

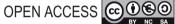
Tropospheric ozone pollution: Implication for food security and crop nutrition in South Asia

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Abstract

The study was conducted on ambient ozone (O₂), the most phyto-toxic air pollutant, and its effects on the growth and quality of okra (Abelmoschus esculentus) and peas (Pisum sativaum) grown in Northern Pakistan during the summer and winter of 2018. Okra was subjected to ambient O₃ levels ranging from 43 to 63 ppb during the summer, with a mean O₃ concentration of 55 ppb, while peas experienced lower winter concentrations of 15–25 ppb, with a mean O3 concentration of 19 ppb. The results indicated significant impacts on the growth and nutritional quality of crops, especially okra. Anti-ozonant ethylene diurea (EDU) was used for soil drenching to protect okra and green peas from O₂ damage. Okra showed notable enhancements of 20%, 20%, 29%, and 13% in ash, protein, fiber, and non-fiber carbohydrates (NFE), respectively. Increase in plant height, leaf numbers, pod length, and dry weight was observed in EDU-treated okra plants. Conversely, peas exhibited less variation, although melioration was observed in plant height, pod numbers, length, and weight with EDU treatment. It was concluded that the concentration of ambient O3 in Peshawar is toxic enough to cause significant damage to crop growth and production. The stark difference in O₃ impact during different seasons suggests that higher summer concentrations could severely compromise crop quality. This elicits significant concerns regarding food security in South Asia, especially for summer crops that can jeopardize future food security. It is recommended that further research be conducted on the effects of O₃ on other regional crops to assess fully its implications for agricultural sustainability in the area.

Keywords: air pollution; ethylene diurea; foliar injury; food security; nutritional quality

Introduction

One of the main secondary pollutants in the atmosphere is tropospheric ozone (O2), which affects both humans and plants significantly. It is mostly emitted by primary pollutants, such as NO_v, So_v and carbonaceous volatile organic compounds (VOCs), from various industries. The mean monthly concentrations (the average amount of ozone present in the troposphere over a month that is typically measured in parts per billion (ppb) or micrograms per cubic meter (µg/m³). have increased from 10 ppb in the late 1800s to approximately 50 ppb at present (Ramya et al., 2023). Tropospheric O₂ is a major threat to crops in Pakistan (Nowroz et al., 2024). According to Ahmad et al. (2013), O₃ is quite high in the peri-urban areas (city outskirts) of Peshawar city, thus affecting crop growth and yield. Therefore, it was important to assess the damage caused by O₃ to agriculture in Peshawar, the oldest in South Asia and a gateway from Central Asia to South Asia, with a current population of about 4.3 million (Pakistan Bureau of Statistics, 2017). Peshawar has a semiarid weather with an average rainfall of around 400 mm. The cultivable area is 75,000 hectares, which is mostly irrigated from the nearby Warsak dam. Major crops are wheat, maize and rice, while plum, apricot and pear are the main fruit crops produced in Peshawar valley (Ihsan *et al.*, 2024; Ahmad *et al.*, 2013).

Peshawar, one of the major cities in Pakistan, is facing rising air pollution due to 10% annual increase in traffic pollution because of population influx (Ghani and Salam, 2023). NO_x , SO_x and Pb are on rise mainly due to two-stroke engine vehicles in the city (Ahmad *et al.*, 2013). Owing to poor road maintenance, high range of suspended dust particles are also produced that further pollutes the environment. The concentration of SO_2 , NO_x and particulate matter (PM) in the atmosphere is approximately 7, 80 and 834 μ g/m³, respectively (Naveed *et al.*, 2024). Because of these emissions, both human health and vegetation are gravely affected in this ever-growing populated city (Ghani and Salam, 2023).

There are around 1500 industries in and around Peshawar, including brick kilns and pharmaceutical and ceramic factories (Yousaf et al., 2024). No rules and regulations and zoning policies exist for managing industries in Pakistan. No primary treatment policy exists in Pakistan for discharging industrial wastes to protect the environment from toxic gases that contaminate water, soil and air (Khan et al, 2024). Industrial wastes are discharged directly into the Kabul River, which is the main source for land irrigation. Untreated toxic substances are absorbed by plants and bio-accumulated in fish, thus affecting the food chain in general (Hayat et al., 2024; Khan et al., 2024). Among the 1500 industries in Peshawar, approximately 400 are brick kilns operating in and around the city that produce around 800,000 bricks per unit annually. These units are regulated poorly as they use low-quality coal, various engine oils and large amount of rubber waste for firing brick kilns, which produces NO_v, CO and carbonaceous volatile organic compounds (VOCs) that are highly toxic and carcinogenic (Ahmad et al., 2012). These gases usually form a thick cover of smoke around the city that travels to long distances depending upon wind speed and direction.

The surface $\rm O_3$ damage to agriculture is recorded with the help of observational and experimental data that revealed different effects of surface $\rm O_3$ on agricultural crops, forests and grasslands (Emberson, 2020). Modeling based on global risk assessment for the year 2000 estimated that loss of staple crop production to $\rm O_3$ pollution was 3–16%, which was equal to US\$14–26 billion economic loss. Concentration of $\rm O_3$ is increasing in the Southern

hemisphere, compared to the Northern because of increased anthropogenic activities (Emberson, 2020).

Ahmad et al. (2013) reported that certain vegetable crops, such as potato, spinach, and tomato, cultivated in Peshawar are sensitive to O₃. Okra (Abelmoschus esculentus), a main summer vegetable, and green pea (Pisum sativaum L. ssp), a prominent winter vegetable, were selected to assess their sensitivity to ambient O₃ using ethylene diurea (EDU). Okra, also called ladyfinger, is a commercial vegetable crop of South Asia. It is mainly cultivated in tropical and subtropical regions of the world. All parts of okra plant are edible and is therefore used for variety of purposes, for example, juvenile fruit of okra plants is consumed as salads, stews, boiled, fried and used in soup (Singh and Pandey, 2024). Okra seeds are considered as a rich source of oil among all other vegetables. They contain linolic acid, proteins, minerals, and fiber gum, and are able to keep the large intestine strong because of rich nutrients. Okra fruit also contains phenolic compounds and has antioxidant activity. Therapeutically, like other medicinal plants okra plant lowers the risk of several diseases, such as diabetes, cancer, heart attack and digestive tract diseases (Pillai et al., 2024; Afsar et al., 2024; Aziz et al., 2024; Benkiran et al., 2024; A. Bacha et al., 2024).

According to the literature, ambient O_3 damages the reproductive part of okra plant, thus decreasing the production of seeds, weight and plant fruit (Ainsworth *et al.*, 2014). The ground-level toxic pollutants contain active form of oxygen that causes severe injuries to okra leaves. It is observed that matured leaves of okra are very sensitive to O_3 , even if exposed for a short duration, while young leaves are tolerant toward tropospheric O_3 . The symptoms that are initially observed on okra matured leaves are flecking (chlorosis, necrosis, bleaching, and bronzing) and stipple (dark brown or red color pigment on the upper surface of leaves; Husen, 2022).

Green pea is a commercial crop grown in the winter season and harvested in the summer season (Getie, 2022). It is observed that seeds of fresh Pisum sativum have a good nutrient value because they contain rich contents of proteins and carbohydrates; moreover, its seeds are canned or frozen all over the world (Chaudhary et al., 2024; Shehzadi et al., 2024; Tulbek et al., 2024). Pea crop exposed to short-term O₃ affects its growth and yield and reduces its tolerance to other environmental stresses (Rusch and Laurence, 1993). Leguminous plants are extremely sensitive to atmospheric pollutants, such as O3. This sensitivity of pea plant and impact of groundlevel O₃ concentration, which has been increasing at a rate of $0.95 \,\mu\text{g/m}^3$ annually, affect plant varieties variably. Higher concentrations of secondary air pollutants damage reproductive cycle and disturb growth and yield of the plant by damaging its biochemical and physiological processes. Peas contain special types of monosaccharide and disaccharide sugars, essential for cell division, cell structure, eukaryotic cell energy, and are a source of carbon. Disaccharide sucrose has a big role in plant photosynthesis (Burbulis *et al.*, 2007).

It was important to assess the impact of tropospheric O_3 on okra and pea in Pakistan because of their economic importance. No study was conducted in the region to examine the negative effects of ambient O_3 on the growth and nutritional quality of okra in summer and peas in winter, as even low O_3 concentration for a longer duration damages the growth and yield of these plants.

Methodology

Site selection

The pot experiment was conducted at the Garden Nursery of the University of Agriculture, Peshawar, Pakistan, to assess the impacts of tropospheric $\mathrm{O_3}$ on okra (*Abelmoschus esculentus*, cv. Pusa Green) and green peas (*Pissum sativum*, cv. Meteor). The pot experiment was carried out on both plant species according to the Male Declaration Report (Swedish International Development Agency, 2009) and the method adopted by Ahmad *et al.* (2013). Okra experiments were conducted from March to May 2018, and the green pea experiments were carried out from early October to December 2018. The experimental site (30×30 m) was away from any industrial site and large buildings. The site was fenced to protect the plants from animals and birds.

Seeds were sown in 20-L volumetric plastic pots. The soil used was selected locally from the irrigation canal in the vicinity of the experimental site. In all, 40 experimental pots were used—20 pots were used for EDU analysis and 20 for non-EDU (NEDU) analysis. The pots were arranged alternatively (EDU/NEDU) in four rows with a space of 1 m between pots and 1.5-m space between rows. Tropospheric O_3 was measured using a photometric O_3 analyzer (Model 400E manufactured by Teledyne API, USA) that was placed on a crop canopy at a level of 1 m near the site throughout the EDU experiment. The temperature and relative humidity (RH) were recorded on daily basis throughout the experiments. Physical observations, for example, number of leaves per plant and plant height, were performed every week until harvested.

Experimental setup

Ethylene diurea was provided by Stockholm Environmental Institute (SEIY, UK) in sufficient quantity.

The day first of EDU application was on day 7 of germination, when the seedlings had emerged to the full. In all, 50% of the plants were treated with EDU, that is, 20 pots were labeled as EDU-treated pots and the rest were irrigated with the same quantity of distilled water. EDU was applied as a soil drenching after every 10 days until harvested, as EDU stays on plant leaves for at least 10 days (Agrawal *et al.*, 2003; Singh *et al.*, 2005; Tiwari and Agrawal, 2009).

Ethylene diurea solution was prepared a day before its application. The prepared solution, with a concentration of 300 ppm, was applied early in the morning before 8 am. The EDU concentration was based on Male crop declaration report (Swedish International Development Agency, 2009) and Ahmad *et al.* (2013), as shown in Table 1.

Okra plants were harvested after 7 weeks, on day 49 in the month of May. Pea plants were harvested on day 52 when they were fully ripped in December. At the time of harvesting, physical characteristics, such as number of leaves, plant height and fruit length, were assessed. Furthermore, the total above-ground biomass of each plant was determined on dry weight basis after harvesting. Okra and pea pods were separated from the rest of the plant marked and labeled in paper bags for each component. The plants were dried at 80°C for 48 h, cooled in desiccators, and stored in sealed paper bags until further chemical analysis.

Qualitative analysis

Plants pods and leaves were analyzed for proximate composition. Nutritional contents, such as moisture, ash, crude proteins, crude fiber, crude fat, and non-fiber carbohydrates (NFE) were analyzed by the method describe by Shahid *et al.* (2023).

Table 1. Ethylene diurea (EDU) with a concentration of 300 ppm used for different applications per 10 days.

Application	Time	EDU concentration @ 300 ppm/application
1st	Day 7	1st application 100 mL/pot (0.6 g/2 L of deionized water
2nd	Day 17	2nd application 100 mL/pot (0.6 g/2 L of deionized water)
3rd	Day 27	3rd application 150 mL/pot (0.9 g/3 L of deionized water)
4th	Day 37	4th application 150 mL/pot (0.9 g/3 L of deionized water)
5th	Day 47	5th application 200 mL/pot (1.2 g/4 L of deionized water)

Statistical analysis

Data analysis (mean and standard error) was carried out using MS Excel, 2010. Statistical analysis was carried out using SPSS 19.0. Data set for all parameters was explored for skewness and kurtosis, and Shapiro–Wilk test was used for normal distribution. Parameters showing major deviation from normal distribution were normalized by using Log 10 transformation. An independent sample *t*-test was used for EDU experiment samples, as comparison of only two treatments was conducted.

Results and Discussion

Okra experiment

Table 2 shows that O_3 increased significantly with increase in weather temperature. Ozone concentration was 45.12 ppb and 43 ppb in week 1 and 2, respectively. The observed range was than increase to 58.86 ppb in week 3. The highest range of O_3 (63.12 ppb) was recorded in week 4 (Table 2). However, from week 5 to week 10, the O_3 concentration was less than 63.12 ppb. The overall mean of O_3 concentrations was recorded as 54.93 ppb and the mean temperature was 32.81°C. Relative humidity (%RH) was recorded as 67.12% in the first week of March, and the overall mean %RH was 35% (Table 2). It was observed that %RH had no effect on O_3 concentration.

 ${
m O_3}$ analyzer was used for the current study instead of passive samplers in order to get peak ${
m O_3}$ concentrations during day time that was not possible using passive samplers. Passive samplers provide mean value for 4 weeks as documented by Wedlich *et al.* (2012), who observed 30% positive biased data using passive sampler, compared to continuous ${
m O_3}$ analyzer.

The active O_3 sampler has lower detection limits, which suggests that active sampler is better than passive sampler (Lurmann *et al.*, 1994). However, passive sampler is inexpensive and a useful method for measuring O_3 concentrations in the absence of O_3 analyzer, particularly for conducting O_3 injury surveys in fields and forest without provision of electricity (Ahmad *et al.*, 2013).

The results of ${\rm O_3}$ analyzer suggested that ${\rm O_3}$ concentration increased gradually in summer from March to June 2018 for okra experiment. The highest ${\rm O_3}$ concentration was 83.12 ppb in the month of May, with an approximate temperature of 40°C. Similar results were also observed by Ahmad *et al.* (2013), who worked on ${\rm O_3}$ effects on crops in Pakistan using ${\rm O_3}$ passive sampler. The authors also reported that ${\rm O_3}$ concentration increased with increase of temperature but was correlated negatively to RH in air that matched with the current study. Previous studies (Agrawal *et al.*, 2003; Tiwari and Agrawal, 2009) have observed the higher ${\rm O_3}$ values of 52–73 ppb in India during the summer season.

The current study suggests that O₃ is higher in summers mainly because of high ultraviolet (UV) radiation of the sun ($h\nu$) that converts NO, SO, and VOC emitted from vehicular and industrial emissions into O₃. The primary pollutants act as a sink for secondary pollutant (O3), and because of low rainfall the atmosphere becomes more polluted. This is an alarming situation because spring and early summer (February to June) in Pakistan is crops cultivation period, and the growth and yield of crops are affected if the concentration of anthropogenic O increases further in the near future. Ozone monitor was used for the first time in Peshawar for okra (March-June 2018) and peas (October-December 2018) using EDU as a soil fertilizer. It was installed near the experimental plot to get ambient O3 readings. A number of studies have used O₃ monitor for such experiments (Elampari et al., 2010; Frei et al., 2010).

All parameters of the proximate composition (ash, protein, fat, fiber and NFE) of NEDU okra seeds were significantly affected by tropospheric ${\rm O_3}$, compared to EDU-treated seeds- of okra, as shown in Table 3. The value of fat was more significant (t-test value = 4.818) as compared to other parameters. Ash, protein, fats, fiber and NFE of the EDU-treated okra seeds were significantly higher by 20%, 20%, 29%, 13% and 9.5%, respectively, compared to the NEDU-treated okra seeds, as shown in Figure 1.

Table 3 shows the ash content of EDU-treated plants was significantly not different from NEDU-treated plants. However, protein, fiber, fats and NFE contents were

Table 2. Mean temperature, O₃, and relative humidity (%RH) from March to May 2018.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Mean
Temp (°C)	22.71	29.5	27	40.67	36.23	31	30.1	35.73	37.24	38	32.81
O ₃ (ppb)	45.12	43	58.86	63.12	58.36	56.99	57.1	57.18	53.23	56.38	54.93
Relative humidity (% RH)	67.12	41.23	45.65	35	28.93	45.89	44.99	28	25.72	25.54	35.10

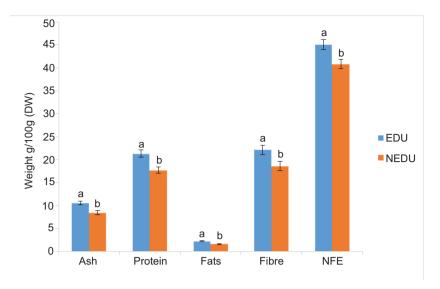


Figure 1. Effect of O_3 on the proximate composition of okra seeds. Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

Table 3. Independent sample t-test and probability values (shown in bold are significant at p = 0.05) for the effect of O₃ on EDU- and NEDU-treated okra seeds. All values are the mean values of 20 replicates.

	EDU mg/100 g	NEDU g/100 g	t-test value	p = 0.05
Ash	10.578 ± 0.337	8.451 ± 0.409	3.821	0.000
Protein	21.3 ± 0.801	17.7015 ± 0.692	3.399	0.002
Fats	2.226 ± 0.096	1.5795 ± 0.093	4.818	0.000
Fiber	22.145 ± 1.045	18.598 ± 1.0281	2.419	0.020
NFE	45.1145 ± 1.111	40.8745 ± 1.061	2.758	0.009

significantly reduced in NEDU-treated plants by 15%, 4%, 8% and 9%, respectively, compared to EDU-treated okra plants (Figure 2). In the current study, the quality of okra was significantly affected by $\rm O_3$. All parameters of proximate composition significant decreased in okra fruit. However, reduction in the crude protein, fats, and fiber contents of okra fruit and leaves due to ambient $\rm O_3$ was of much importance.

In Pakistan, most of the population is malnourished; especially women and children are prone to diseases because of low intake of quality food. Almost 50% of children in Pakistan do not attain their full height because of malnourishment due to the non-availability of nutritious diet. If $\rm O_3$ negatively effects the nutritional quality of okra, an important summer vegetable for lower middle-class population in Pakistan. Then this will directly affect the health and growth of women and young children. $\rm O_3$ significantly reduced all parameters of nutritional quality found in okra. However, a significant reduction in protein and dietary fiber of NEDU-treated okra pods was of prime concern. The current study was the first EDU

analysis to assess the effect of O_3 on the quality of okra in Peshawar. In the past, several studies were conducted to study the effect of O_3 on nutritional quality of mung bean in South Asia (Agrawal *et al.*, 2003, 2009; Ahmad *et al.*, 2013; Tiwari *et al.*, 2017). They concluded that protein, fats, and dietary fiber were significantly lowered in NEDU-treated plants (Table 4).

These findings were similar to the results of the current study. It is also worth mentioning that all the parameters of proximate composition decreased in okra fruit, compared to okra leaves. In leaves, only fat and fiber contents were reduced significantly. This is an important finding of the current study that in terms of nutritional quality, the edible parts of okra were much more sensitive to anthropogenic O_3 , compared to other parts.

No significant difference was found in plant height of EDU- and NEDU-treated plants from week 1 to week 5, as shown in Figure 3 and Table 5. However, the average plant height was significantly higher in the last week before harvest. The highest average height of

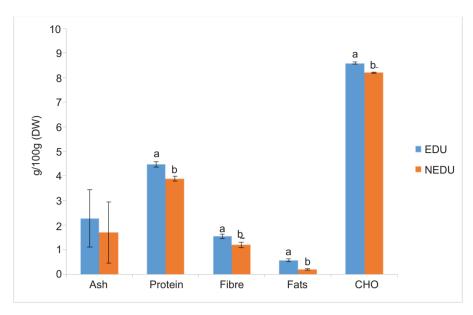


Figure 2. Effect of O_3 on the proximate composition of okra leaves. Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

Table 4. Independent sample t-test and probability values (shown in **bold** are significant at p = 0.05) for the effect of O_3 on EDU- and NEDU-treated okra leaves. All values are the mean values of 20 replicates.

Parameters	EDU (mg/100 g)	NEDU (g/100 g)	t-test value	p = 0.05
Ash	2.279 ± 0.101	1.6955 ± 0.095	4.203	0.796
Protein	4.4705 ± 0.085	3.889 ± 0.105	4.203	0.046
Fats	0.5705 ± 0.047	0.2045 ± 0.024	4.304	0.001
Fiber	1.548 ± 0.058	1.1985 ± 0.038	4.304	0.013
NFE	8.591 ± 0.090	8.205 ± 0.088	6.931	0.050

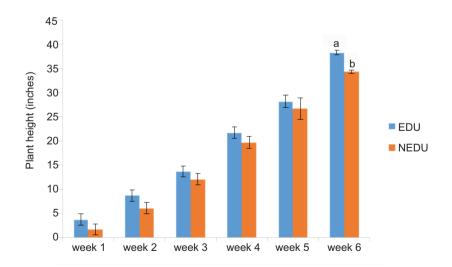


Figure 3. Effect of O_3 on okra plant height (inches) per plant. Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

Table 5. Independent sample t-test shows the weekly average of okra plant height in case of EDU- and NEDU-treated plants. All values are the mean values of 20 replicates (p = 0.05).

EDU	NEDU	t-test value	p = 0.05
3.75 ± 0.16	1.7 ± 0.16	8.673	0.888
8.7 ± 0.14	6.1 ± 0.16	11.596	0.611
13.7 ± 0.14	12.05 ± 0.18	6.762	0.434
21.7 ± 0.14	19.65 ± 0.22	7.481	0.282
28.15 ± 0.26	26.65 ± 0.22	4.15	0.116
38.25 ± 0.48	34.3 ± 0.38	6.175	0.045
	3.75 ± 0.16 8.7 ± 0.14 13.7 ± 0.14 21.7 ± 0.14 28.15 ± 0.26	3.75 ± 0.16 1.7 ± 0.16 8.7 ± 0.14 6.1 ± 0.16 13.7 ± 0.14 12.05 ± 0.18 21.7 ± 0.14 19.65 ± 0.22 28.15 ± 0.26 26.65 ± 0.22	3.75 ± 0.16 1.7 ± 0.16 8.673 8.7 ± 0.14 6.1 ± 0.16 11.596 13.7 ± 0.14 12.05 ± 0.18 6.762 21.7 ± 0.14 19.65 ± 0.22 7.481 28.15 ± 0.26 26.65 ± 0.22 4.15

EDU: ethylene diurea; NEDU: non-ethylene diurea.

Table 6. Independent sample t-test shows the weekly average of okra leaf numbers per plant in case of EDU- and NEDU-treated plants. All values are the mean values of 20 replicates (p = 0.05).

Time	EDU	NEDU	t-test value	p = 0.05
Week 1	3.25 ± 0.09	1.5 ± 0.22	6.9	0.634
Week 2	6.6 ± 0.15	3.5 ± 0.11	16.23	0.630
Week 3	14.85 ± 0.27	9.75 ± 0.22	14.168	0.032
Week 4	19.85 ± 0.36	14.3 ± 0.39	10.812	0.047
Week 5	23.7 ± 0.44	19.25 ± 0.44	6.924	0.530
Week 6	27.9 ± 0.48	22.8 ± 0.48	7.141	0.043

EDU-treated plant at the time of harvesting was 38.2 in and that of NEDU-treated plant 34.3 in. This was 11% lower in NEDU-treated plants, compared to EDU-treated plants, as shown in Figure 3.

Table 6 shows comparison in the number of leaves per plant in EDU- and NEDU-treated plants. Except for the first 2 weeks, the leaf number was significantly higher in EDU-treated plants, as shown in Table 5. Maximum leaves (EDU = 27.9 and NEDU = 22.8) were found in week 6 of the experiment, and were 19.2% higher in EDU-treated okra plants, compared to NEDU-treated plants, as shown in Figure 4.

Table 7 shows that the overall mean length and dry weight of pods were significantly high in EDU-treated plants, compared to NEDU-treated plants. The meannumber of pods were 36.45 and 28.55 cm in EDU- and NEDU-treated plants, respectively, which was 24.7% higher. The mean okra pod length was 8.65 cm and 7.21 cm in EDU- and NEDU-treated plants, respectively, with a significant difference of 17.7 cm.

Similarly, the average dry weight of EDU-treated plant was 28.55 g and that of NEDU-treated plant 22.1 g (Figure 5), which showed that the dry weight of NEDU-treated okra pod was significantly lowered by 25.2%,

compared to the dry weight of EDU-treated okra pod. In the current study, no negative effects of O_3 were observed on height in NEDU-treated okra plants except in the last week just before harvesting. This might be due to the increased concentration of O_3 (50 ppb), which was lower in the previous weeks of the experiment.

The study was in line with the study conducted by Ahmad (2010), who worked on Pakistani spinach and onion species in OTC experiments and observed that the plant height and leaf numbers were decreased significantly in 60-ppb and 90-ppb fumigation chambers. The leaf number of okra was also significantly reduced in the later stages of experiment, showing positive correlation with increase in O₃ concentrations, with peak O₃ concentration being more than 60 ppb during day time. Pod length, numbers, and dry weight (biomass) were also significantly reduced in NEDU-treated okra plants, demonstrating that O₃ concentration increased in April-May onwards and directly affected the physiological characteristics and biomass of okra crop. Similar results were obtained by Wahid (2006a, 2006b) and Wahid et al. (1995a, 1995b, 2001), who studied the effects of O₃ on wheat, rice and soya bean in Lahore. The authors reported significant reduction in yield (biomass) and growth (pant height and leaf number) due to high O₃ concentrations in Lahore during the growing season

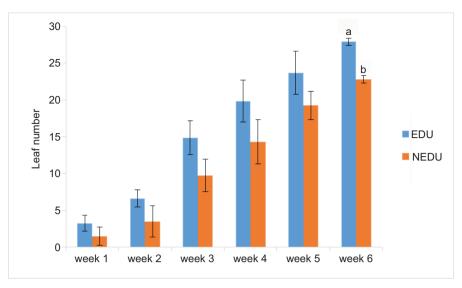


Figure 4. Effect of O_3 on leaf numbers per okra plant. Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

Table 7. Independent sample *t*-test shows the weekly average of okra pods per plant in case of EDU- and NEDU-treated plants. All values are the mean values of 20 replicates (*p* = 0.05).

Pods	EDU	NEDU	t-test value	p = 0.05
No. of pods	36.55 ± 0.63	28.45 ± 0.89	7.363	0.045
Total length	8.65 ± 0.30	7 ± 0.21	8.171	0.034
Dry weight	28.35 ± 1.30	22.3 ± 1.17	3.459	0.050

40 35 30 25 b 20 ■ EDU ■ NEDU 15 10 5 0 No. of Pods Length (cm) Dry weight (gm) Okra Pods

Figure 5. Effect of O_3 on okra pods per plant. Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

of winter. This marked reduction in physiological and nutritional properties suggests that $\rm O_3$ directly affects the nutritional food security of the country.

Green peas experiment

Photometric O_3 analyzer was used for measuring O_3 concentration from October to December 2018. Table 8 shows that O_3 concentration decreased significantly with decrease in temperature. The O_3 concentration was 24.5 ppb and 24.2 ppb in week 1 and 2, respectively. Then it decreased to 14.9 ppb in the last week of December. Maximum O_3 concentration (24.5 ppb) was recorded in the 1st week of October, with temperature (33.3°C) being at its peak during the experimental period.

It was observed that O₂ concentration decreased gradually with decrease in temperature from week 5 to week 10, as shown in Table 8. The mean O₃ concentration for the whole experimental period was 19 ppb with the mean temperature being 25°C. The highest %RH (77%) was observed in the 3rd week of October, and the mean %RH was 64%, as shown in Table 8. The O_3 concentration was not that much higher for peas experiment in winter, compared to the okra experiment in the summer of 2018. The lowest value was 14.5 ppb in December (last week of the experiment) and the highest value was 24.5 ppb in October 2018. This suggests that high O₃ concentration depends upon increased sun light and atmospheric temperature. O₃ is a secondary pollutant that develops from primary pollutants, that is, vehicular and industrial emissions (NO_x, SO_x and VOCs). Modelling based on global risk assessment for the year 2000 estimated that loss of staple crop production to $\rm O_3$ pollution was 3–16%, which was equal to US\$14–26 billion economic losses. In the atmosphere, primary pollutants are converted into $\rm O_3$ in the presence of ultraviolet rays of sun. Hence, $\rm O_3$ concentration was higher in summers, compared to winters in Peshawar. Ahmad *et al.* (2013) also observed similar results for $\rm O_3$ concentrations for summer and winter seasons in 2008.

Table 9 shows that $\rm O_3$ has no significant effect on ash, protein and NFE contents of pea seeds. However, fat and fiber contents of NEDU-treated pea seeds were 5% and 9% lower, respectively, than EDU-treated pea seeds, as shown in Figure 6.

Table 10 shows that no significant difference was observed in ash, protein and NFE values of pea leaves except fiber and fat values, which were significantly higher by 6% and 15%, respectively, in EDU-treated pea leaves, compared to NEDU-treated pea leaves, as shown in Figure 7. Fat and fiber contents were significantly reduced in pea pods and leaves of NEDU-treated plants. However, this didn't entail that pea pods and leaves were more resistant to O₃ as compared to okra plant. As okra plant was exposed to high $O_{\scriptscriptstyle 3}$ concentration in summer, it was affected more from O₃ toxicity, compared to pea plants. However, significant reduction in fat and fiber contents (5% and 9%, respectively) in pea pods was not much higher, compared to the reduction of alarming level in okra plant. Fiber and fats from vegetables remain an essential food source for the lower-income population. If background O₃ concentrations increase in winter, the negative effects of O₃ on

Table 8. Mean temperature, O₂ and relative humidity from October to December 2018.

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	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Mean
Temp (°C)	33.3	31.4	33.9	25.45	30.4	29.3	25.1	21.2	17.3	15.4	25
O3 (ppb)	24.5	24.2	25.4	20.5	24.6	23.7	19.5	18.4	15.5	14.9	19
Relative Humidity (% RH)	55.4	56.7	55.7	77.1	57.7	58.9	57.6	63.2	74.8	75.3	64

Table 9. Independent sample t-test and probability values (shown in bold are significant at p = 0.05) for the effect of O₃ on EDU- and NEDU-treated pea seeds. All values are the mean values of 20 replicates.

	EDU (mg/100 g)	NEDU (g/100 g)	t-test value	p > 0.05
Ash	3.1945 ± 0.071	2.8475 ± 0.083	4.203	0.796
Protein	21.785 ± 0.088	21.202 ± 0.103	4.304	0.476
Fats	1.32 ± 0.032	1.1095 ± 0.020	6.931	0.001
Fiber	2.425 ± 0.007	1.935 ± 0.005	5.026	0.013
NFE	61.559 ± 0.180	61.2825 ± 0.193	3.066	0.749
EDI le otherland dien	rea: NEDU: non-ethylene diurea: NEE:	non filos coulo los duntos		

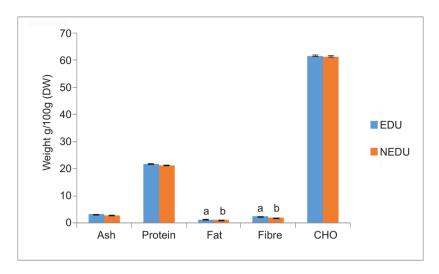


Figure 6. Effect of O_3 on the proximate composition of pea seeds. Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

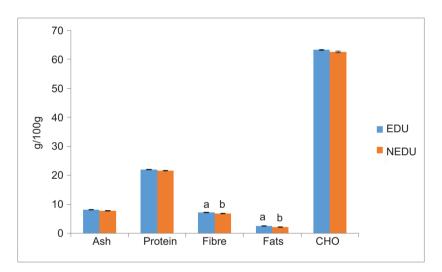


Figure 7. Effect of O_3 on the proximate composition of pea leaves. Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

Table 10. Independent sample t-test and probability values (shown in bold are significant at p = 0.05) for the effect of O_3 on EDU- and NEDU-treated pea leaves. All values are the mean values of 20 replicates.

	EDU (mg/100 g)	NEDU (g/100 g)	t-test value	p > 0.05
Ash	8.1665 ± 0.076	7.807 ± 0.086	3.119	0.279
Protein	21.9815 ± 0.133	21.578 ± 0.121	2.241	0.472
fats	2.6355 ± 0.046	2.233 ± 0.031	7.151	0.018
Fiber	7.2685 ± 0.033	6.8365 ± 0.073	5.394	0.007
NFE	63.2295 ± 0.174	62.5475 ± 0.195	2.611	0.466

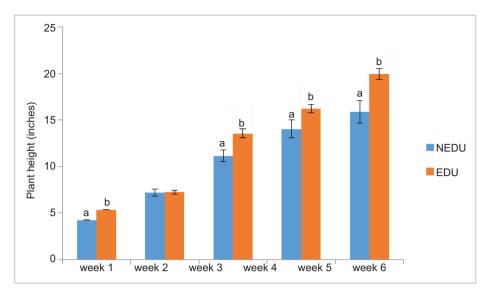


Figure 8. Effect of O_3 on pea plant height (inches). Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

the nutritional quality of these vegetables may also rise to a significant level, directly harming the nutritional status of the local population.

The height of pea plants was significantly not different in the first week of the experiment as they were too small. However, the height of EDU-treated plants showed significant increase, compared to NEDU-treated plants (Table 11). The plant height was not significant in the 3rd week of the experiment (Figure 8), but in EDU-treated plants, it showed significant increase from week 4 until the plants were harvested in week 6 (Figure 8). The maximum height of EDU-treated plants at the time of harvest was 15.9 inches, compared to 20 inches for NEDU-treated plants, representing a significant 22.8% increase in height for the EDU-treated plants. as shown in Figure 8 and Table 11.

Except for week 2, leaf numbers of pea plants were significantly not different from each other (Table 12). However, with the passage of time, an increasing trend in plant height was observed in EDU-treated pea plants, compared to NEDU-treated pea plants. Maximum leaf numbers (EDU-treated = 113 and NEDU-treated = 99.9) were observed in the 6th week of the experiment, as shown in Figure 9.

As shown in Table 13, the overall mean numbers, length and dry weight of pea pods were significantly high in EDU-treated plants, compare to NEDU-treated plants (Figure 10). The mean number of pods was 25.5 and 20.2 in EDU- and NEDU-treated plants, respectively, which were 23% higher in EDU- than that in NEDU-treated plants. The mean pod length in EDU-treated plants was

Table 11. Independent sample t-test shows the weekly average of pea plant's height in case of EDU- and NEDU-treated plants. All values are the mean values of 20 replicates (p = 0.05).

Time	EDU	NEDU	<i>t</i> -test value	p = 0.05
Week 2	4.35 ± 0.34	5.25 ± 0.19	0.047	0.003
Week 3	7.2 ± 0.63	7.25 ± 0.49	0.217	0.29
Week 4	13.15 ± 0.97	11.55 ± 0.44	1.027	0.001
Week 5	14.05 ± 1.19	15.2 ± 0.57	0.594	0.001
Week 6	19.9 ± 1.41	15.95 ± 0.64	2.359	0.003
EDU: ethy	lene diurea; NEDU	J: non-ethylene diu	rea.	

5.05 cm and in NEDU-treated plants 4.15 cm, with a difference of 17.3%. Similarly, the mean dry weight of EDU-treated pods was 32.7% higher, compared to dry weight of NEDU-treated pea pods.

The dry weight of EDU-treated pods was 18.3 g and that of NEDU-treated pods was 13.15 g, as shown in Figure 10. Height of pea plants was significantly affected in NEDU at later stages. However, no marked decrease was observed in leaf numbers of pea plants throughout the experiment. Pod numbers, length and dry weight were affected significantly. This suggested that the pea varieties used in Peshawar were sensitive even to low concentrations of $\rm O_3$ in terms of plant biomass. The pea varieties grown in winter could have shown more manifestations of $\rm O_3$ damage if the $\rm O_3$ concentration were higher enough as that during the summer season.

Several studies have reported the effects of O_3 concentration, both chronic and acute, on reduced plant

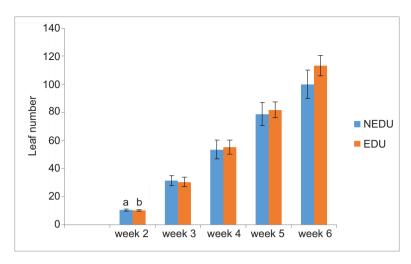


Figure 9. Effect of O_3 on leaf numbers per pea plant. Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

Table 12. Independent sample t-test shows the weekly average of pea leaf numbers per plant in case of EDU- and NEDU-treated plants. All values are the mean values of 20 replicates (p = 0.05).

Time	EDU	NEDU	t-test value	p = 0.05
Week 2	10.4 ± 0.70	10.15 ± 0.47	0.573	0.021
Week 3	31.3 ± 3.38	30.4 ± 3.29	0.404	0.91
Week 4	55.4 ± 6.61	53.3 ± 5.03	0.004	0.331
Week 5	81.8 ± 8.19	78.75 ± 5.62	0.081	0.099
Week 6	113.1 ± 10.08	99.9 ± 7.22	0.856	0.097

Table 13. Independent sample *t*-test shows the weekly average of pea pods per plant in case of EDU- and NEDU-treated plants. All values are the mean values of 20 replicates (*p* = 0.05).

	EDU	NEDU	t-test value	p = 0.05
No. of pods	25.25 ± 0.61	20.2 ± 0.48	6.45	0.048
Total length	5.05 ± 0.169	4.15 ± 0.13	4.194	0.050
Dry weight	18.3 ± 0.62	13.15 ± 0.59	5.97	0.030

respiration, photosynthesis, and changes in photosynthetic activities, which ultimately caused negative effects on the physiology and biomass of crops (Grulke and Heath, 2020; Yi *et al.*, 2016).

As this was the first study on okra and peas conducted in Pakistan using EDU as anti-ozonant, we observed that both crops showed sensitivity to O_3 , with okra showing more signs of O_3 damage, compared to peas in their respective growing conditions. However, this does not mean that pea, being the winter crop, are not sensitive to O_3 pollution, compared to summer crops. The predicted

high O_3 concentration for the future will also affect the winter crops as they are affecting the summer crops.

The impact of $\mathrm{O_3}$ on future food security must be considered, in addition to other the stress factors causing global changes, particularly in case of developing economies (Ramya *et al.*, 2023; Teixeira *et al.* 2011). Population increase in emerging economies poses a danger of food security, because unplanned urbanization and industrialization release precursors to create optimal conditions for ground-level $\mathrm{O_3}$ pollution (Pachauri *et al.*, 2014). As oxidizing air pollution, $\mathrm{O_3}$ has a negative impact on

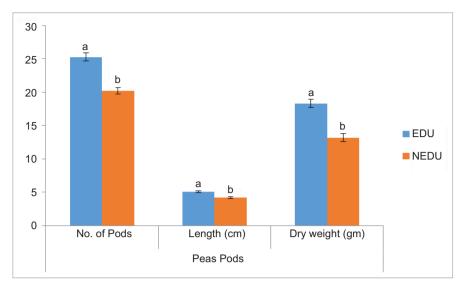


Figure 10. Effect of O_3 on pod numbers, length and dry seed weight of pea plants. Error bars indicate standard error. Bars sharing different letters differ significantly at p = 0.05.

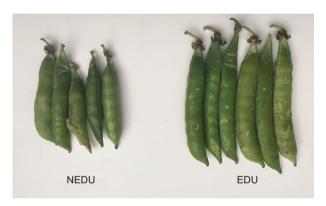


Figure 11. Visible effects of O_3 concentration on the biomass of pea pods.

living beings (vegetation, animals, and humans) as well as materials (rubber, textiles, surface coatings, dyes, metals, and construction materials). Important food crops, such as wheat, rice, maize, and soybean, show a considerable drop in yield, which clearly demonstrates that the current O_3 concentration is endangering food security, which may increase in the future (Hassan et al., 2024; Feng *et al.*, 2022; Mukherjee *et al.*, 2021).

In the current study, single variety of both okra and peas was subjected to O_3 EDU experiment. It is worth mentioning that different varieties show different responses to O_3 pollution because of varied environmental conditions and genetic makeup. Different crop species respond differently to the same O_3 concentration (Emberson *et al.*, 2018). Therefore, it is imperative to subject all government-approved and commercial varieties of local crops to O_3 screening either through open top chamber (OTC) studies or by conducting dose–response experiments under

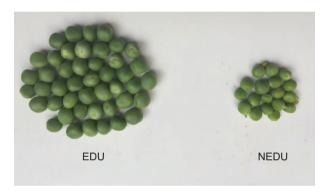


Figure 12. Visible effects of O_3 concentration on the biomass of pea seeds.

field conditions. This would help to identify the best resistant variety both quantitatively and qualitatively because both crops in the present study had reduced biomass due to high $\rm O_3$ concentrations (Figures 11 and 12).

There were several limitations to the study. The $\rm O_3$ concentration was measured only for few months in both seasons; this might have hampered the clearer description of $\rm O_3$ pollution. The $\rm O_3$ concentration must be measured throughout the year at multiple sites in and around the city to evaluate its true impact on agricultural crops.

Conclusion and Recommendations

The study was concluded to analyze the threat of surface O_3 to local crops' growth and yield in the Peshawar region of Pakistan. The study revealed that summer crops are more vulnerable to O_3 pollution, compared to winter

crops, as O_3 concentration is high from March to June because of high UV rays from the sun that expedite the formation of O_3 from primary pollutants; this O_3 concentration is significantly high to affect local agriculture. It was also concluded that okra (a summer crop) was more affected by O_3 pollution than peas (a winter crop) because of low O_3 concentration in winter. However, it does not mean that winter crops are resistant to O_3 concentration, as their sensitivity to O_3 could increase if O_3 concentration mounts in winter. Although EDU is expensive, it is the best anti-ozonant to evaluate the effects of O_3 concentration on different local crops.

The current study also revealed that O₃ not only affects the growth of local crops but is also responsible for reducing the nutritional quality of crops, as observed in okra. Any negative effect of O₂ on the quality of okra weakens its nutritional status. It is recommended that O₂-resistant varieties of both okra and peas should be cultivated to overcome impact of O₃ on crop yield. Although peas showed low response to O3 than okra, the expected rise in O₃ concentration in the future may affect winter crops as well. The air pollution control policies should be revised to control emissions and confront high O₃ concentration. In order to avoid reduced yield, the recently developed crop varieties must be subjected to air pollution screening to identify resistant varieties prior to introducing the same to market. It is important to note that the current Food Composition Table for Pakistan is outdated. A revision is recommended to account for the introduction of new crop varieties, as well as the impact of environmental stressors such as climate change, increased air pollution, and other abiotic factors on these verities should be investigated.

Therefore, to enhance food security, more detailed studies should be conducted to determine the effects of O₃ on crops/vegetation with respect to climate, nutrient, and water availability (Ramya et al., 2023). It is recommended to develop flux models to assess the amount of O₂ that is actually taken by plants through their stomata. In the current study, only the crude forms of proteins, fats and fiber were analyzed. It is suggested to subject cereals to amino acid and fatty acid fraction to determine the effect of O₃ on complete profile of protein and fatty acids to access their nutritional aspects. Screening experiments with different okra and pea bean cultivars should be carried out to initiate breeding of O₃-resistant cultivars. EDU experiments should be conducted all over the country to study the economic and nutritional aspects of O₃ pollution at regional levels.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Conceptualization, Muhammad Nauman Ahmad; methodology, Sadaf Qasim; software, Afia Zia; validation, Sahib Alam; formal analysis, Muhammad Riaz.; investigation, Nureen Zahra; resources, Tariq Aziz.; data curation, Majid Alhomrani.; writing—original draft preparation, Sadaf Qasim; writing—review and editing, Majid Alhomrani; visualization, Walaa F Alsanie, Validation: Abdulhakeem S Alamri; supervision, Muhammad Nauman Ahmad; project administration, Tariq Aziz, Funding Acquisition: Tariq Aziz

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