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EVALUATION OF AMBIENT AIR POLLUTION EFFECTS ON THREE CULTIVARS OF SESAME (*SESAMUM INDICUM* L.) BY USING ETHYLENEDIUREA

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Abstract

Study was conducted in the suburbs of Lahore city of Pakistan to ascertain the protective effects of ethylene diurea (EDU) on three cultivars of sesame plants against ambient air pollutants. Seasonal mean 10 hr pollution levels at the site remained very high (O_3: 91 ppb; NO_2: 38 ppb; SO_2: 10 ppb). It was found that plants treated with highest EDU concentration (500 ppm) showed increases in stomatal conductance (52%), transpiration rate (53%) and net photosynthesis rate (61%) compared with non-EDU treated plants. EDU treated plants depicted luxurious vegetative growth with reduced rate of leaf senescence compared to control plants. EDU protection was remarkable on different biochemical attributes with increases recorded in total chlorophyll by 31%, carotenoids by 15%, protein by 62%, and ascorbic acid by 65%. Total dry biomass was increased from 147-197% and root/shoot ratio from 29-37% in EDU-treated plants compared to plant in non-EDU. Seed yield was greater by 33-43% in different sesame cultivars treated with highest EDU concentration than non-EDU plants demonstrating the efficacy of EDU in preventing air pollutants induced yield losses. The results have wider implications in understanding the injurious effects of air pollutants on agroeconomic husbandry in Pakistan.

Introduction

Air pollution is a product of anthropogenic activities of man and a wide range of pollutant gases (SO_2, NO_2, NO, CO_2, HCs) are being discharged into the atmosphere by motor vehicles, factories, power plants, home furnaces and waste incineration plants that can adversely affect both plants and humans (Emberson *et al*., 2001). Once in atmosphere, pollutants often undergo chemical reactions and produce additional harmful compounds like ozone (O_3), peroxyacetyl nitrate (PAN) and hydrogen peroxide (Fuhrer *et al*., 1997). These diverse emissions into the atmosphere bring about quantitative and qualitative changes in the normal composition of the air (Faiz & Sturm, 2000). Air pollution is often subject to weather patterns that can blow it across the globe to damage pristine environments far from its original source (Agrawal *et al*., 2003; Ashmore *et al*., 2004).

Plants are often more sensitive to ambient air pollutants than other organisms as they are stationary, and are always exposed to the natural environment. They reduce the pollutants concentrations in the air through absorption, adsorption, detoxification, metabolization and accumulation of pollutant compounds exhibiting various types of foliar injuries resulting in reduced productivity due to chlorophyll loss (Wahid 2006a), but some times growth and yield losses occur without appearance of visible injury symptoms (Wahid *et al*., 1995a,b; Maggs *et al*., 1995).

Air pollution is now considered as major environmental threat to crop yield in urban, peri-urban and rural areas (Wahid *et al.,* 2001). Major field studies on the direct impact of atmospheric pollutants on agricultural crops carried out in India, Pakistan, Mexico, China, Japan, Taiwan, Egypt, Australia, Europe and North America has shown significant growth and yield losses in agricultural crops due ambient air pollutants; especially due to tropospheric O_3 (Emberson *et al*., 2003, 2009; Ashmore 2005; Agrawal, 2005; Agrawal *et al*., 2006) that is found both in deep rural areas as well as in urban-industrial locations (Fowler *et al*., 1999). Elevated levels of tropospheric O_3 may cause foliar injury in susceptible plants, accelerated leaf senescence, reduction in photosynthetic activity and affect plants metabolism, thereby reducing plants productivity (Farage & Long 1999; Calatayud *et al*., 2004; Wahid, 2006b). Reduced plant performance has been directly related to the concentration of pollutants to which they were exposed and to the duration of exposure as well (Torsethaugen *et al*., 1999). For detection, quantification and interpretation of plant responses to pollutants, symptoms of injuries, changes in growth habitat, and reduction in quantity of the biomass and yield are the main parameters to be determined and correlated with pollutant concentrations (Wahid 2003; Zhao *et al*., 2011).

Several experimental protocols have been employed in assessing the effects of air pollutants on plants and among them, ethylenediurea or EDU (*N*-2-[2-{oxo-1-imidizolidimyl}ethyl]-N^1-phenylurea) is widely used to suppress acute and chronic effects of air pollutants, in particular O_3 (Carnahan *et al*., 1978) on a variety of plant species without confounding effects of its own (Manning, 2000; Agrawal & Agrawal, 2000): for instance, in potato (Eckardt & Pell 1996), wheat (Tiwari *et al*., 2005), tomato (Varshney & Rout, 1998), radish and turnip (Hassan *et al*., 1995; Pleijel *et al*., 1999), soybean (Wahid *et al*., 2001), tobacco plants (Nakjima *et al*., 2002), beans (Tonneijck & Van Dijk, 2002), clover (Ball *et al*., 1998) popular (Ainsworth *et al*., 1996; Bortier *et al*., 2001), pine (Kuehler & Flagler 1999; Manning *et al*., 2003) and mung bean (Agrawal *et al*., 2005). Studies have also been carried out on the mode of action of EDU but the exact mechanism of action is still a controversial matter (Gatta *et al*., 1997; Lee *et al*., 1997). EDU is systemic in plants and applied as foliar spray, stem injections or soil drenches that protects the plants from premature senescence due to O_3 and maintains the nutrient levels to allow successful growth and productivity (Tiwari *et al*., 2005).

Sesame (*Sesamum indicum* L.) is among the most ancient crops cultivated for its oil in Pakistan. The oil extracted from sesame seeds contains good semi-drying oil, mostly with oleic and linoleic triglycerides along with 26% protein, and is used in making margarine. Its seeds are cooked on breads, and baby-leaves are eaten as stews and soaps in Asia. Present research program was undertaken to assess the effects of air pollution on three cultivars of sesame grown in ambient field conditions using four rates of applications of EDU as soil drenches. Selected biochemical, physiological, growth and yield parameters were used to explain the EDU-induced protection in sesame plants from air pollutants of concern.

Hypothesis of study: Dose-response studies are essential to determine proper EDU-concentration that is effective in reducing crop growth and yield losses from atmospheric pollutants (Kostka-Rick & Manning 1993; Manning 2000).

Materials and Methods

Description of site, climate, and pollution monitoring: Rice Research Institute at Kala Shah Kaku (KSK) was chosen for this experimentation, located 35 km north of Lahore city; a rural site surrounded by thousands of acres of lands famous for growing of cereals and oil-seed crops. This site is near to the national highway connecting Lahore to Islamabad and is dominated by heavy commercial vehicles, buses and cars/wagons round the clock. Ozone, NO_2 and SO_2 levels were monitored from seed germination to crop maturity. Photosynthetically active radiations were measured with a portable light meter, while temperature and relative humidity with a temperature-humidity probe. Wind speed was recorded using a portable anemometer. Data of rainfall was collected from a nearby meteorological station (Table 1).

Table 1. Mean monthly pollutant concentrations and ambient climate during sesame growth season-2005.

Months	Ambient climate					Pollutants conc. (ppb)		
	Temp.	PAR	R.H.	Rainfall	Wind	O_3	NO_2	SO_2
August	41.2	55.8	54.3	11.2	6.2	78.9	28.7	6.8
September	39.5	54.4	51.1	5.4	5.4	88.5	36.5	9.3
October	34.3	61.3	42.1	3.2	9.5	97.6	41.4	11.4
November	29.6	59.2	32.8	1.5	10.6	99.3	44.8	13.2
Seasonal avg.	**36.2**	**57.7**	**45.1**	**5.3**	**7.9**	**91.1**	**37.9**	**10.2**

Pollutant levels of a month represent average of daily readings taken from 0800 – 1800 hrs; Temp. (°C), PAR (µmol m^{-2} s^{-1}), R.H. (%), Rainfall (mm), Wind speed (km/hr)

Source of plant material: Three recommended sesame (*Sesamum indicum* L.) cultivars (Ts-3, Til-93, S-17), for growing in the agricultural fields of Punjab were selected for the present research work, and the seeds were provided by National Agricultural Research Centre, Islamabad. All the cultivars were resistant to fungal-pathogen diseases having similar phenology and harvesting time (110-120 days), but varied in their yield, ranged from 1000-1200 kg/ha for Ts-3, 1100-1250 kg/ha for Til-93, and 1200-1400 kg/ha for S-17.

Experimental design and growing of plants: Soil in the agricultural fields was sandy loam in texture which was most appropriate for the growth of sesame crop. Field plots were developed at the end of July 2008 using standard agronomic practices i.e. 2-3 ploughings up to a depth of 30 cm followed by 1-2 planking to ensure fine level seed beds and sufficient moisture, and addition of recommended dose of commercial fertilizers during preparation of field plots (Urea: 80 kg/ha; Nitrophosphate: 60 kg/ha). Seeds were hand sown on 24th of July 2008 in rows (40-45 cm apart) to a depth of approximately 3.5 cm for satisfactory germination in 45 plots of 1.5 x 1.5 m size. The experiment had five treatments of EDU viz., Non-EDU (**NEDU** or control), EDU-125 (**E-1**), EDU-250 (**E-2**), EDU-375 (**E-3**), EDU-500 ppm (**E-4**), and each treatment were replicated 3 times. The design of the experiment was a split plot with cultivars as the whole plot and EDU treatments as subplots randomized with the whole plots. Seedling emergence was observed after 5-6 days of sowing on 1st August, 2008, and then manual thinning was done after a week by keeping one plant every 25-30 cm apart according to recommended agricultural protocol. After thinning and hoeing, there were approximately 40 plants/plot and all the plants were almost of equal size. Immediately after thinning of plants, 100 ml of EDU treatment was applied as soil drenches to plants between 9.00-11.00 A.M. on weekly basis up to 4 weeks of growth, and thereafter, quantity of EDU treatments was increased (200 ml of EDU/plant) up to 8 weeks, followed by further increase (400 ml EDU/plant) till crop maturity (16 weeks). EDU-application was initially less due to small size of plants and was gradually increased as they grew up with time. Experimental fields were carefully observed regularly to avoid any extra moisture and waterlogged conditions, not recommended for the growth of sesame crop. All the plants were kept under similar water regimes. Each application of EDU was freshly prepared in tap water and control plants received similar quantity of normal tap water.

Measurement of photosynthetic parameters: After 8-week of growth of plants, different parameters of growth physiology such as stomatal conductance (g_s), transpiration rate (E), calculated internal CO_2 concentrations (CI) and net photosynthetic rate (P_N) of randomly selected 8 leaves per treatment per cultivar were measured with an Infrared Gas Analyser. Measurements were carried out between 0800-1200 hours in the field under natural light conditions. The photon flux density incident on the cuvette was maintained in the range of 490±20 µmol m^{-2} s^{-1}. Readings were recorded on the data logger after enclosing the leaf in the chamber for at least 2 minutes. Leaf temperature of the plants sampled ranged between 28-30C° with air relative humidity of 32-35% in the cuvette.

Measurement of biochemical attributes: Selected biochemical attributes were determined at the age of 8-week of growth by taking 4 random leaf samples of plants/treatment/cultivar for various analyses. For the estimation of chlorophyll and carotenoid contents, 0.5 g fresh leaf sample was placed in 10 ml cold 80% acetone in a stoppered tube for over night at 4°C in a refrigerator. They were then homogenized and centrifuged at 6000x g for 15 minutes. Optical densities of leaf extract were taken at 663nm, 645nm and 652nm for the determinations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll, respectively on a UV-visible range spectrophotometer (Hitachi, Model U-1100, Japan). The amount of chlorophyll *a*, *b*, and total chlorophyll were calculated by using the formulae developed by Arnon (1949), and of carotenoid by Duxbury & Yentsch (1956). For protein extraction, fresh leaves were homogenized in tris buffer (0.1 M) followed by mixing of TCA (10%) and then dissolved into 0.1 N NaOH following the method of Lowry *et al*., (1951). For the estimation of ascorbic acid contents, method of Keller & Schwager (1977) was used: leaf samples were homogenized in oxalic acid and Na EDTA extraction solution using 2, 6-dichlorophenolindophenol dye to develop colour and the absorbance was taken at 520nm. After bleaching the colour by 1% ascorbic acid, the difference between absorbance was used to determine the ascorbic acid contents.

Biomass assessment: A mid-season harvest of 8-week-old plants of various EDU treatments was taken by randomly selecting half number of plants of each cultivar per plot. Plants were then brought to the laboratory in labeled paper bags for their respective treatment, cultivar and replicate number in order to assess the effects of EDU on different parameters viz., shoot and root lengths, fresh shoot and root weights, dry shoot and root weights, total dry biomass, root/shoot ratio and number of green and senescent leaves. After taking fresh weights of shoots and roots separately, plants dry weights were recorded by oven drying at 80 °C for 48 hours to constant dry weights.

Harvesting of crop: Sesame crop was harvested well in time after maturity (120 days) to avoid losses in yield due to shattering, and various parameters of yield and yield components were recorded as: number of pods/plant, pod weight/plant, number of seeds/pod, seed weight/plant, 1000-seed weight, above-ground dry biomass, and harvest index (ratio of economic yield to biological yield).

Statistical analysis: Analysis of variance followed by Duncan's Multiple Range Test (Steel & Torrie, 1960) was carried on various parameters to check significance differences between treatment means. The data was also subjected to a 2-way ANOVA test using treatments and cultivars as two factors to depict F-values for Treatments, Cultivars, and Interactions between treatments+cultivars. The statistical analyses were performed using the R-software version 2.2.1 (2005) as stated in Crawley (2002).

Results

Climatology and atmospheric pollutants: Ambient climatic conditions at the experimental site for sesame growth season 2008 (Table 1) were not similar; months of August and September were most humid (54% and 51% respectively) with high ambient temperatures (41 and 40°C) and bright sunshine (56 and 54 µmol m^{-2} s^{-1}). However, temperature and relative humidity gradually decreased with due course of time. Rainfall was 11.2 mm in August with occasional rainy showers (1.5 mm) at the end of season. Wind speed varied slightly from months to months with minor fluctuations. Overall weather conditions at the site were quite hot with long sunny days that became slightly comfortable only towards completion of the experiment. Table 1 also described concentrations of ambient air pollutants. O_3 concentrations was quite high in the beginning of the experiment (79 ppb) and gradually became much higher (99 ppb) in November, while NO_2 and SO_2 levels were comparatively lower (28 and 7 ppb, respectively) in August which also became remarkably higher (45 and 13 ppb, respectively) with gradual increases towards November. Seasonal mean 10 hr pollution levels were very high (O_3: 91 ppb; NO_2: 38 ppb; SO_2: 10 ppb) during sesame growth season-2005.

Effects on photosynthesis, biochemical aspects and vegetative growth: Various parameters of photosynthesis were appreciably increased in EDU treatments than NEDU (Fig. 1). Highly significant increases were found in higher EDU-treatments while NEDU and E-1 treatment were almost similar. Stomatal conductance was increased by 13-16% in E-2, 33-39% in E-3, and 47-52% in E-4 treatment compared to NEDU-treated ones in different cultivars. Transpiration rate was also higher in treatment E-2 (11-15%), E-3 (23-36%), and E-4 (43-53%) than NEDU treatment plants. Similarly net photosynthetic rate was increased by 25-35%, 47-52%, and 56-61% in E-2, E-3 and E-4 treatments, respectively depicting their luxurious vegetative growth. Internal CO_2 concentrations showed an opposite trend with reductions of 4-6% in E-2, 8-10% in E-3, and 9-12% in E-4 treatment plants than their counterparts grown in NEDU in different sesame cultivars. Highly significant effects of 2-way ANOVA test on EDU treatments on photosynthetic parameters of three cultivars of sesame are also given in Table 2.

Biochemical parameters of sesame cultivars were significantly increased due to application of EDU treatments. However, there was no significant effect of E-1 treatment on various measured parameters in all three cultivars when compared with NEDU. Fig. 2 showed highly pronounced increases in photosynthetic pigments due to applications of EDU than NEDU. Chlorophyll *a* was increased by 5-7% in E-2, 13-14% in E-3, and 17-21% in E-4 treatment compared to NEDU. In general, increase in chlorophyll *b* was much higher than chlorophyll *a*, for instance, 13-21% in E-2, 40-45% in E-3, and 58-66% in E-4. Total chlorophyll contents were similarly higher in corresponding higher EDU-treatments in all three cultivars of sesame. Carotenoid contents were also appreciably increased in higher EDU applications (E-2 to E-4) in all cultivars; ranged from 7-10%, 11-12%, and almost 15%, than NEDU. Similar trend was found in protein contents of EDU-treatment plants (17-18% in E-2, 34-38% in E-3, 58-62% in E-4), and ascorbic acid contents (24-37% in E-2, 44-52% in E-3, and 60-65% in E-4) than NEDU. Overall, effects of EDU-treatment on biochemical attributes of 3 sesame cultivars are presented in Table 3.

Table 2. F-ratio and level of significance of 2-way ANOVA test for different parameters of growth physiology of 7-week-old three sesame cultivars.

Parameter	Treatments	Cultivars	Treat. x Cult.
Stomatal conductance (g_s)	939.614 ***	91.429 ***	0.615 ns
Transpiration rate (*E*)	76.389 ***	15.158 ***	0.767 ns
Internal CO_2 conc. (IC)	339.546 **	37.775 *	0.552 ns
Photosynthesis rate (P_N)	657.569 ***	59.597 ***	3.394 **

Level of significance: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; ns = not significant. gs: millimol m^{-2} s^{-1}; *E*: millimol m^{-2} s^{-1}; IC (ppm); P_N: micromol m^{-2} s^{-1}

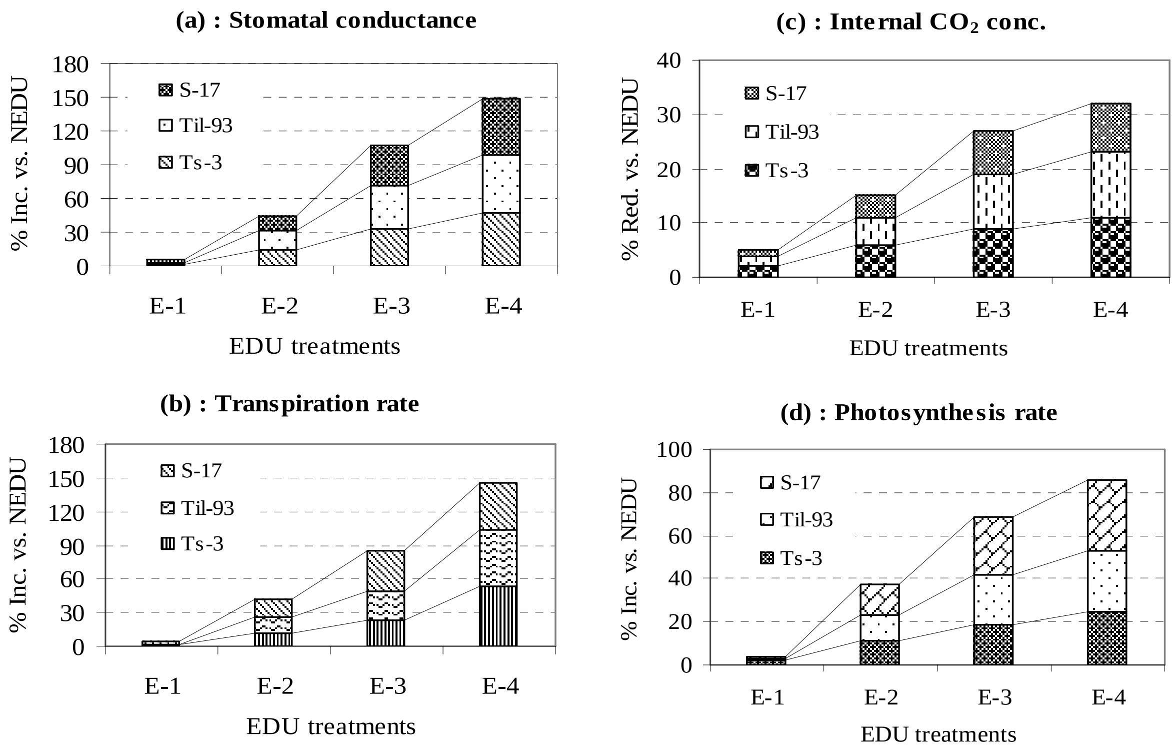

Fig. 1. Effects of EDU-treatments on photosynthetic attributes of 8-weeks-old three cultivars of sesame.

Table 3. F-ratio and level of significance of 2-way ANOVA test for some biochemical aspects of 8-week-old three cultivars of sesame.

Parameter	Treatments	Cultivars	Treat. X Cult.
Chlorophyll a	296.944 ***	6.076 **	1.515 ns
Chlorophyll b	496.511 ***	5.069 *	1.244 ns
Total chlorophyll	689.414 ***	8.546 **	1.065 ns
Carotenoid contents	386.123 ***	8.584 **	1.401 ns
Protein contents	329.248 ***	17.529 *	0.198 ns
Ascorbic acid	439.271 ***	8.779 ***	2.402 *

Level of significance: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; ns = not significant

Parameters of biomass assessment are shown in Table 4 that shows positive influence of EDU applications on vegetative growth of sesame than NEDU, but the effect of E-1 concentration remained non-significant through out experimentation in all the cultivars. It may be noted from the data in Table 4 that increases in shoot and root length, fresh shoot and root weights, dry shoot and root weights, and total dry biomass were highly significant ($p<0.001$) in different cultivars of sesame due to EDU-treatments. Root:shoot ratio was statistically significant in E3 and E-4 treatments in all cultivars and was non-significant in rest of the EDU-treatments (E1 and E2) compared with NEDU. Number of green leaves was significantly ($p<0.001$) increased in E-2, E-3, and E-4 treatment plants, while senescent leaves per plants were significantly reduced in EDU-treatments (E-2, E-3, and E-4) than their counterparts grown in NEDU depicting the protective effects of EDU from early leaf senescence. Percentage increases or reductions in biomass compared with NEDU plants are also shown in Table 4 for three cultivars of sesame, and are statistically highly significant ($p<0.001$) due to EDU-treatments, cultivars and interactions of treatment x cultivars.

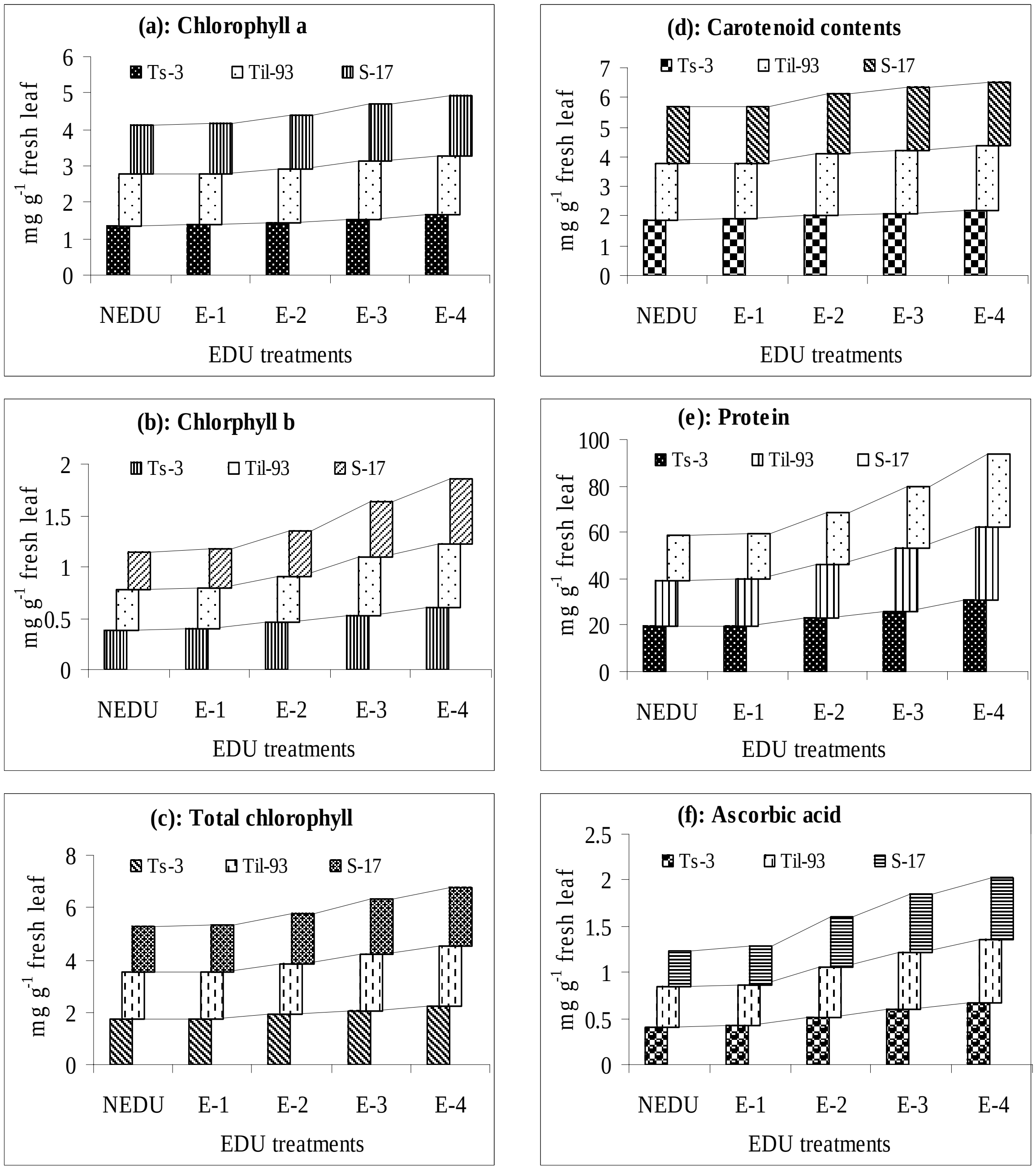

Fig. 2. Impact of EDU treatments on biochemical parameters of 8-weeks-old, three cultivars of sesame.

Effects on yield and yield components: Yield parameters showed positive impact of EDU applications in all 3 cultivars studied during the present course of investigation (Table 5) except for lower EDU-treatment (E-1). Higher number of pods was found in E-2 (11-14%), E-3 (36-47%), and E-4 (54-70%), while pod weight was improved only in E-3 (9-14%) and E-4 (17-27%) compared with NEDU in sesame cultivars. Number of seeds per pod was increased by 19-26% in E-3 and 31-37% in E-4 and was statistically highly significant, but in other EDU treatments it was non-significant except for 13% increase in cultivar S-17 in E-2 treatment compared with NEDU. Seed weight per plant showed remarkable effects of EDU application in all the cultivars with increase of 6-14% (E-2), 20-27% (E-3), and 33-43% (E-4), while there was no effect of lower concentration of EDU (E1) on this parameter than that of NEDU-treated plants. 1000-seed weight was although slightly higher in EDU-treated plants than NEDU but increases were statistically non-significant. Above-ground dry biomass was significantly improved in EDU-treated plants (5-13% in E-2; 19-25% in E-3; 31-40% in E-4) than NEDU while it was non-significant in E-1 treatment plants. Harvest index was although slightly higher in EDU-treated plants but the increases were statistically non-significant. In general, significant increases in number of pods per plants along with number of seed per pod contributed to overall higher economic yield.

Table 4. Growth performance of three cultivars of sesame after 6-week of EDU application.

Parameters • 2-way ANOVA: F-ratio	EDU-treatment	Sesame cultivars		
		Ts-3	**Til-93**	**S-17**
Shoot length (cm)	NEDU	41.8d	43.2d	47.2d
Treatment: 346.25 ***	E-1	41.9d (+0.2)	43.5d (+0.7)	47.3d (+0.2)
Cultivars: 154.23 ***	E-2	44.9c (+7.4)	46.9c (+8.6)	51.6c (+9.3)
Treatment x Cultivars: 1.93 ns	E-3	49.8b (+19.1)	51.3b (+18.7)	56.5b (+19.7)
	E-4	55.3a (+32.3)	59.4a (+37.5)	64.6a (+36.9)
Root length (cm)	NEDU	17.9d	18.9d	20.2d
Treatment: 656.29 ***	E-1	18.1d (+1.1)	19.1d (+1.1)	20.4d (+1.0)
Cultivars: 208.36 ***	E-2	19.1c (+6.7)	21.2c (+12.2)	24.2c (+19.8)
Treatment x Cultivars:14.41 ***	E-3	23.7b (+32.4)	25.0b (+32.3)	29.7b (+47.0)
	E-4	28.4a (+58.7)	31.8a (+68.2)	37.4a (+85.1)
Fresh shoot wt. (g)	NEDU	42.4d	38.8d	33.4d
Treatment: 423.42 ***	E-1	43.6d (+2.8)	39.5d (+1.8)	33.8d (+1.2)
Cultivars: 609.81 ***	E-2	45.6c (+7.5)	41.4c (+6.7)	35.6c (+6.6)
Treatment x Cultivars: 3.07 *	E-3	50.3b (+18.6)	44.5b (+14.7)	39.6b (+18.6)
	E-4	58.4a (+37.7)	51.1a (+31.7)	46.0a (+37.7)
Fresh root wt. (g)	NEDU	7.6d	6.5d	4.9d
Treatment: 442.87 ***	E-1	7.6d (+1.3)	6.8d (+4.6)	5.0d (+2.0)
Cultivars: 156.34 ***	E-2	9.6c (+28.0)	8.4c (+29.2)	6.7c (+36.7)
Treatment x Cultivars: 0.36 ns	E-3	11.9b (+58.7)	11.1b (+70.8)	8.8b (+79.6)
	E-4	15.0a (+100.0)	14.0a (+115.4)	12.1a (+146.9)
Dry shoot wt. (g)	NEDU	5.7d	4.7d	3.1d
Treatment: 894.71 ***	E-1	5.8d (+1.7)	4.9d (+4.3)	3.3d (+6.4)
Cultivars: 433.45 ***	E-2	7.4c (+29.8)	6.6c (+40.4)	4.7c (+51.6)
Treatment x Cultivars: 8.01 ***	E-3	9.5b (+66.7)	8.6b (+83.0)	6.9b (+122.6)
	E-4	13.0a (+128.1)	12.0a (+155.3)	8.6a (+177.4)
Dry root wt. (g)	NEDU	1.7d	1.4d	1.1d
Treatment: 615.49 ***	E-1	1.8d (+5.9)	1.4d (+7.7)	1.1d (0.0)
Cultivars: 71.85 ***	E-2	2.3c (+35.3)	2.0c (+53.8)	1.8c (+63.6)
Treatment x Cultivars: 4.67 ***	E-3	3.3b (+94.1)	3.0b (+130.8)	2.9b (+163.6)
	E-4	5.3a (+211.8)	4.7a (+261.5)	3.9a (+254.5)
Total dry biomass (g)	NEDU	7.4d	6.0d	4.2d
Treatment: 1388.31 ***	E-1	7.6d (+2.7)	6.3d (+5.0)	4.4d (+4.8)
Cultivars: 462.261 ***	E-2	9.7c (+31.1)	8.6c (+43.3)	6.5c (+54.8)
Treatment x Cultivars: 11.42 ***	E-3	12.8b (+73.0)	11.6b (+93.3)	9.8b (+133.3)
	E-4	18.3a (+147.3)	16.7a (+178.3)	12.5a (+197.6)
Root/shoot ratio	NEDU	0.30b	0.29c	0.35bc
Treatment: 16.88 ***	E-1	0.31b (+3.3)	0.29c (0.0)	0.35bc (0.0)
Cultivars: 6.81 **	E-2	0.32b (+6.7)	0.31c (+6.9)	0.38abc (+8.6)
Treatment x Cultivars: 0.72 ns	E-3	0.35ab (+16.7)	0.35b (+20.7)	0.42ab (+20.0)
	E-4	0.41a (+36.7)	0.39a (+34.5)	0.45a (+28.6)
Green leaves	NEDU	25.3d	21.7d	18.0d
Treatment: 179.56 ***	E-1	26.0cd (+2.8)	23.0cd (+6.0)	18.d (+3.9)
Cultivars: 196.35 ***	E-2	28.0c (+10.7)	24.7c (+13.8)	21.7c (+20.6)
Treatment x Cultivars: 0.96 ns	E-3	31.7b (+25.3)	28.0b (+29.0)	25.3b (+40.6)
	E-4	36.7a (+45.1)	31.3a (+44.2)	28.7a (+59.4)
Senescent leaves	NEDU	12.7a	13.4a	10.7a
Treatment: 59.61 ***	E-1	12.4a (-2.6)	13.1a (-2.2)	10.6a (-0.9)
Cultivars: 25.48 ***	E-2	11.0a (-13.3)	12.0a (-10.6)	8.7b (-18.2)
Treatment x Cultivars: 0.47 ns	E-3	8.73b (-31.4)	8.9b (-40.0)	6.6c (-38.1)
	E-4	6.5b (-48.9)	6.5c (-52.0)	5.1c (-52.5)

Treatment means of every parameter followed by different letters in each column of sesame cultivars are statistically significant according to Duncan's multiple range test at p=0.05. NEDU (Non-EDU); E-1 (EDU-125); E-2 (EDU-250); E-3 (EDU-375); E-4 (EDU-500 ppm). Values in parenthesis are % increases (+) or decreases (-) as compared to NEDU. Levels of significance: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; ns = not significant

Table 5. Effect of EDU-treatments on yield and yield components of three cultivars of sesame.

Parameter	EDU treatments (ppm)					L.S.D. (P=0.05)	F-value
	NEDU	E-1	E-2	E-3	E-4		
Sesame cv. Ts-3							
Pods per plant	8.67d	8.73d	9.60c	11.80b	13.33a	0.88	54.43 ***
Pod wt. per plant (mg)	181.82d	182.87d	185.08c	198.35b	215.08a	2.26	398.32 ***
Seeds per pod	16.50c	16.70c	17.33c	19.65b	21.53a	0.97	50.38 ***
Seed wt. per plant (mg)	78.50d	79.53d	82.84c	94.14b	104.51a	1.29	734.69 ***
1000-seed wt. (g)	4.759a	4.765a	4.781a	4.794a	4.856a	0.23	0.398 ns
Dry biomass (g)	11.57c	11.70c	12.13bc	13.73b	15.17a	0.93	46.42 **
Harvest index	6.79ab	6.81ab	6.83ab	6.86ab	6.92a	0.03	5.64 ns
Sesame cv. Til-93							
Pods per plant	8.70b	8.77b	9.95b	12.77a	14.13a	1.37	31.88 ***
Pod wt. per plant (mg)	182.80d	183.02d	187.74c	202.34b	220.04a	1.93	682.65 ***
Seeds per pod	17.23c	17.33c	18.20c	21.33b	23.23a	1.82	21.68 ***
Seed wt. per plant (mg)	79.83d	80.33d	84.38c	99.24b	108.31a	1.99	406.87 ***
1000-seed wt. (g)	4.635a	4.637a	4.643a	4.662a	4.676a	0.43	0.12 ns
Dry biomass (g)	12.10c	12.20c	12.80bc	14.93b	16.10a	1.05	51.89 **
Harvest index	6.60ab	6.61ab	6.62ab	6.68ab	6.74a	0.04	6.03 ns
Sesame cv. S-17							
Pods per plant	9.77c	9.83c	10.83c	13.50b	16.57a	1.16	63.19 ***
Pod wt. per plant (mg)	183.30d	183.77d	191.10c	208.33b	232.09a	2.62	623.45 ***
Seeds per pod	18.37b	18.47b	20.77b	23.07a	25.13a	2.23	17.28 ***
Seed wt. per plant (mg)	81.06d	81.72d	92.18c	102.55b	115.51a	2.38	374.32 ***
1000-seed wt. (g)	4.431a	4.434a	4.452a	4.459a	4.559a	0.52	0.18 ns
Dry biomass (g)	12.30d	12.37d	13.90c	15.40b	17.27a	1.091	67.65 **
Harvest index	6.62a	6.62a	6.63a	6.69a	6.70a	0.02	9.36 ns

Treatment means of parameters in rows within cultivars followed by different letters are statistically significant according to Duncan's multiple range test. NEDU (Non-EDU); E-1 (EDU-125); E-2 (EDU-250); E-3 (EDU-375); E-4 (EDU-500 ppm). Level of significance: ** = p<0.01; *** = p<0.001; ns = not significant

Discussion

Air pollution posses a serious threat to agricultural production all around the globe. In the present study carried out at a rural site, 35-km north of Lahore city, where seasonal 10 hr mean O_3 concentration remained very high (91 ppb), along with NO_2 (38 ppb) and SO_2 (10 ppb). In an early study carried out during 1996-97 at the same site has reported 6 hr seasonal mean O_3 concentrations of 48 ppb along with 27 ppb of NO_2 (Wahid *et al*., 2001). It is a matter of serious concern that in the last one decade O_3 concentrations have shown two-fold increase in the rural agricultural background of Pakistan, while NO_2 showed 40 % increase. SO_2 concentration was not found during 1996-97 experimentation in that particular site but now it was present at the site (Wahid, 2003). Actually, higher ambient temperature, bright sunny days and low winds along with high precursor emissions in the ambient climate of Lahore are responsible for the formation of secondary air pollutants like O_3 that is usually found in higher concentrations in rural backgrounds (Ashmore *et al.*, 2004). Agrawal *et al.*, (2006) have also reported higher O_3 concentrations during summer than winter months in peri-urban areas of Varanasi, India. Many workers (Wahid *et al*., 1995aandb; have also reported >50 ppb O_3 concentrations in urban areas during summer and have found positive correlation between O_3 and high temperature, higher light levels, low humidity and relatively still wind movements. The behaviour of atmospheric pollutants, especially O_3, in urban fringe and rural areas of North America, Western Europe and Asia suggests that O_3 concentrations could be higher in rural areas outside major cities (Emberson *et al*., 2001; Vingarzan, 2004; Agrawal *et al*., 2006) with consequent risk to crops and wild vegetation (Ashmore, 2005).

The enormous surface area of expanded leaves of terrestrial plants acts as a natural sink for gaseous pollutants and stomata are the main avenues through which pollutants diffuse into the plant leaves causing various types of foliar injuries leading to losses in growth and yield (Wahid, 2003; 2006a,b). In present study, growth physiology parameters (g_s, E, and P_N) was considerably higher in EDU-treated plants than that of NEDU (Fig. 2) under very high stress of atmospheric pollutants (Table 1) in the ambient field conditions, while internal CO_2 concentration was lower in EDU-treated plants suggesting that CO_2 is being properly fixed into assimilates in these plants leading to their healthier growth. According to Calatayud & Barreno (2001), photosynthesis has been shown to be particularly sensitive to O_3 and exposure of this gas produces a variety of effects in plants ranging from stomatal closure, inhibition of one or more steps in CO_2 fixation, to a decrease in electron transport rate resulting in reduced plant growth and this has been found in NEDU and low-level EDU-treatment plants of present study with sesame crop. Stomata control access of CO_2 and O_3 to photosynthetic cells and guard cells of the stomata are not fully cuticularized. Following exposure to O_3 there is decline in stomatal conductance. But stomatal conductance is closely linked with photosynthetic activity, and in healthy leaves it is closely linked to photosynthetic rate (Long, 1985). Stomatal conductance could therefore decline as an indirect result of O_3 damage to photosynthetic capacity within the mesophyll cells or as direct response to O_3 by impairing K^+ uptake to channels of protoplasts through guard cells (Torsethaugen *et al*., 1999). Many other studies (Wahid, 2006 a,b) carried out in charcoal filtered air has shown higher values of gs, E and P_N in the pollution free environment, and magnitude of response is dependent on plant species, concentration and duration of pollutants exposure. According to Cooley and Manning (1987), O_3 is known to reduce photosynthesis and cause a shift in partitioning of photosynthate in susceptible plants. Partial closure of stomata, reduced transpiration and photosynthesis in response to high O_3 has also been confirmed in many other studies (Guidi *et al*., 1999; Calatayud *et al*., 2004), and are in lines with the present research work.

Photosynthetic pigments showed protective effects of EDU-treatments than NEDU-treated ones in the present study and were significantly higher in EDU-treated plants (Fig. 2). A number of studies (Kostka-Rick and Manning, 1993; Manning *et al*., 2003) have also described that different concentrations of EDU proved to be effective in preventing damage to biochemical attributes of crop plants due to O_3 induced yield losses. Higher chlorophyll and carotenoid contents were also found in a variety of plant species (Eckardt and Pell, 1996; Lee *et al*., 1997; Agrawal *et al*., 2003). EDU-treatment in sesame cultivars may have counteracted the formation of oxyradicals due to elevated O_3 (>90 ppb) in present study leading to maintenance of higher levels of photosynthetic pigments for longer period of time as suggested by Lee *et al*., (1997) and Gatta *et al*., (1997). Protein and ascorbic acid contents were also significantly higher in EDU-treated plants of sesame cultivars than NEDU (Fig. 2) in the present study. Astorino *et al*., (1995) reported reduction of 36% in chlorophyll *a* leading to reduction in photosynthesis by 50% in *Phaseolus vulgaris*. According to Brunschon-Harti *et al*., (1995b), major effects of O_3 on plant metabolism are membrane destruction as a result of radical generation, and therefore, lipid peroxidation, decreased photosynthesis; disabled carbon allocation leads to decrease growth due to decreased enzyme function. Alaiz *et al*., (1999) reported that O_3 cause oxidative stress and induces degradation of many biologically important molecules like amino acids, proteins and carbohydrates due to release of malondialdehyde. Lee & Chen (1982) found that EDU preserved protein content longer by regulating protein catabolism in leaves by acting like cytokinin to retard chlorophyll degradation. Lee (1991) and Van Hove *et al*., (2001) reported a significant positive relationship between O_3 sensitivity of the tissue and ascorbic acid content. Increase in ascorbic acid contents of many other plants was also recorded by Gillespie *et al*., (1998) and Lyons *et al*., (2000). According to Manning *et al*., (2003), higher concentrations of ascorbic acid protect the plants from O_3 injury. In an elaborated study, Tiwari *et al*., (2005) reported higher contents of chl. *a*, *b*, total chlorophyll, carotenoids, protein and ascorbic acid contents in EDU-treated leaves of wheat plants than NEDU ones. According to them, EDU application, perhaps maintained a higher level of ascorbic acid by reducing production of free radicals in the presence of elevated O_3 levels. This published literature is well consistent with the results of biochemical parameters of sesame cultivars in the present research work.

Vegetative growth of sesame plants was enhanced by the application of different concentrations of EDU than NEDU plants (Table 4). However lower EDU-treatment (E-1: 125 ppm EDU) was similar to NEDU in biomass assessment. Number of green leaves was enhanced by 44-59% in highest EDU-treatment-level (E-4: 500 ppm EDU) along with reduced senescence rate observed in this treatment (Table 4), which depicts that leaves of EDU-treated plants remained green for a longer period of time and hence more assimilate was formed that that of NEDU-treated ones that showed accelerated leaf senescence leading to poor growth. Results of the present study clearly indicated that ambient atmospheric concentrations of pollutants (>90 ppb O_3, 44 ppb NO_2, 23 ppb SO_2) at the experimental site reduced vegetative growth of NEDU-treated sesame cultivars significantly. Protective effects of EDU against O_3 effects on plants have been widely reported in a number of studies (Carnahan *et al*., 1978; Hassan *et al*., 1995; Lee *et al*., 1997; Pleijel *et al*., 1999; Tonneijck & Van Dijk, 2002). Bisessar & Palmer (1984) found 17% increase in biomass of EDU-treated tobacco plants, while Ensing *et al*., (1985) reported 73% increase in leaf-drop in NEDU plants than that of EDU-treated ones. A reduction of 22% in shoot biomass of *Raphanus sativus* L. was also reported by Pleijel *et al*., (1999) when grown in NEDU. Wahid *et al*., (2001) reported highly significant increases in vegetative growth of EDU-treated soybean plants viz., plant length, number of green leaves, oven dry biomass, root:shoot ratio and lower leaf senescent rates at seasonal mean O_3 levels of 48 ppb. Manning *et al*., (2003) reported more heights of loblolly pine seedlings in response to EDU-treatment. Tiwari *et al*., (2005) and Agrawal *et al*., (2005) also found significant increases in growth parameters of EDU-treated wheat and mungbean crops at O_3 levels of 40 ppb and >60 ppb, respectively as compared to NEDU control and both studies also reported early senescence of leaves in NEDU plant than EDU-treated ones. In general, O_3 accelerates leaf senescence in plants but EDU delays O_3 induced senescence promoting higher vegetative growth (Manning *et al*., 2003).

The protective effect of EDU treatments on three cultivars of sesame was further supported by progressive increases in various yield parameters as shown in Table 5. Number of pods per plant was increased in all the cultivars of sesame in response to the application of EDU, but it remained highly significant in E3 and E4 treatments (375 and 500 ppm EDU, respectively) than NEDU showing increases of 36-54%, 47-62%, 38-70% in sesame cultivars Ts-3, Til-93, and S-17, respectively. Similarly, pod weight per plant was also higher in these cultivars. Number of seeds (19-31% in cv. Ts-3; 24-35 in cv. Til-93; 26-37% in cv. S-17 in EDU treatments of E3 and E4, respectively), and seed weight per plant (20-33% in cv. Ts-3; 24-36% in cv. Til-93; 27-43% in cv. S-17 in E3 and E4 treatments, respectively) also showed the similar response as recorded in earlier yield parameters. Above-ground dry biomass also maintained the preceding trend. However, minor increases were recorded in 1000-seed weight and harvest index in response to EDU-treatments. EDU application of 125 ppm (E-1) did not proved effective in protecting the plants from ambient atmospheric pollutants, and EDU-treatment of 250 ppm (E-2) was although effective but could not protected plants productivity of sesame crop completely. However, EDU-applications viz., E-3 (375 ppm) and E-4 (500 ppm) remained highly effective, especially E-4 (500 ppm EDU) in the field conditions of Pakistan.

Increase in yield of EDU-treated beans were recorded by many workers, viz., 36% in navy beans (Hofstra *et al*., 1978), 24% in white beans (Temple & Bisessar, 1979), and 30% in *Phaseolus vulgaris* (Toivonen *et al*., 1982). Legassicke and Ormrod (1981) showed yield increase up to 30% for tomatoes, while Ensing *et al*., (1985) found an increase in yield of 17% for peanuts in EDU-treated plants. EDU increased pod weight of *Phaseolus vulgaris* by 31-65% (Brunschon-Harti *et al*., 1995a), 52% (Astorino *et al*., 1995), 14% (Vandermeiren *et al*., 1995), and 20% (Tonneijck & Van Dijk, 1997) as compared to NEDU plants. Tonneijck & Van Dijk (2002) also found significant increases in green and mature pods of *Phaseolus vulgaris* treated with EDU. Wahid *et al*., (2001) in an extensive study carried out at three widely separated sites (urban, rural, roadside rural) evaluated the effects O_3 on soybean plants by using EDU and found increased pod numbers (38-109%), seed numbers per pod (9%), seed weight per plant (47-170%), and only small increase in 1000-seed weight at two sites except for 15% increase at a deep rural site during experiment in 1996. During second experiment in 1997, the effects of EDU on pod number per plants (85-155%) seed numbers per pod (9-14%), and seed weight per plant (113-285%) were, in contrast, much large and significant. However, 1000-seed weight also varied from (16-33%) at two rural sites. The increase in yield was primarily due to increase number of pods per plant in EDU-treatment plant (400 ppm) along with higher number seeds per pod than in non-EDU-treated ones at O_3 concentrations of 40-48 ppb along with 14-27 ppb NO_2 during 1996 season, while in 1997 seasonal O_3 was 63-75ppb, and NO_2 was 26-34 ppb. These result showed that soybean cultivar (NARC-I) was extremely sensitive to O_3 pollution. Tiwari *et al*., (2005) working on wheat has also described that seed yield was increased by 19-21%, and 25-70% in two wheat cultivars; M-533 and M-234, respectively in EDU-treated plants than NEDU-treated. In another recent study, Agrawal *et al*., (2005) reported that in EDU-treated plants, number of pod and dry weight of pods was increased by 52% and 26%, respectively, while yield was increased by 32% in mungbean plant (*Vigna radiata*) in response to O_3 concentrations of >60 ppb. Results of above referred to literature confirmed that growth and yield losses in three cultivars of sesame in present study are due to higher ambient O_3 concentrations (>90 ppb) prevailing in the rural agricultural fields of Lahore as have been reported earlier (Wahid *et al*., 2001). All cultivars of sesame showed positive response to various concentrations of EDU, and their sensitivity trend may be summarized in the order as: S-17>Til-93>Ts-3. The mechanism of action of EDU in alleviation O_3 effects is suggested to be biochemical and biophysical (Lee & Bennett, 1982). EDU detoxifies O_3 in apoplastic region of the cell and does not act directly as an antioxidant. EDU helps in maintaining higher levels of cellular antioxidants associated with protection during O_3 stress (Lyons *et al*., 2000). The results on EDU-induced maintenance of higher levels of antioxidants and activities of protective enzymes in various plants during elevated levels of O_3 stress are however, inconsistent (Lee *et al*., 1997; Brunschon-Harti *et al*., 1995b; Agrawal *et al*., 2005). Present study depicted the relative effectiveness of various EDU-applications on sesame crop and demonstrated that lower concentrations of EDU (125 ppm and 250 ppm) were not successful in complete plant protection from O_3 damage, but instead, higher levels of EDU (375 and 500 ppm) proved to be quite successful in alleviating O_3 induced damage in all cultivars of sesame crop. At the current time, application of known concentrations of EDU to plants is an effective technique that can be used to ascertain the injurious effects of atmospheric pollutants (especially ozone) which otherwise go unnoticed.

Conclusions: The results of this experiment clearly demonstrated alarming effects of O_3 on the growth and productivity of three cultivars of sesame grown at a rural agricultural site of Lahore city of Pakistan. Present study confirmed that EDU can be successfully employed for assessing O_3 induced growth and yield losses in agricultural crops in ambient environment. However, its dose should be standardized to ascertain full effects on plants community. Because the protection provided by EDU may not be 100 %, the real impact of ozone at this site may be even larger. Any how, the results of this study provide clear support for the hypothesis that the impacts of ozone on the yield of agricultural crops are larger in rural areas than might be predicted, as pollutants may travel 50-1000 km from the point of origin (Bell & Cox 1975; Ashmore & Marshall, 1999; Wahid, 2003; Vingarzan, 2004), covering vast tracts in the direction of the prevailing atmospheric turbulence, and penetrate deep into rural areas (Wahid *et al*., 2001; Ashmore, 2005). Many workers (Bell & Treshow, 2002; Emberson *et al*., 2003; Zhao *et al*., 2011) have urged the need for such studies in Asian developing countries to establish the O_3 concentrations in rural areas, especially outside major cities and to determine their impact on agricultural crops.

Future research priorities: Application of EDU chemical to plants has proved a reliable technique as a soil drench in assessing the direct impacts of atmospheric pollutants on crop growth performance than that of open-top chambers (OTC) technique for ascertaining effects of atmospheric pollutants on plants. In developing countries, OTC are difficult to operate in the rural backgrounds due to security reasons in field, shortage of trained staff, and difficulty in electricity supply in remote rural areas. Data obtained from OTC is also difficult to interpret due chamber effects by modifying climatic conditions. Hence, EDU dose-response studies should be carried out in rural agricultural areas to establish critical levels of O_3 in predicting economic losses due to agronomic crops.

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