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ASSESSING THE IMPACT OF AMBIENT OZONE POLLUTION ON BIOCHEMICAL CHARACTERISTICS OF *VIGNA RADIATA* L. PLANTS BY USING ETHYLENEDIUREA

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ABSTRACT

Increased tropospheric O_3 concentrationismain factor affecting world agriculture production. In present investigations it has been investigated the affectivity of ethylene diurea (N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N-phenylurea; EDU) treated mungbean plants (*Vigna radiate* L.)against ambient ozone and to assess the effects of ambient ozone by atmospheric pollution tolerance index (APTI) on mungbean plants grown under field conditions and onsome biochemical parameters. During the experimental period, monthly mean O_3 concentration varied between 79.4 ppb and 105.2 ppb. Substantial reduction in mungbean crop productivity under ambient conditions in contrast to EDU and N-EDU-treated plants was recorded. Significantly goodresults by the application of EDU were seen in order to observe photosynthetic pigments and air pollution tolerance index (APTI). The study validated that EDU lightens the unfavorable O_3 effects on mungbean crop and EDU can be utilized as a very useful toolto investigate agricultural crop reduction due to higher O_3 concentration.

Key words: Tropospheric, EDU, Antiozonant, Mungbean, Pakistan.

INTRODUCTION

Pollution in air has become a main problem arising mostly from industrialization and urbanization during the last few decades. Air pollution is now considered as main environmental problem to crop yield in all areas urban, peri-urban and rural (Wahid *et al.*, 2001). Plants are often more sensitive to ambient air pollutants than other organisms because they are motionless, and are always visible to the natural environment. They reduce pollutants concentrations in the air through absorption, adsorption, purification, metabolization and gathering of pollutant compounds showing various types of foliar injuries on plant surfaces resulting in chlorophyll loss and reduced productivity (Wahid 2006a), but sometimes growthand yield losses occur without presence of visible injury symptoms (Wahid *et al*., 1995a,b; Maggs *et al*., 1995).

Several experimental protocols have been employed in gauging the impacts of air pollutants on different crops and among them, ethylenediurea or EDU (*N*-2-[2-{oxo-1-imidizolidimyl}ethyl]-*N1*-phenylurea) is widely used to suppress acute and chronic effects of air pollutants, inparticular O_3 (Carnahan *et al*., 1978) on a different types of plantspecies without mystifying effects of its own (Manning,2000; Agrawal and Agrawal, 2000): for instance, in potato (Eckardt and Pell 1996), wheat (Tiwari *et al*., 2005), soybean (Wahid *et al*., 2001), tobacco plants (Nakajima *et al*., 2002), beans (Tonneijck and Van Dijk, 2002) and mung bean(Agrawal *et al*., 2005).Determining the factors of resistance and vulnerability can be understood by biochemical responses of plants to air pollution (Seyyednejad *et al.,* 2011). Air pollution patience and sensitivity of plants varies with change in total chlorophyll, ascorbic acid, leaf pH and relative water content (Chouhan *et al.,* 2012).

Air pollution tolerance index (APTI) is natural quality of plants to face problem of air pollution stress, now in present days it is most important especially in industrial and non-industrial areas. Therefore, APTI of the plants needs checked properly especially of economically important plant species that are present in the polluted and non-polluted areas.Meteorological conditions in Pakistan are encouraging for the formation of ozone.In Pakistan, very few laboratories have equipment of O_3 monitoring, so EDU used as an alternate economical research tool. In the present study, aptness of EDU as soil drench to evaluate the effects of ambient ozoneby Atmospheric Pollution Tolerance Index (APTI) in a suburban area of Lahore on mungbean crop have elevated O_3 levels.

MATERIALS AND METHODS

Description of experimental site: The experiment was arranged at the Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore (31^{o}29-00-N, 74^{o}17-00-E), Pakistan, on a suburban site 20x14 m next to agricultural fields. The main road was 1500 m away from experimental site, industrial area was 7 km and main city center was about 7.5 km.

Metrological Data: To study the effects of metrological parameters on ozone formation and their concentration, data collected from Pakistan Metrological Department, Lahore. Different parameters like temperature, precipitation, relative humidity and sunshine are summarized in Figure-1.

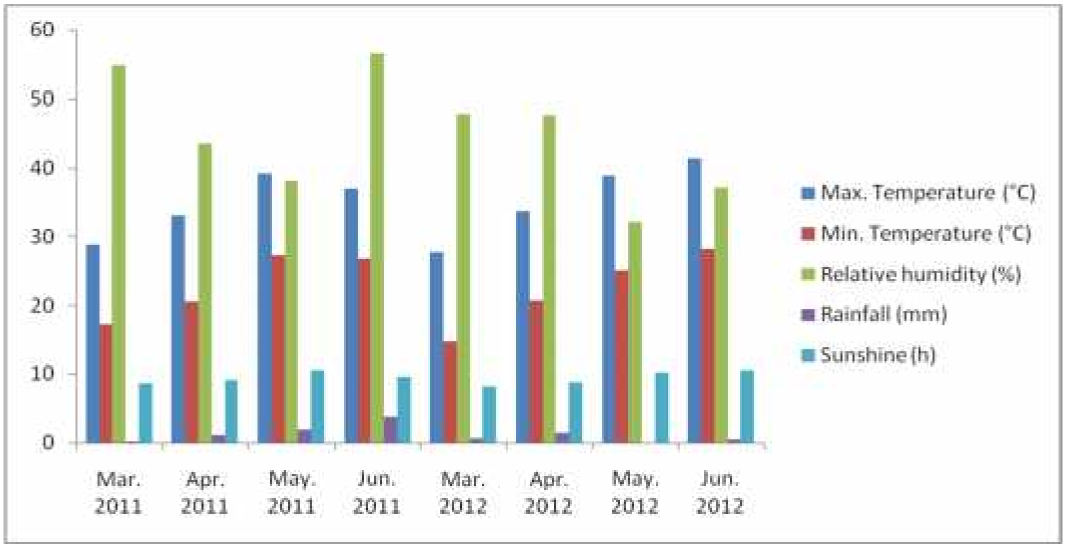


Fig: 1. Ambient Climate: monthly and seasonal summaries for temperature, light intensity and relative humidity values on any particular day of the month during two successive mungbean growth seasons 2011 and 2012.

Ambient ozone monitoring: The 400E model ozone monitor was used for ozone concentrations measurements between 9.00 to 17.00 h during the whole growth period of the plant at different stages.

Procurement of Plant materials: Two mungbean cultivars seeds (NIAB-2006 & AZRI-2006) were obtained from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan and Arid Zone Research Institute (AZRI), Bhakkar, Pakistan.

Raising of plants: In this study pots of size 36 cm diameter were used. The field soil was used during pot experimentation. This soil was mixed sieved composted farmyard manure with thoroughly ratio by volume 6:1. For germination of mungbean plants suitable moisture was maintained in these pots.One week old seedling of mungbean at weekly interval up to 90 days were treated with EDU solution (200, 300, 400 & 500 ppm) by Randomized Complete Block Design (RCBD). Deionized water was used for fresh preparation of EDU solution and 100 ml of EDU solution was applied as a soil drench to each mungbean plant. Control plants received only deionized water (100 ml plant^{-1}). Plants were subjected to same water supply both for EDU-treated and N - EDU-treated ones.

Crop Harvest: Five mungbean plants were selected at random per treatment from each replicate pot for biomass determination and growth analysis at 30 and 60 days after germination (DAG). Plant samples were analyzed for shoot and root lengths, number of leaves and leaf area.

Photosynthetic pigment: Fresh leaf sample were used for determination of Chl and Car by the method of Hiscox and Israelstam (1979).

Extraction: Fresh leafswere kept on a moist filter paper, washed these leafs with cold distilled water. Cut equal size of leaf discs from both side of the midrib for the determination of chlorophyll a, b and carotenoid content. 7 ml of dimethyl sulfoxide (DMSO) were taken in vial with 100 mg of the chopped leaf material in triplicates. These vials were then kept in an oven at 65°C for 1 h for complete filtering of the pigments.

Thereafter, after that volume make up to 10 ml with DMSO. After that immediately, measured chlorophyll content.

Estimation: Take2 ml of the extract from vial and then transferred to a cuvette to take absorbance at 480, 510, 645 and 663 nm using a spectrophotometer against DMSO as a blank. Optical densities values were used for determination of Chl a, Chl b, total Chl and Car contents by using the formula given by Maclachlan and Zalik (1963) for Chl a, Duxbury and Yentsch (1956) for Chl b, Arnon (1949) for total Chl and Barnes *et al.*, (1992) for Car contents:

$$Chl'a'(mg\ g^{-1}\ fr.wt.) = \frac{[12.3(oD_{663}) - 0.86\ (oD_{645})]}{(D \times 1000 \times W)} \times V$$

$$Chl'b'(mg\ g^{-1}\ fr.wt.) = \frac{[19.3(oD_{645}) - 3.60\ (oD_{663})]}{(D \times 1000 \times W)} \times V$$

$$Total\ 'Chl'(mg\ \ g^{-1}\ fr.wt.) = \frac{[20.2\ (oD_{645})+8.02\ (oD_{663})]}{(D\times1000\times W)}\times V$$

$$Carotenoids\ (mg\ \ g^{-1}\ fr.wt.) = \frac{[7.6\ (O.D_{480}) - 1.49\ (O.D_{510})]}{(D\ \times 1000\ \times W)}\times V$$

Where:
D: Distance travelled by the light path
W: Wt. of the leaf material
V: Volume of the extract
OD: Optical Density.

Determination of Air pollution tolerance index: APTI of experimental plants was calculated by the Singh and Rao (1983):

APTI = A (T+P)+R/10

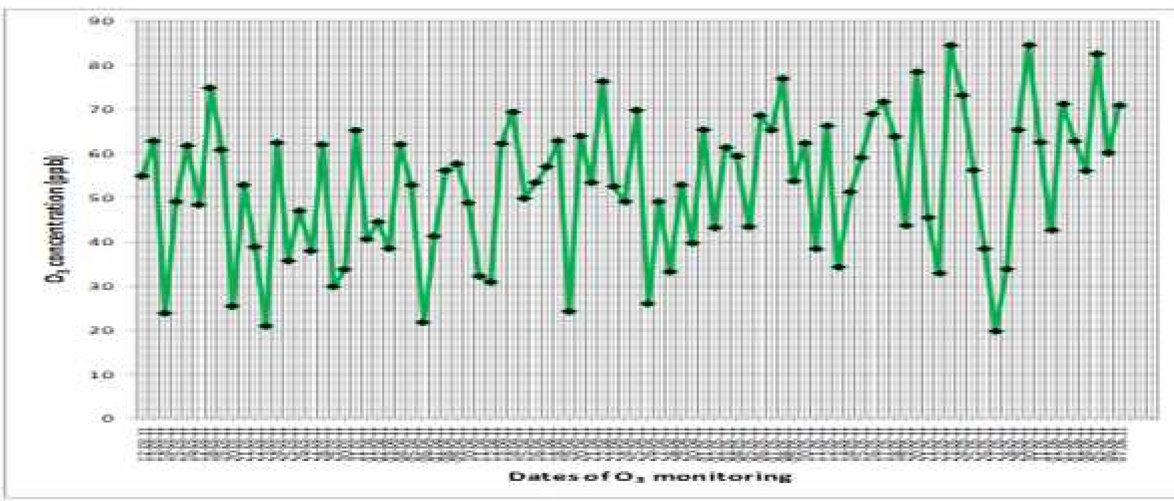
Where;
Ascorbic acid content of leaf (mg/g dry weight): A

Total chlorophyll content of leaf (mg/gdry weight):T

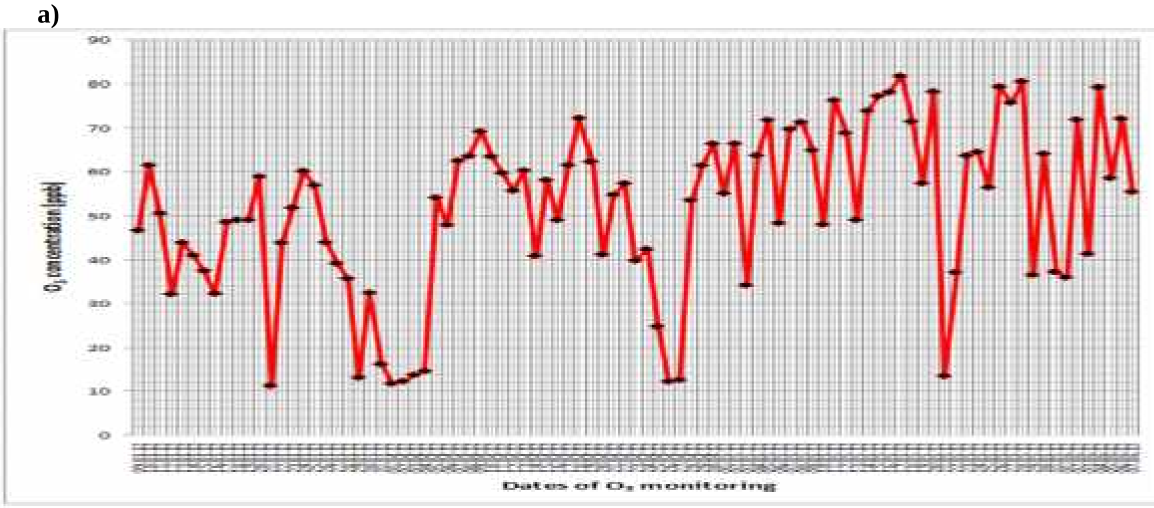
leaf extract pH: P
% relative water content of leaf: R

RESULTS AND DISCUSSION

Monthly mean O_3 concentration values on every day of a particular month for both the mungbean seasons are summarized in Fig 2. During 2011 mungbean season, monthly mean O_3 concentration in ambient air was much higher i.e., 101.3 ppb, 93.81 ppb and 79.4 ppb for the months of May, June and July respectively, while O_3 levels decreased for the months of March, 54.0 ppb and August, 65.5 ppb. Almost similar pattern of ozone behavior was noted in the second mungbean experimental season. Mean 8 h O_3 concentration during 2012 mungbean season 105.2 ppb, 95.5 ppb and 93.5 ppb for the months of May, June and July was relatively higher than that of March, 64.3 ppb and August 65.3 ppb respectively (Figure-2 a & b).



a)



b)

Fig 2. Variation in ozone concentration during the experimentations (a & b).

Changes in plant pigments of two cultivars of mungbean (NIAB-06 & AZRI-06) after 60 days of growth during mungbean growth season 2011 and 2012 at experimental site was observed by applying different concentration of ethylenediurea (EDU). The chl a, chl b, carotenoids, total chlorophyll, relative water content, ascorbic acid, pH and air pollution tolerance index (APTI) data areshown in Table 1.Chl a, chl b, carotenoids and total chlorophyll content were found to be high mungbean plants treated with 400-EDU in both varieties after two growth seasons, while NIAB-06 show higher total chlorophyll content as compared to AZRI-06. Photosynthetic reactions take place due to Chlorophyll a, Chlorophyll b and carotenoid. Photosynthetic activity disturbed badly due to significant role of different pollutants to block the photosynthesis that may show in the form of plantweakening of plant pigments in the leaves of crop plants (Chauhan and Joshi, 2008).

Table: 1. Changes in some biochemical characteristics of two cultivars of mungbean after 60 days of growth during mungbean growth season 2011 and 2012 at experimental site.

Cultivar	Treatment	Biochemical Characteristics (mg / g fresh green tissue)							
		Chl a	Chl b	Total Chl	Carotenoid	Ascorbic acid	RWC	Leaf pH	APTI
		Growth season – 2011							
NIAB - 2006	NEDU	1.54de	0.27ef	1.03f	0.42c	0.92c	72.98d	6.49a	7.99c
	EDU-200 ppm	1.73bcd	0.35de	1.42e	0.56c	1.00bc	74.41cd	6.11bcd	8.19c
	EDU-300 ppm	2.00bc	0.42cd	1.63cd	0.72b	1.11bc	80.36abc	6.03cd	8.89b
	EDU-400 ppm	2.61a	0.61ab	2.86a	1.02a	1.62a	83.84a	5.96d	9.81a
	EDU-500 ppm	2.03b	0.52bc	1.76c	0.75b	1.06bc	77.14bcd	6.24b	8.56bc
	L.S.D.	**0.30**	**0.10**	**0.15**	**0.14**	**0.20**	**5.67**	**0.17**	**0.60**
AZRI – 2006	NEDU	1.35e	0.23f	1.04f	0.44c	0.98bc	72.54d	6.62a	8.00c
	EDU-200 ppm	1.68cde	0.36de	1.40e	0.54c	1.07bc	73.27d	6.19bc	8.14c
	EDU-300 ppm	1.97bc	0.45cd	1.48de	0.74b	1.11bc	81.13ab	6.13bcd	8.96b
	EDU-400 ppm	1.94bc	0.65a	2.09b	1.08a	1.59a	84.55a	6.02cd	9.75a
	EDU-500 ppm	1.89bc	0.48c	1.70c	0.79b	1.19b	76.82bcd	6.30b	8.63bc
	L.S.D.	**0.30**	**0.10**	**0.15**	**0.14**	**0.20**	**5.67**	**0.17**	**0.60**
		Growth season – 2012							
NIAB - 2006	NEDU	1.24bc	0.29e	1.01f	0.39cd	0.98c	70.78df	6.12ab	7.91a
	EDU-200 ppm	1.53cd	0.30ab	1.49d	0.59f	1.09abc	73.91cde	6.19bd	8.10b
	EDU-300 ppm	2.05c	0.41cd	1.73d	0.70b	1.07c	79.16ac	6.00cd	8.59b
	EDU-400 ppm	2.75ad	0.68b	2.81a	1.22ad	1.60a	82.59ae	5.77df	9.72a
	EDU-500 ppm	2.13bd	0.50b	1.49df	0.71ab	1.21ac	77.01bd	6.31b	8.36ac
	L.S.D.	**0.28**	**0.12**	**0.18**	**0.11**	**0.21**	**5.42**	**0.15**	**0.62**
AZRI – 2006	NEDU	1.44ae	0.29cf	1.01b	0.41ac	0.80bc	71.44ad	6.42cd	8.04c
	EDU-200 ppm	1.59de	0.39e	1.44ae	0.51c	1.03ac	73.17cd	6.21bc	8.15ac
	EDU-300 ppm	1.87ac	0.43cd	1.38ade	0.71ab	1.15bc	81.08ad	6.02bd	8.88a
	EDU-400 ppm	1.96c	0.61ab	2.19bc	1.03ab	1.49a	84.65ac	6.12acd	9.59d
	EDU-500 ppm	1.65abd	0.42d	1.64bc	0.78b	1.09ab	76.61bd	6.41cb	8.61c
	L.S.D.	**0.28**	**0.12**	**0.18**	**0.11**	**0.21**	**5.42**	**0.15**	**0.62**

Treatment mean followed by different letters in each column within cultivars are significantly different from one another at P= 0.05 according to Duncan's multiple range test; L.S.D.: Least Significant Difference at P= 0.05; NEDU: Non EDU
RWC: Relative Water Contents
APTI: Atmospheric Pollution Tolerance Index

Chlorophyll content was reduced by acidic pollutants formation like SO_2 by chlorophyll acidification (Rao and LeBlanc, 1966). The carotenoids content of NIAB-06was found to be high and it was low for AZRI-06. In some plants carotenoid contents were found to decrease in replay to SO_2 (Nandi, 1984).

Ascorbic acid content of varieties with different EDU doses between 0.92 to 1.62 mg/g in NIAB-06 and between 0.80 to 1.59 mg/g in AZRI-06. A resistance to pollution and ascorbic acid contents have relationship present in plants (Varshney and Varshney, 1984). The natural detoxicant is ascorbic acid, which may prevent the plant tissues from damaged caused by air pollutants (Singh *et al.*, 1991) and favors pollution tolerance of this substance in plants (Lee *et al.*, 1984).

The pH observed in mungbean varieties NIAB-06 and AZRI-06 have more alkaline when applied 200, 300, 500 ppm and NEDU while more acidic in nature when 400 ppm EDU concentration was applied in both growing seasons. The results reported during present investigation are soundly supported by the earlier many researchers that the photosynthetic efficacy of crop plant strongly depends upon the pH of plant leaf. The pH value of NIAB-06 with 400 ppm dose was found to be 5.96 and 5.77 in 2011-12 and similar results were reported by Krishnaveni *et al.* (2012). Relative water content of different plantsconsequential in early senescence of leavesassociated with permeability of protoplasm in plant cells which causes loss of water and dissolved nutrients (Masuch *et al.,* 1988). The relative water content mungbean plant treated with 400 ppm EDU in both varieties (NIAB-06 & AZRI-06) was set up to be high.

The APTI are very effective and important for selection ofcrop plants in order to check their susceptibility against any environmental stress like air pollution. This is a simple and easy method to adopt on different types of field conditions and reduced the use of costly environmental monitoring equipment's. The APTI was found to be above 9 in NIAB-06 and AZRI-06 by applying 400 ppm EDU concentration. While lowest values less than 8 were observed in mungbean plants with N-EDU. Generally, crop plants with low APTI values were sensitive to air pollutants. The APTI value estimated using the four biochemical parameters in plant leaves namely RWC, total chlorophyll content, pH and ascorbic acid value can be used as a predictor of air quality. Plants having higher APTI index value are air pollution tolerant while others with less index value show low tolerance (Singh and Rao, 1983). According to different geographical area conditions specific plants behave differently depends on environmental conditions (Raza *et al.,* 1985).

Conclusion: The results of present investigation suggested that ambient O_3have unfavorableeffects on various physiological and biochemical characteristics on agricultural crop (mungbean) in and around sub-urban agricultural areas.Treatment of 400 ppm EDU was found be an efficient dose to protect the mungbean crop from ambient O_3.

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