Impacts of Elevated Ozone and Ozone protectanton Plant Growth, Nutrients, Biochemical and Yield properties of Turnip (Brassica rapa)

Boomiraj Kovilpillai
Tamil Nadu Agricultural University
Sethupathi Nedumaran
Indian Agricultural Research Institute
Sudhakaran Mani (✉ saintsudha@gmail.com)
Tamil Nadu Agricultural University
Jayabalakrishnan Raja Mani
Tamil Nadu Agricultural University
Sritharan Natarajan
Tamil Nadu Agricultural University
Jagadeeswaran Ramasamy
Tamil Nadu Agricultural University

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Abstract

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 elevated ozone and ozone protectants spray on plant growth, nutrients, biochemical and yield properties of turnip crop in a factorial completely randomized block design replicated thrice. The elevated ozone exposure significantly reduces the plant height, tuber size, tuber weight, Chlorophyll ‘a’, Chlorophyll ‘b’, Total chlorophyll, total nitrogen, total potassium, total Manganese, Iron, Zinc, Copper inturnip. Meanwhile, the elevated ozone exposure significantly increased the total phosphorous, catalase and peroxide activity inturnip. However, ozone protectants played a major role to nullify the tropospheric ozoneeffect on growth, physiology, development and yield of turnip and among them panchagavya performed well followed by neem oil and ascorbicacid.

Introduction:

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). Tropospheric Ozone (O_3) is one of the prominent secondary air pollutantsin the world.Currently, ground level ozone has been considered as mostdamaging to vegetation and agricultural crops (Rathore and Chaudhary, 2019). Surface ozone is not only a greenhouse gas, which is next to CO_2 and Methane but 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). Ozone concentrations arepredicted to continue to increase in developing regions in thefuture unless precursor emissions are further controlled (Turnock et al., 2018).The anthropogenic emissions of O_3precursors such as nitrogen oxides and volatile organic compounds (VOC) across Asia also increased, in particular over the Indian and Chinese region (Ohara *et al.*,2007).

In India, urban and rural agriculture is mainly affected by the ozone pollution (Bell et al., 2011). In our atmosphere, the tropical regions are mostly referred as active photochemical region for production of ozone (Kunhikrishnan et al., 2004). The highest concentration of ozone generally measured during March to June, whereas the lowest values were observed during June to September (Ali et al., 2012; Ghude et al., 2008; Jain *et al.*,2005).

Higher level of ozone affects plants in different mechanism such as premature leaf fall, reduction in photosynthetic activity and biomass loss (Tetteh et al., 2015) and also physiological and growth changes occur in the absence of foliar injury (Rai 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 (Fiscus et al., 2005; Morganet al., 2006, Li et al., 2017).

Ozone can reduce agricultural yield by a variety of mechanisms. The first of those mechanism is acute visible injury in many horticultural crops, can cause an obvious and immediate loss of economic value (Ashmore, 2005). Ozone injury is also reported in various crops *viz.*, radish (*Raphanus sativus*) and turnip (*Brassica rapa*) in Egypt (Hassan et al., 1995), Potato (*Solanum tuberosum)* in India (Bambawale 1986; Suganthy2014) it is affirmed that the ozone concentration is increasing in the atmosphere and it has potential threat to agricultural production and sustainability.Understanding the mechanism of the effect of ozone on agricultural crop could control the global yield losses by the year 2030 (Chaudhary and Rathore, 2020). There are no sufficient information and studies on quantifying the effect of ozone on growth and yield of turnip. Hence, the purpose of the study is to identify the critical issues related to the effects of ozone exposure on turnip crop and to identify antiozonant to prevent the ozone induced losses.In view of the above, presented study was conducted to assess their impacts of elevated ozone level on plant growth, nutrients, biochemical and yield propertiesof turnip ii) impacts of ozone protectants on chlorophyll contents and anti-oxidant enzymes of turnip.

Materials And Methods:

Study area:

The Indian Space Research Organization (ISRO) Climate Change Observatory is located at Woodhouse house farm, Horticultural Research 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 approximately5 -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.

Experimental Design:

A pot culture experiment was conducted to study the effect of elevated levels of ozone and ambient ozone level on turnip under the open-top chamber.The polycarbonate glass covered with steel skeleton structured and fabricated as open-top chamber of size 3m X 3m X 3m area was constructed. The open-top chamber was provided with ground cable electrical supply to connect with corona discharge type ozone generator to conduct experiment with elevated levels of ozone fumigation. The Ozone Generator Model A-series No: A4G with inbuilt oil-free air compressor and corona discharge type was installed nearby open-top chamber. The ozone generator produce maximum of 4g hr-1ozone with flow meter 0.5-13L per minute to generate different levels of ozone.The ozone generator was an ISO-9001 certified (CE) model with dimensions of (L x W x H) 410x280x470mm weighing about 12 kg. For the purpose of experiment, the elevated levels of O_3on turnip *viz.*, Ambient, 150 and 200ppb were selected.AOT40 is calculated as the sum of differences between the hourly mean concentrations (O_3) and 40 ppb for hours when $O_3 > 40$ ppb, for each daylight hour with global radiation ≥ 50 Wm-2 over a 3 months period.

For this experiment, mud pots with 10 liter volume, 25cm surface diameter and 45cm height were filled with soil, sandand compost mixture (1:1:1 ratio). Turnip crop was maintained uniformly up to the most critical stage *viz.*, the tuber initiation and curd initiation stage respectively outside the chamber. Only during this critical stage *viz.*, tuber initiation stage, curd initiation three different levels of ozone Ambient (March–76ppbvandapril 81ppbv), 150 and 200 ppb were fumigated @4 hours/day for 7 days inside the chamber separately. Aset of untreated plants were also maintained as control for the purpose of comparing with treated plants. Crop protection and management practices were carried out as per the TNAU crop production techniques for horticultural crops.

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.

Measurement of growth and yield parameters:

Data on plant height, no of leaves/ plant were recorded during the crop stand for turnip. At the time of harvesting the yield parameters tuber and curd size, weight were recorded for replicated plants and also for the treated and untreated turnip expressed in SI units (Sadia et al., 2013).

Measurement of Plant nutrients and Biochemical parameters:

During the experiment, plant nutrients such as total nitrogen (TN)was analyzed by micro-kjeldahl method (Shang *et al.*,2017). Total phosphorous (TP)was estimated by triple acid extraction (HNO_3: H_2SO_4: $HClO_4$in 9: 2: 1 ratio) method (Fangmeier et al., 2002). Total potassium (TK) was analyzed by flame photometry methodJackson (1973). Micronutrients such as manganese (Mn), Iron (Fe), zinc (Zn) and copper (Cu) were analyzed by atomic absorption spectrophotometer(Yabo et al., 2017).

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).

Data analysis

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 plant nutrients and biochemical properties of turnip 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:

Growth and yield parameters:

Our experimental result showed that the treatment T_{12} (Ambient ozone level + 3% Panchagavya) had significantly higher value of plant height (50.00 cm) compared with all other treatments and lowest plant height (37.33 cm) was observed in treatment T_3 (Elevated ozone exposure @ 200 ppb) (Table 1).

Table 1
Effect of ambient and elevated ozone on tuber size and tuber weight in turnip

Treatments	**Plant Height (cm)**	**No. of Leaves / Plant**	**Tuber Size (cm)**	**Tuber Weight (gm)**
T_1	48.33 ± 1.67ba	10.67 ± 1.20a	18.50 ± 1.89ba	196.35 ± 0.39c
T_2	38.33 ± 3.84d	8.33 ± 0.67ba	11.40 ± 0.31c	60.60 ± 0.63j
T_3	37.33 ± 2.96d	7.00 ± 0.58b	5.83 ± 1.20d	27.50 ± 2.34b
T_4	41.00 ± 3.51dcb	9.00 ± 0.33ba	12.33 ± 1.20c	121.40 ± 0.31f
T_5	42.00 ± 2.08dcb	9.00 ± 1.53ba	18.00 ± 0.58ba	134.53 ± 0.55e
T_6	42.33 ± 0.33dcba	9.33 ± 0.33ba	12.80 ± 1.30c	93.37 ± 0.38g
T_7	39.33 ± 3.18d	8.67 ± 0.88ba	12.00 ± 2.08c	92.18 ± 0.33g
T_8	38.33 ± 3.84d	8.33 ± 0.67ba	12.00 ± 1.16c	80.50 ± 0.61h
T_9	40.33 ± 2.96dc	9.00 ± 1.53ba	12.00 ± 0.52c	70.60 ± 0.30i
T_{10}	47.33 ± 1.20cba	10.33 ± 0.33ba	15.27 ± 1.16cb	139.60 ± 0.33d
T_{11}	47.33 ± 1.67cba	9.67 ± 0.67ba	21.35 ± 2.19a	285.00 ± 0.92a
T_{12}	50.00 ± 1.16a	11.00 ± 1.53a	14.67 ± 3.18cb	137.93 ± 1.17d
P value	**0.006**	**0.390**	**0.000**	**0.000**

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

The protectants spray against the ozone exposure had significant effect on plant height. Treatment T12 (Ambient ozone level + 3% Panchagavya) had higher (50.00 cm) plant height followed by T_{10}, $T_{11} > T_6 > T_5 > T_4 > T_9 > T_7$and the lowest height (38.33 cm) was observed in T_8(Elevated ozone exposure @ 200 ppb + 3% Neem Oil) among the ozoneprotectants (Table 1).

Plant height was showed a significance difference among the all treatments.T_{12}(Ambient ozone level + 3% Panchagavya) had significantly higher number of leaves (11.0) per plant and a smaller number of leaves (7.0) was observed in treatment T_3(Elevated ozone exposure @ 200 ppb). Number of leaves per plant was not showed any significant difference among the all treatments. Among the elevated ozone exposures with protectant spray, the treatment T_6(Elevated ozone exposure @ 150 ppb + 3% Panchagavya) had higher (9.33) number of leaves(Table 1).

The present study showed that treatment T11 (Ambient ozone level + 3% Neem Oil) had significantly higher tuber size (21.35 cm) compared with all other treatments and lower tuber size (5.33 cm) was observed in treatment T3 (Elevated ozone exposure @ 200 ppb) (Table 1). Tuber size was showed a significance difference among the all treatments. Thus, the tuber size in turnip among the treatment was in the order of T_{11} > T_1 > T_{10}> T_{12}> T_5> T_6> T_4> T_7, T_8 & T_9 > T_2 > T_3. Tuber weight was significantly higher(285 gm) in T_{11}(Ambient ozone level + 3% Neem Oil) when compared with all other treatments and lower tuber weight (27.50 gm) was observed in treatment T_3 (Elevated ozone exposure @ 200 ppb).Among the elevated ozone level @ 200 ppb treatments (T_7, T_8, T_9), T_7(Elevated ozone @ 200 ppb + 0.1% Ascorbic Acid) had higher (92.18gm) tuber weight (Table 1).

Plant nutrients and micronutrients:

Plant nutrients such as total nitrogen, phosphorus and potassium were showed a significant difference among the treatments. Total nitrogen (27.25 %) content was significantly higher in T_{12} (Ambient ozone level + 3% Panchagavya) and lower (15.34 %) in treatment T_3 (Elevated ozone exposure @ 200 ppb).The elevated zone exposure compared with ambient ozone treatments, the elevated ozone exposure was significantly reduces the total nitrogen content in turnip(Table 2). Treatment T_2(Elevated ozone exposure @ 150 ppb) had significantly higher amount of total phosphorous (3.971 %) compared with all treatments and lowest value of (1.851 %) in T_1(Ambient level ozone).Compared with elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure was significantly increasing the total phosphorous in turnip(Table 2). When compared the all treatments, T_{12}(Ambient ozone level + 3% Panchagavya) had significantly higher amount of total potassium (26.060 %) and lower (12.330 %) in treatment T_3(Elevated ozone exposure @ 200 ppb). Compared with elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure was reduced the totalPotassium in turnip(Table 2).

Table 2
Effect of ambient and elevated ozone on major nutrients and micronutrients in turnip

Treatments	Total N (%)	Total P (%)	Total K (%)	Mn (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)
T_1	23.14 ± 0.15d	1.85 ± 0.08^{d}	22.88 ± 0.38dcb	1.41 ± 0.22b	2.39 ± 0.19a	0.308 ± 0.032dc	0.111 ± 0.007d
T_2	18.72 ± 0.38i	3.97 ± 0.19^{a}	18.40 ± 0.16f	0.73 ± 0.01e	0.12 ± 0.01e	0.026 ± 0.002fg	0.013 ± 0.001i
T_3	15.34 ± 0.08j	2.55 ± 0.14^{c}	12.33 ± 0.52g	0.22 ± 0.01f	0.08 ± 0.01e	0.011 ± 0.005ed	0.011 ± 0.003i
T_4	21.06 ± 0.24f	3.63 ± 0.17^{ba}	22.51 ± 0.31dcb	0.98 ± 0.01dc	0.91 ± 0.05c	0.260 ± 0.040ed	0.089 ± 0.004e
T_5	22.10 ± 0.32e	3.28 ± 0.13^{b}	22.42 ± 0.31dc	0.96 ± 0.02edc	1.51 ± 0.13b	0.281 ± 0.110edc	0.085 ± 0.002e
T_6	20.61 ± 0.10f	2.28 ± 0.75^{dc}	22.60 ± 0.48dcb	0.91 ± 0.01edc	0.45 ± 0.06d	0.302 ± 0.012dc	0.053 ± 0.002f
T_7	19.24 ± 0.20ih	3.47 ± 0.42^{ba}	21.86 ± 0.45d	0.76 ± 0.01ed	0.25 ± 0.05ed	0.036 ± 0.002fg	0.028 ± 0.003h
T_8	20.48 ± 0.10f	3.74 ± 0.14^{ba}	19.52 ± 0.31e	0.84 ± 0.02edc	0.44 ± 0.06d	0.153 ± 0.026fe	0.036 ± 0.002hg
T_9	19.83 ± 0.12hg	3.43 ± 0.24^{ba}	19.61 ± 0.21e	0.77 ± 0.02ed	0.13 ± 0.01e	0.121 ± 0.002fg	0.041 ± 0.001g
T_{10}	24.95 ± 0.22c	2.35 ± 0.13^{dc}	23.54 ± 0.21b	1.83 ± 0.02a	1.74 ± 0.14b	0.450 ± 0.052cb	0.256 ± 0.005a
T_{11}	25.68 ± 0.36b	2.39 ± 0.10^{dc}	23.16 ± 0.28cb	1.26 ± 0.02b	2.57 ± 0.06a	0.508 ± 0.023b	0.144 ± 0.002b
T_{12}	27.25 ± 0.16a	2.58 ± 0.21^{c}	26.06 ± 0.32a	1.02 ± 0.01c	1.53 ± 0.02b	0.767 ± 0.052a	0.132 ± 0.005c
P value	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

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

Our result showed that, micronutrients such as manganese (Mn), Iron (Fe), zinc (Zn) and copper (Cu) weresignificant differences among the treatments(Table 2).Treatment T_{10} (Ambient ozone level + 0.1% Ascorbic Acid) was had significantly higher amount of total manganese (Mn) (1.83 ppm) compared with all other treatments and lowest amount of Mn (0.238 ppm) was observed in treatment T_3(Elevated ozone exposure @ 200 ppb). When compared the all treatments, T_{11}(Ambient ozone level + 3% Neem Oil) had significantly higher amount of total Fe (2.570 ppm) and lowest amount of total Fe (0.079 ppm) was observed in treatment T_3(Elevated ozone exposure @ 200 ppb). T_{12}(Ambient ozone level + 3% Panchagavya) had significantly higher amount of total Zn (0.767 ppm) compared with all other treatments in the experiments and lower amount of Total Zn (0.767 ppm) was observed in treatment T_3 (Elevated ozone exposure @ 200 ppb). Compared with elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure was significantly reduced the total Zn content in turnip(Table 2).$T1_0$(Ambient ozone level + 0.1% Ascorbic Acid) had significantly higher amount of total copper (Cu) (0. 256 ppm) and lowest value of total Cu (0.011 ppm) was observed in treatment T_3 (Elevated ozone exposure @ 200 ppb). Compared with ozone exposure and ambient treatments, ozone exposure was significantly decreases the total Cu content in turnip(Table 2).

Plant chlorophyll content and Biochemical properties:

Our result showed that, treatment T_{12}(Ambient ozone level + 3% Panchagavya) had significantly higher amount of chlorophyll ‘a’ (0.260 mg/g), chlorophyll ‘b’ (0.260 mg/g) and total chlorophyll (0.412 mg/g)compared with all treatments and lowest chlorophyll ‘a’ (0.040 mg/g), chlorophyll ‘b’ (0.040 mg/g) and total chlorophyll (0.071 mg/g) were observed in treatment T_3 (Elevated ozone exposure @ 200 ppb).Thus the Chlorophyll ‘a ’, chlorophyll ‘b’ and total chlorophyll content in turnip among the treatment was in the order ofT12 > T1 > T10 > T11, T6 > T5 > T4 > T8, T9 > T7 > T2 > T3 (Table 3).

Table 3

Effect of ambient and elevated ozone on Plant chlorophyll content and Biochemical properties

Treatments	**Chlorophyll a (mg/g)**	**Chlorophyll b (mg/g)**	**Total chlorophyll (mg/g)**	**Catalase ($H_2 O_2$ / g / min)**	**Peroxidase ($H_2 O_2$ / g / min)**
T_1	0.209 ± 0.046a	0.210 ± 0.006b	0.349 ± 0.006b	10.20 ± 0.45d	1.84 ± 0.19ed
T_2	0.049 ± 0.002b	0.060 ± 0.005f	0.119 ± 0.004e	35.70 ± 1.27ba	6.52 ± 0.57b
T_3	0.040 ± 0.001b	0.040 ± 0.003g	0.071 ± 0.001f	41.50 ± 1.75a	20.12 ± 0.87a
T_4	0.090 ± 0.002b	0.091 ± 0.005e	0.179 ± 0.005d	15.30 ± 0.57dc	3.08 ± 0.537dc
T_5	0.098 ± 0.005b	0.120 ± 0.006c	0.189 ± 0.005dc	15.30 ± 0.47dc	2.64 ± 0.22edc
T_6	0.099 ± 0.002b	0.160 ± 0.004d	0.200 ± 0.012c	13.60 ± 0.54dc	3.56 ± 0.39c
T_7	0.079 ± 0.005b	0.079 ± 0.003e	0.168 ± 0.004d	24.65 ± 0.57dcb	3.80 ± 0.50c
T_8	0.088 ± 0.002b	0.089 ± 0.005e	0.179 ± 0.004d	27.20 ± 0.19cba	3.96 ± 0.43c
T_9	0.088 ± 0.002b	0.089 ± 0.001e	0.171 ± 0.004d	24.65 ± 0.61dcb	3.80 ± 0.50c
T_{10}	0.209 ± 0.052a	0.171 ± 0.006d	0.341 ± 0.006b	11.90 ± 0.46dc	1.430 ± 0.14d
T_{11}	0.201 ± 0.052a	0.160 ± 0.006d	0.340 ± 0.006b	7.65 ± 0.36d	1.76 ± 0.25ed
T_{12}	0.260 ± 0.005a	0.260 ± 0.011a	0.412 ± 0.012a	8.50 ± 0.42d	1.400 ± 0.07d
P value	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

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

The present study showed that treatment T_3(Elevated ozone exposure @ 200 ppb) had significantly higher amount of catalase activity (41.500 $H_2 O_2$/ g / min) compared with all treatments and lower catalase activity (7.650 $H_2 O_2$/ g / min) was found in T_{11} (Ambient ozone level + 3% Neem Oil). Catalase activity was showed a significance difference among the all treatments. Thus, the catalase activity in turnip among the treatment was in the order of$T_3 > T_2 > T_8 > T_9$, $T_7 > T_4$, $T_5 > T_6 > T_{10} > T_1 > T_{12} >$

T_{11}.Compared with elevated ozone exposure and ambient ozone treatments, the elevated ozone exposure was significantly increased the catalase activity in turnip(Table 3).

When compared all the twelve treatments, T_3 (Elevated ozone exposure @ 200 ppb) had significantly higher amount of peroxidase activity (20.12 H_2 O_2/ g / min) and lowest activity (1.400 H_2 O_2/ g / min) was observed in treatment T_{12}(Ambient ozone level + 3% Panchagavya). Peroxidase activity (Table 3) was showed a significance difference among the all treatments. Thus, the Peroxidase activity in turnip among the treatment was in the order of $T_3 > T_2 > T_8 > T_7$, $T_9 > T_6 > T_4 > T_5 > T_1 > T_{11} > T_{10} > T_{12}$. Compared withelevated ozone exposure and ambient ozone treatments, the elevated ozone exposure was significantly reduced the peroxidase activity in turnip(Table 3).

Discussion

Effects of ozone exposure on plant growth parameters and chlorophyll content in turnip (Brassica rapa L.)

Our experimental study revealed that plant height, tuber height, tuber weight, chlorophyll ‘a’, ‘b’& total chlorophyll contents were significantly reduced under elevated ozone exposure condition in turnip (*Brassica rapa* L.) (Tables 1 & 3). However, number of leaves per plant was not affected by ozone exposure in turnip. The same results were obtained by (Pandey et al., 2018) in wheat cultivars.The no change in number of leaves in clones of cotton wood when, they were exposed under elevated ozone level, was due to the enhanced amount of plant defense mechanism against ozone stress (Shang et al., 2018), this might be the reason for not changing leaf number in turnip also in our crop. Yendrek et al. (2013) also reported that production of number leaves with smaller leaf area in polluted environment. (Black et al., 2000) and (Singh et al., 2012) were revealed that plants growth, chlorophyll content and yield parameters were reduced under ozone exposure. In our study showed that turnip was more sensitive to elevated ozone pollution.

Earlier studies also suggested that sensitivity to ozone pollution can be associated with various biochemical and physiological changes in field crops (Broberg et al., 2017). Our study also indicated that turnip plant was highly sensitive to ozone pollution. Wittig et al. (2009), Hoshika et al. (2013) and Fares et al. (2013) studies also reported that ozone exposure affects important metabolic processes leading to the reduction in carbon assimilation, plant growth, leaf area, transpiration and dry matter production in field crops. Present study showed that, still some supplementary plant physiological traits such as chlorophyll content and plant height may also play significant role in determining elevated ozone sensitivity in plants, which is in line with (Hoshika *et al.*,2013).

Yamoriet al. (2011) reported that Chlorophyll contents were highly correlated with plant growth parameters. In our study chlorophyll (a, b & total) and plant growth parameters were reduced in turnip. The decreased in the chlorophyll contents in turnip 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, which may leads to less protein content in tuber crops.

During the study period higher number of necrotic lesions leaves was observed in turnip under 150 & 200 ppb elevated ozone level. It is indicated that turnip was more sensitive to ozone pollution. The visible injury observed on the upper side of leaves of the turnip due to elevated level of ozone exposure. The visible injury of leavesas tiny yellow, black or purple-red spots might be due to the collapse of the palisade cells resulted in the formation of lesions on the upper surfaces between leaf veins and become chlorotic followed by progressive necrosis and in the most severecases, defoliation and accelerated death of the plant and these visible injuries primarily to short term exposure to elevated ozone, which was observed by (Sharps *et al.*, 2021). In addition, ozone pollution induced damage may facilitate entry by foliar pathogen, thereby, reduced yield as a result of secondary infections (Oksanen et al., 2013). Moreover, it was observed that the tuber crops showed the most severe visible necrotic injury symptoms near the bottom and middle leaves of the canopy than tip leaves (Broberg et al., 2017). The same results was observed in sugarcane, due to acute exposure to high ozone levels, there was a correlation between visible injury and reductions in growth and physiological effects of ozone exposure include reduced photosynthesis, increased turnover antioxidant systems damage to physiological process, lowered carbon transport to roots (Moura *et al.*,2018).

In tuber crop like turnip, visible leaf damage also contributed for reduced yield by reducing the amount of leaf area available for carbon fixation for further biomass growth and tuber maturation. Loss of photosynthetic capacity is an early effect of ozone exposure, which is due to accelerated senescence with down regulation of photosynthetic genes. Inhibition of CO_2assimilation can also be resulted from direct or indirect inhibition of stomatal opening (Burke et al., 2001). Reductions in carbon storage are leads to reduction of whole plant biomass, inducing yield reduction in crops by reducing the availability of leaf area to fix and provide carbon for reproductive parts, which is stemming reduced photosynthetic efficiencies and or stomatal conductance. Ozone induced reductions in yield of potato tubers is the direct consequence of reductions and allocation source in to the reproductive structures (Caregnato et al., 2013).

Our experimental results showed that chlorophyll a, b and total chlorophyll contents were reduced in turnip under elevated ozone level (Table 3). Ozone induced reductions in photosynthetic potential occurred mainly after vegetative phase, during tuber initiation was a key factor in determining final tuber yield (Oksanen et al., 2013). Moreover, exposure to elevated ozone may alter biomass partitioning between the above and below ground components by reducing assimilate production within the leaves. Reductions in source strength of soluble strength availability of soluble sugars for export, as reported for cereals (Giacomo *et al.*,2010).

Effects of ozone exposure on plant nutrients in turnip (Brassica rapa L.)

Previous studies were reported that ozone exposure increasing the P, Zn, Mn and Fe elements in plants. (Cao et al., 2016; Wang et al., 2014; Zhang et al., 2018). In our study elevated ozone exposure was strongly reduced the N, K, Cu, Zn, Mn and Fe nutrient levels in turnip plants (Table 2). In contrast phosphorus content of turnip was not affected by elevated ozone exposure. The increasingconcentration 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 was 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 (Tanaka and Tanaka, 2007). Kou et al. (2017) also reported a strong correlation between the plant nutrients and chlorophyll contents in rice cultivars.

Elevated ozone decrease photosynthesis even at comparatively low concentrations followed by decreasing the nitrogen concentration in the leaves. Our findings also agreedthat the elevated ozone directly affect the chlorophyll content, which may lead to reduce the N and K in turnip. Since photosynthetic substrates are essential for iron reduction by roots, it is predictable that lower carbohydrate in roots may result in lower Fe, Zn and Cu uptake in plants. Similar results were observed in this study, which showed lower Cu, Zn, Mn and Fe nutrient levels in turnip.

Moreover, redox potential is closely related to root exudes and thus the ozone induced changes in root exudes also affect bioavailability and speciation of micronutrients. Thus, ozone stress is attributed to changes of the bioavailability of micronutrients in turnip (Karan et al., 2014). Besides, elevated ozone inhibitsthe plant physiology and thus affects in nutrient uptake and transport. The partitioning and translocation of carbohydrates between leaves and sink organs (tuber) are regularly disturbed. Our findings of reduced 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). Further studies should be carried out to identify the plant chlorophyll contents associated with nutrient transport (Feng *et al.*,2016).

Effects of ozone exposure on peroxidase and catalase in turnip (Brassica rapa**L.**)

The present study demonstrated that elevated ozone was significantly increased the catalase and peroxidase activities in turnip compared with ambient ozone level (Table 3). Scebbaet al. (2006) revealed from their research that distinct patterns of enzyme activities in differentially in ozone sensitive and tolerant plants as shown for clover and tobacco. Pasqualini et al., (2007) experimental research revealed thatthe mature leaves suffer more severely comparatively than younger leaves under elevated ozone exposure condition. 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 (Di Baccio et al., 2008). During the study period higher number of necrotic lesions leaves was observed in elevated ozone compared than ambient ozone level. These higher catalase and per oxidase activities in turnip might be due to stress created in plant by elevated ozonelevel.

The mechanism of formation of reactive oxygen species under elevated O3 exposure are, when ozone that has passed leaf internal air spaces dissolves in aqueous layer produces hydroxyl(OH), peroxyl (OH2) and superoxide (O2) radicals (Torres et al., 2007). This oxidative process also produces H2O2 and aldehydes in a humid environment. The internal air spaces within the leaf are a potential site for O3 reactions and formations of toxic compound within the cellular spaces (Vandenabeele et al., 2003). The plasma membranes are protected by enzymes such as catalase and peroxidase. For H2O2 detoxification, Catalase and peroxidase are the important enzymes in plants under ozone stress. Plants contain various types of H2O2-degrading enzymes;however, catalase and peroxidase activities are essential for maintaining the redox balance to escape further oxidative stress (Sharma et al., 2012). This might be the reason for enhanced peroxidase and catalase enzyme activities of our experimental crops such as turnip under elevated ozone level.

Performance of various ozone protectants under elevated ozone on plant growth parameters of turnip (Brassica rapa**L.**)

Our 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) were significantly reduces the ozone sensitivity of turnip leaves and they acted as good physical barriers. The physical barrier which was created and acted against the free radial system of the plant such 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_3response (Fathiet 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 potato genotypes to elevated O_3level (Sudhakar *et al.*,2008).

The simplest explanation for the observed improved yield and reduced effect of elevated ozone exposure level 150 ppb & 200 ppb in turnip was due to the fact that 0.1% Ascorbic Acid, 3% Neem Oil and 3% Panchagavya protects turnip plants against O_3damage. In addition to the well-known its chemical characteristics able to show protective effects on photo inhibition seem to be associated to its singlet oxygen quenching capacity. Singlet oxygen (Reactive oxygen species) formed due to O_3stress is considered an intermediatory in photo inhibition (Vander Heyden *et al.*, 2001). The result in the present study demonstrates that foliar application of 0.1% Ascorbic Acid, 3% Neem Oil and 3% Panchagavya to and after the tuber initiation stage of turnip improved the antioxidant enzymes activity which significantly increased the tuber yield in turnip.

The foliar application of organic liquid mixtures viz., 3% panchagavya and 1 % Neem oil were showed efficiency in showing significant improvement in plant growth parameters, increased photosynthetic rate,, enzyme (catalase and peroxidase) activity, average tuber fresh weight and tuber size than ozone exposure 150 & 200 ppb in turnip. Meanwhile foliar application of 0.1% Ascorbic Acid showed significant improvement in micro nutrients in turnip under elevated ozone with foliar spray treatments. The sensitivity to O3 stress seems to be associated with an increased ROS production (superoxide radical and

H_2O_2) which creates a pro-oxidative environment inside the cells. Apparently, enzymatic activities (Catalase and Peroxidase) are unable to maintain the intracellular redox homeostasis, which leads to oxidative damages to structural molecules such as the membrane lipids (Caregnato *et al.*,2013).

The protective nature of both the organic liquid sprays was due to their bio-stimulant nature to different environment conditions which was in agreement with the studies conducted by (Pandey et al., 2015) in rice cultivars. The presence of vitamin A, vitamin B, calcium, fat and glycocides in panchgavya which protects wounds from biotic or biotic stress and improved the biological efficiency of potato genotypes. The effective microorganism present in the 3% panchagavya solution synthesize phytoharmones, auxins and growth regulators, which were significantly increase the green leaf area and photosynthetic rate and stomatal response (Gunasekar *et al.*,2018).

In panchagavya, effective microorganisms such as *Lacto bacillus*, *Sacchoromyces, Streptomyces* and photosynthetic bacteria (*Rhodoseudomonas*) acted as physical protectant / barrier on the leaf surface acted as scavenging agent to the elevated potato genotypes than control. Moreover, the presence of macro and micronutrients in panchagavya improved the N, P, K and micro nutrient content of leaves of turnip compared than ozone exposure 150 & 200 ppb. Even though, the organic ozone protectants viz., the liquid organic sprays (1% Neem oil, 3% panchagavya) and 1% ascorbic acid which were selected based on the local availability and cost-effective nature acted as better protectant against tropospheric ozone in turnip.

Conclusion:

From our study we conclude that the elevated ozone has detrimental effect on growth, physiology, development and yield of turnip. The elevated ozone exposure significantly reduces the plant height, tuber size, tuber weight, Chlorophyll ‘a’, Chlorophyll ‘b’, Total chlorophyll, total nitrogen, total potassium, total Manganese, Iron, Zinc, Copper inturnip. Meanwhile, the elevated ozone exposure significantly increased the total phosphorous, catalase and peroxide activity inturnip. However, ozone protectants played a major role to nullify the tropospheric ozoneeffect on growth, physiology, development and yield of turnip and among them panchagavya performed well followed by neem oil and ascorbicacid. Future research may be focused to study the tropospheric ozone impact studies on different crops and identify the best ozone protectants to nullify the tropospheric ozone impact for sustainable agriculture.

Declarations

Ethical Approval: Not Applicable

Consent to Participate: All the authors are contributed to preparation and improvement of the research article

Consent to Publish: All the authors agree to publish the paper in *Environmental Science and Pollution Research*

Authors Contribution:

Dr. Boomiraj Kovilpillai: Objective formulation and overall guidance for the research work.

Mr. Sethupathi Nedumaran: All laboratory analysis work and preparation of manuscript.

Dr. Sudhkaran mani: Statistical analysis of the research data.

Dr. Jayabalakrishnan Raja Mani: Ozone protectants study and fine tuning of the manuscript

Dr. Sritharan Natarajan: Contributed to crop physiological parameter analysis and manuscript fine tuning

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Tables

Table 1: Effect of ambient and elevated ozone on tuber size and tuber weight in turnip

Treatments	Plant Height (cm)	No. of Leaves / Plant	Tuber Size (cm)	Tuber Weight (gm)
T_1	48.33 ± 1.67ba	10.67 ± 1.20a	18.50 ± 1.89ba	196.35 ± 0.39c
T_2	38.33 ± 3.84d	8.33 ± 0.67ba	11.40 ± 0.31c	60.60 ± 0.63j
T_3	37.33 ± 2.96d	7.00 ± 0.58b	5.83 ± 1.20d	27.50 ± 2.34b
T_4	41.00 ± 3.51dcb	9.00 ± 0.33ba	12.33 ± 1.20c	121.40 ± 0.31f
T_5	42.00 ± 2.08dcb	9.00 ± 1.53ba	18.00 ± 0.58ba	134.53 ± 0.55e
T_6	42.33 ± 0.33dcba	9.33 ± 0.33ba	12.80 ± 1.30c	93.37 ± 0.38g
T_7	39.33 ± 3.18d	8.67 ± 0.88ba	12.00 ± 2.08c	92.18 ± 0.33g
T_8	38.33 ± 3.84d	8.33 ± 0.67ba	12.00 ± 1.16c	80.50 ± 0.61h
T_9	40.33 ± 2.96dc	9.00 ± 1.53ba	12.00 ± 0.52c	70.60 ± 0.30i
T_{10}	47.33 ± 1.20cba	10.33 ± 0.33ba	15.27 ± 1.16cb	139.60 ± 0.33d
T_{11}	47.33 ± 1.67cba	9.67 ± 0.67ba	21.35 ± 2.19a	285.00 ± 0.92a
T_{12}	50.00 ± 1.16a	11.00 ± 1.53a	14.67 ± 3.18cb	137.93 ± 1.17d
P value	**0.006**	**0.390**	**0.000**	**0.000**

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

Table 2: Effect of ambient and elevated ozone on major nutrients and micronutrients in turnip

eatments	Total N (%)	Total P (%)	Total K (%)	Mn (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)
	23.14 ± 0.15d	1.85 ± 0.08^{d}	22.88 ± 0.38dcb	1.41 ± 0.22b	2.39 ± 0.19a	0.308 ± 0.032dc	0.111 ± 0.007d
	18.72 ± 0.38i	3.97 ± 0.19^{a}	18.40 ± 0.16f	0.73 ± 0.01e	0.12 ± 0.01e	0.026 ± 0.002fg	0.013 ± 0.001i
	15.34± 0.08j	2.55 ± 0.14^{c}	12.33 ± 0.52g	0.22 ± 0.01f	0.08 ± 0.01e	0.011 ± 0.005ed	0.011 ± 0.003i
	21.06 ± 0.24f	3.63 ± 0.17^{ba}	22.51 ± 0.31dcb	0.98 ± 0.01dc	0.91 ± 0.05c	0.260 ± 0.040ed	0.089 ± 0.004e
	22.10 ± 0.32e	3.28 ± 0.13^{b}	22.42 ± 0.31dc	0.96 ± 0.02edc	1.51 ± 0.13b	0.281 ± 0.110edc	0.085 ± 0.002e
	20.61 ± 0.10f	2.28 ± 0.75^{dc}	22.60 ± 0.48dcb	0.91±0.01edc	0.45 ± 0.06d	0.302 ± 0.012dc	0.053 ± 0.002f
	19.24 ± 0.20ih	3.47 ± 0.42^{ba}	21.86 ± 0.45d	0.76 ± 0.01ed	0.25 ± 0.05ed	0.036 ± 0.002fg	0.028 ± 0.003h
	20.48 ± 0.10f	3.74 ± 0.14^{ba}	19.52 ± 0.31e	0.84 ± 0.02edc	0.44 ± 0.06d	0.153 ± 0.026fe	0.036 ± 0.002hg
	19.83± 0.12hg	3.43 ± 0.24^{ba}	19.61 ± 0.21e	0.77 ± 0.02ed	0.13 ± 0.01e	0.121 ± 0.002fg	0.041 ± 0.001g
0	24.95 ± 0.22c	2.35 ± 0.13^{dc}	23.54 ± 0.21b	1.83 ± 0.02a	1.74 ± 0.14b	0.450 ± 0.052cb	0.256 ± 0.005a
1	25.68 ± 0.36b	2.39 ± 0.10^{dc}	23.16 ± 0.28cb	1.26 ± 0.02b	2.57 ± 0.06a	0.508 ± 0.023b	0.144 ± 0.002b
2	27.25± 0.16a	2.58 ± 0.21^{c}	26.06 ± 0.32a	1.02 ± 0.01c	1.53 ± 0.02b	0.767 ± 0.052a	0.132 ± 0.005c
alue	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

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

Table 3: Effect of ambient and elevated ozone on Plant chlorophyll content and Biochemical properties

eatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Catalase (H_2 O_2 / g / min)	Peroxidase (H_2 O_2 / g / min)
	0.209 ± 0.046a	0.210 ± 0.006b	0.349 ±0.006b	10.20 ± 0.45d	1.84 ± 0.19ed
	0.049 ± 0.002b	0.060 ± 0.005f	0.119 ± 0.004e	35.70 ± 1.27ba	6.52 ± 0.57b
	0.040 ± 0.001b	0.040 ± 0.003g	0.071 ± 0.001f	41.50 ± 1.75a	20.12 ± 0.87a
	0.090 ± 0.002b	0.091 ± 0.005e	0.179 ± 0.005d	15.30 ± 0.57dc	3.08 ± 0.537dc
	0.098 ± 0.005b	0.120 ±0.006c	0.189 ± 0.005dc	15.30 ± 0.47dc	2.64 ± 0.22edc
	0.099 ± 0.002b	0.160± 0.004d	0.200 ± 0.012c	13.60 ± 0.54dc	3.56 ± 0.39c
	0.079 ± 0.005b	0.079 ± 0.003e	0.168 ± 0.004d	24.65 ± 0.57dcb	3.80 ± 0.50c
	0.088 ± 0.002b	0.089 ± 0.005e	0.179 ± 0.004d	27.20 ± 0.19cba	3.96 ± 0.43c
	0.088 ± 0.002b	0.089 ± 0.001e	0.171 ± 0.004d	24.65 ± 0.61dcb	3.80 ± 0.50c
	0.209 ± 0.052a	0.171 ± 0.006d	0.341 ± 0.006b	11.90 ± 0.46dc	1.430 ± 0.14d
	0.201 ± 0.052a	0.160 ± 0.006d	0.340 ± 0.006b	7.65 ± 0.36d	1.76 ± 0.25ed
	0.260 ± 0.005a	0.260 ± 0.011a	0.412 ± 0.012a	8.50 ± 0.42d	1.400 ± 0.07d
alue	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

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