	0.279 ± 0.005^{fe}	0.681 ± 0.012^{e}
T_6	0.309 ± 0.006^{cb}	0.298 ± 0.002^{ed}	0.780 ± 0.006^{dc}
T_7	0.250 ± 0.005^{dc}	0.270 ± 0.005^{f}	0.681 ± 0.006^{e}
T_8	0.180 ± 0.046^{e}	0.151 ± 0.004^{h}	0.418 ± 0.004^{g}
T_9	0.219 ± 0.005^{ed}	0.182 ± 0.006^{g}	0.509 ± 0.112^{f}
T_{10}	0.331 ± 0.006^{b}	0.358 ± 0.006^{b}	0.850 ± 0.024^{b}
T_{11}	0.329 ± 0.029^{b}	0.330 ± 0.006^{c}	0.848 ± 0.029^{b}
T_{12}	0.410 ± 0.006^{a}	0.541 ± 0.012^{a}	1.160 ± 0.052^{a}
P Value	0.000	0.000	0.000

±: Standard Error, Values followed by same letters with in columns are not significantly different at P ≤ 0.05

Moreover, redox potential is closely related to root exudes and thus, the ozone induced changes in root exudes also affect bioavailability and speciation of nutrients. Besides, elevated ozone inhibit the plant physiology and thus affects nutrient uptake and transport. The partitioning and translocation of carbohydrates between leaves and sink organs (curd) are regularly disturbed. The findings of decreased plant growth correspond with previous studies. The lower ratio of plant growth in response to elevated ozone confirms the inhibition of ozone on carbohydrate translocation to roots in rice cultivars (Wang *et al.*, 2014).



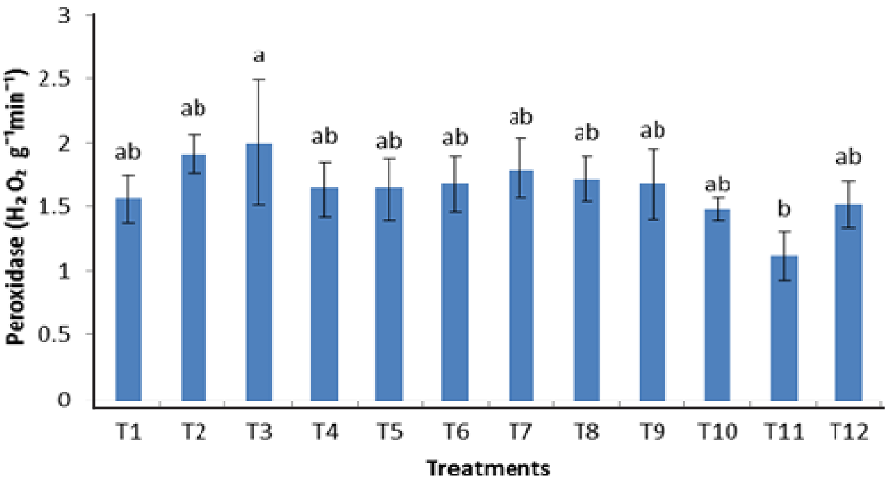
Same letters with in each bars are not significantly different at P ≤ 0.05
Fig. 1. Effect of ozone exposure on catalase in cauliflower

Chlorophyll content

The treatment T_{12} (Ambient ozone level + 3% Panchagavya) had significantly higher amount of Chlorophyll 'a' (0.410 mg g^{-1}) compared with all other treatments and lowest Chlorophyll 'a' (0.160 mg g^{-1}) content was observed in treatment T_3 (Elevated ozone exposure @ 200 ppb). Chlorophyll 'a 'content showed a significance difference among the treatments. Thus, the Chlorophyll 'a' content in cauliflower plant among the treatment was in the order of $T_{12} > T_{10} > T_{11} > T_1 > T_6 > T_4 > T_5 > T_7 > T_9 > T_8 > T_2 > T_3$. Compared with elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure significantly reduces the Chlorophyll 'a 'content in cauliflower plant (Table 2).

Chlorophyll 'b' content of the cauliflower plant also showed similar trend, treatment T_{12} (Ambient ozone level + 3% Panchagavya) had significantly higher amount (0.541 mg g^{-1}) compared with all other treatments and lower content (0.111 mg g^{-1}) was observed in treatment T_3 (Elevated ozone exposure @ 200 ppb). Chlorophyll 'b 'content showed significant difference among the treatments. Thus, the Chlorophyll 'b 'content in cauliflower plant among the treatment was in the order of T_{12}> T_{10}> T_{11}> T_1> T_6> T_4> T_5> T_7 > T_9> T_8> T_2> T_3. Compared with elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure showed effects on the Chlorophyll 'b' content. Among the elevated ozone exposures with protectant spray, the treatment T_6 (Elevated ozone exposure @ 150 ppb + 3% Panchagavya) had higher amount (0.298 mg g^{-1}) of Chlorophyll 'b (Table 2).

Total chlorophyll content of cauliflower plant also showed similar trend, treatment T_{12} (Ambient ozone level + 3% Panchagavya) had significantly higher amount of Total chlorophyll (1.160 mg g^{-1}) compared with treatments and lower amount of Total chlorophyll (0.378 mg g^{-1}) was observed in treatment T_3 (Elevated ozone exposure @200 ppb). Total chlorophyll content showed a significant difference among the treatments. Thus, the total chlorophyll content in cauliflower plant among the treatment was in the order of T_{12}> T_{10}> T_{11}> T_1> T_6> T_4> T_5, T_7> T_9> T_8> T_2> T_3. Compared with elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure significantly reduced the total chlorophyll content in cauliflower plant (Table 2).



Same letters with in each bars are not significantly different at P ≤ 0.05

Fig. 2. Effect of elevated ozone on peroxidase in cauliflower

The experimental results showed that chlorophyll a, b and total chlorophyll contents were decreased in cauliflower plant under elevated ozone level. Ozone induced decrease in photosynthetic potential after vegetative phase, during tuber initiation was a key factor in determining final tuber yield (Oksanen *et al.*, 2013). The decreased chlorophyll contents in cauliflower plant under elevated ozone treatments directly inhibit the plant growth and yield parameters. The research study by Caregnato *et al.,* 2013 also revealed that decreased chlorophyll contents under ozone exposure, may lead to less protein content in tuber crops.

Anti-oxidant enzymes

The present study showed that treatment T_3 (Elevated ozone exposure @ 200 ppb) had significantly higher amount of catalase activity (80.24 H_2O_2 g^{-1} min^{-1}) compared with treatments and lower catalase activity (13.09 H_2O_2 g^{-1} min^{-1}) was found in treatment T_{11} (Ambient ozone level + 3% Neem Oil). Catalase activity showed a significance difference among all the treatments. Thus, the catalase activity in cauliflower plant among the treatment was in the order of T_3> T_2> T_8> T_7> T_9> T_6> T_5> T_4> T_1> T_{10}> T_{12} > T_{11}. Compared with elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure significantly increased the catalase activity in cauliflower plant (Fig. 1)

When comparing all the treatments of the experiment, the treatment T_3 (Elevated ozone exposure @ 200 ppb) had significantly higher amount of peroxidase activity (2.0 H_2O_2 g^{-1} min^{-1}) and lower peroxidase activity (1.12 H_2O_2 g^{-1} min^{-1}) was found in treatment T_{11} (Ambient ozone level + 3% Neem Oil). Peroxidase activity showed a non-significance difference among the treatments. Thus, the Peroxidase activity in cauliflower plant among the treatment was in the order of T_3> T_2> T_7> T_8> T_9, T_6> T_5, T_4> T_1> T_{12}> T_{10}> T_{11} (Fig. 2)

The present study demonstrated that elevated ozone significantly increased the catalase and peroxidase activities in cauliflower plant when compared with ambient ozone level. In the sensitive varieties, both H_2O_2 and catalase accumulate in leaf margins following ozone exposure and the peroxidase has been correlated with the appearance of leaf necrotic lesions. During the study period, higher number of necrotic lesions in leaves was observed in elevated ozone than ambient ozone level. These higher catalase and peroxidase activities in cauliflower plant might be due to stress created in plant by elevated ozone level.

The experimental results revealed that the foliar spray of 0.1% Ascorbic Acid, 3% Neem Oil and 3% Panchagavya within elevated ozone exposure level (150 ppb & 200 ppb) significantly decreased the ozone sensitivity of cauliflower leaves by acting as good physical barriers. The physical barrier which was created and acted against the free radial system of the plant reactive oxygen species(ROS) namely superoxide, hydrogen peroxide and hydroxyl radical are thought to be associated with the initial breakdown of O_3 in the leaf apoplast and these free radicals are involved in the early stages of O_3 response (Fathi *et al.,* 2017). The variability in their efficiency of response was due to the localized ROS formation, either hydrogen peroxide or superoxide depending on the sensitivity nature of cauliflower crop to elevated O_3 level.

In panchagavya, effective microorganisms such as *Lacto bacillus, Sacchoromyces*, *Streptomyces* and photosynthetic bacteria (*Rhodoseudomonas*) acted as physical protectant or barrier on the leaf surface by acting as scavenging agent to the elevated O_3 than control. Moreover, the presence of macro and micronutrients in panchagavya improved leaf chlorophyll contents of cauliflower plant which withstood higher ozone exposure. The organic ozone protectants namely the liquid organic sprays (3% Neem oil, 3% panchagavya) and 0.1% ascorbic acid, which were selected based on the local availability, cost effective nature and acted as better protectant against tropospheric ozone in cauliflower crop.

Conclusion

From the present study, it is conclude that tropospheric ozone has detrimental effect on N, P, K, chlorophyll contents, catalase and peroxidase levels of cauliflower plant. Further the ozone protectants played a major role to nullify the tropospheric ozone effect on N, P, K, chlorophyll contents, catalase and peroxidase of cauliflower plant. Among the treatments, panchagavya and neem oil performed well followed by ascorbic acid. Since, the tropospheric ozone has been identified as potential air pollutant by IPCC, the future research may be focused to study the impact of tropospheric ozone on different crops and identify the best ozone protectants to nullify the impact of tropospheric ozone for sustainable agriculture and ensure food security for growing population with shrinking land and water resources under changing climate.

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