Impact of ambient ozone pollution on yield attributes of *Pisum sativum* L. plants by using ethylenediurea

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Abstract

Ozone (O_3) is a widespread secondary photochemical air pollutant, which occurs naturally at ground level in low concentration and is regarded as the most significant air toxicant throughout the world. The current research was conducted to evaluate the potential effect of ethylenediurea (EDU) on the yield of two pea cultivars (Meteor and Sprinter). *Pisum sativum* L. plants were exposed to O_3 stress using two methods, namely soil drench and foliar spray application during two consecutive years. The monthly mean concentration of O_3 during the experimental period varied between 29.4 and 60.7 ppb. Ozone stress reduced yield characteristics of *P. sativum.* Application of EDU enhanced yield parameters (number of pods, pod weight, number of seeds, and straw weight) of *P. sativum*. Application of EDU concentration of 450 ppm via soil drenching significantly enhanced yield parameters of *P. sativum.* Current research reveals that EDU ameliorates the deleterious effects of O_3 on pea crop and it can be used as an effective tool to investigate agricultural crop reductions due to higher O_3 concentrations.

Keywords: tropospheric ozone, EDU, pea, yield, harvest index, soil drench, foliar spray

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Introduction

Now-a-days, a very well-known and arising problem is the tropospheric ozone suppressing the productivity of plants on a wider scale (Oksanen et al., 2013). The magnitude and extent of the ozone produce high levels of risks to the agricultural crops resulting in high levels of yield losses up to 20% in the case of the most important cash crops experienced due to elevated levels of ozone (Emberson et al., 2009). The current environmental levels of ozone have produced damaging effects on crops causing the overall global economic damage of 14 to 26 billion US Dollars (Mills and Harmens, 2011); (Avnery et al., 2013).

It is the need of hour to resolve the issue of ozone damaging yield of plants. The transboundary nature of ozone produces complication in controlling the damaging and toxic effects of ozone by any type of control methods (Agathokleous et al., 2016a). Therefore, there is a need to develop biotechnologically modified crops and their cultivars in order to reduce yield loses because of the ozone tolerant and resistant crops produced. In this aspect, various substances and compounds have been employed in order to test their phyto-protective efficiency. These compounds show variable characteristics under

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testing process and at the same time they are not considered as efficient substances (Agathokleous et al., 2016b); (Singh et al., 2015).

Pea (*Pisum sativum* L.), the leguminous crop belonging to the Leguminoseae family, is highly rich in proteins specifically the proteins containing lysine amino acid (Nawab et al., 2008). Pea is a vegetable high in nutrition and one of the most cultivated crop in the whole world. The total amount of trading in pulses is about 40% of the pea vegetable and it is recognized as the cash crop, highly exported in the world (Ali et al., 2002). It is the most extensively grown crop in the world, ranked the 2nd in terms of cultivation after soybean (Smýkal et al., 2012). In case of grains production, it comes out to be at fourth number producing 441.53 thousand tons after soybean, groundnut, and beans at the land area of about 528.71 thousand hectares in the whole world (Ashraf et al., 2011); (Khan et al., 2013).

In the above scenario, the present experiment was conducted to assess the effects of environmental ozone stress on yield attributes by selecting two cultivars of pea under various EDU applications by two different methods.

Materials and Methods

Description of experimental site

The experiment was conducted in wire fenced area of the Botanical Garden at Department of Botany, University of Punjab, Quaid-e-Azam Campus, Lahore (31°.497185-N, 74°.298172-E), Pakistan. It was 1 km far from the nearest main road, 7.5 km from the nearest industry, and about 7 km from the city center.

Meteorological parameters

To study the effects of metrological parameters on ozone formation and their concentration, various data concerned with meteorological parameters including temperature (T max and T min ℃), relative humidity (RH max and RH min %), total rainfall (mm), as well as day and night span of the experimental site were collected from Pakistan Metrological Department, Lahore.

The means of ambient temperature, light intensity, and relative humidity with maximum and minimum values during the crop growing months of pea cultivars of both growing seasons are shown in Fig. (I). November was the hottest month in both pea seasons with the mean temperature of 18.6 and 20.5 ℃. December was the rainiest month with maximum sunshine hours in the first growth season while January was the rainiest month with least sunshine hours in the second growth season.

Ambient ozone monitoring

Concentration of ambient ozone present in the experimental site was recorded on daily basis for 12 hours (6:00 AM - 18:00 PM) using a UV absorption photometric O_3 analyzer (Horiba, APOA-370, Ozone Monitor, Japan). Monitoring of O_3 was done during the entire experimental period.

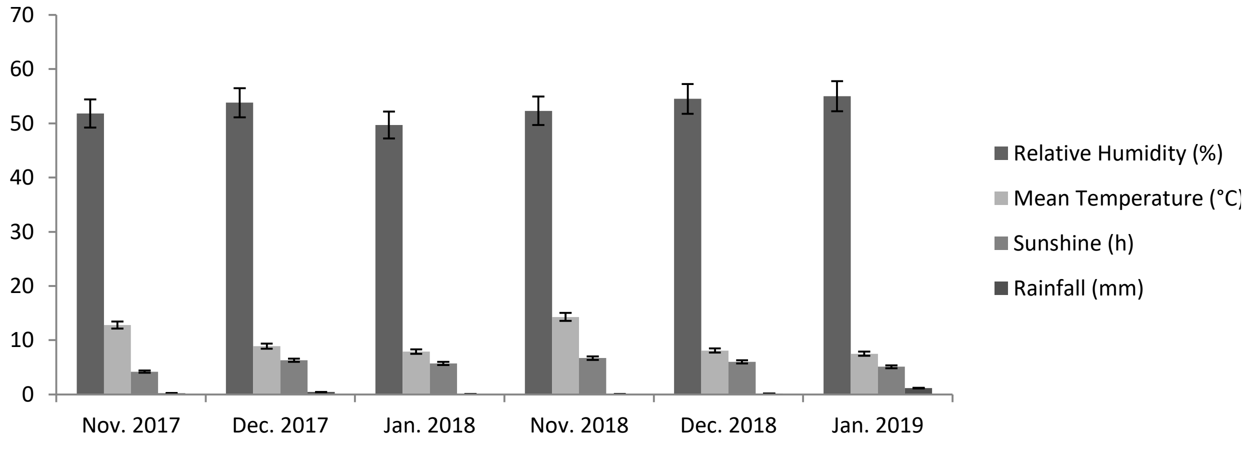

Fig. I. Ambient climate: monthly means of relative humidity (%), temperature (°C), sunshine hours and rainfall (mm) during two successive pea growth seasons 2017-18 and 2018-19.

Procurement of plant material

Seeds of certified pea (*Pisum sativum* L.) cultivars, Meteor and Sprinter, were obtained from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Viable and healthy seeds were separated from the desiccated one and used for further experimental work. Selected seeds were surface-sterilized using a mixture of 70% ethanol and H_2O_2 (1:1 by v:v) and washed with distilled water several times. Then, seeds were soaked in a beaker filled with distilled water for 24 hours.

Plants raising

Clay pots with a diameter of 36 cm were employed in order to grow the pea varieties. Pots were prepared using unsterilized loamy soil along with sand (medium-coarse, mined and sieved through 2 mm mesh size) in 3:1 ratio. The ratio of mixing soil with farmyard manure was 6:1. Four pre-soaked seeds were sown in each pot. Number of seedling was kept as one per pot specifically in the middle of pot after complete germination. The seedlings were provided treatments of EDU (150, 300, 450, and 600 ppm) solutions after a gap of every 10 days for up to 80 days (final harvest) by RCBD (Randomized Complete Block Design). Solutions of EDU were applied using two methods, one as soil drench (100 ml $plant^{-1}$) and the other as foliar spray (10 ml $plant^{-1}$) to the two sets of plants, respectively. The plants were treated early in the morning at 8:30 AM on a ten-day interval basis. Control plants were not treated with EDU but received only distilled water as 100 ml per plant. Both plants treated and not treated with EDU were watered with the tap.

Plant sampling

Random sampling of both pea cultivars was undertaken in three replicates from each treatment at 80 DAS for taking up the final yield.

Yield attributes

After the complete germination and establishing vegetative growth, plants were harvested in order to note the yield components, e.g. Number of pods/ plant, average pod size/ plant (cm), average weight of pod/ plant (g), seed weight/ pod (g), average number of seeds/ pod, number of seeds/ plant, yield of seeds/ plant (g), 100 seed weight (g), average pod straw weight (g), and weight of straw/ plant (g).

Harvest Index

The harvest index of the pea plants was determined by using the formula as given below:

$$\text{Harvest Index} = \frac{\text{Grain dry mass}}{\text{Total aerial biomass}}$$

Statistical Analysis

The descriptive statistical analysis was performed for calculation of standard deviation and the expression of data was done in mean ± S. E. of 3 replicates each. The statistical analyses were performed by applying ANOVA and DMRT (Duncan's Multiple Range Test) by SPSS (Version 20) in the computer system.

Results

Monitoring of ozone

The estimated range for the ambient ozone during the first growth year (2017-18) was 29.4 to 57.3 ppb with mean concentration of ambient ozone as 44.4 ppb. During the second growth year of 2018-19, the mean estimated concentration for ozone was 46.4 ppb with the range 32.5 - 60.7 ppb (Fig. II). The increased ambient levels of ozone in the city of Lahore were estimated to be due to increase in temperature day-by-day, long, shiny, sunny days, less level of winds along with the high emissions of precursors of ozone-promoting substances and mostly highlighted in the backgrounds of the rural areas. Monitored air quality data showed up the lower levels of ozone concentrations along with the AOT40 value during the January due to lower temperature ranges and less shiny days in contrast to the month of February. The maximum value of ozone was

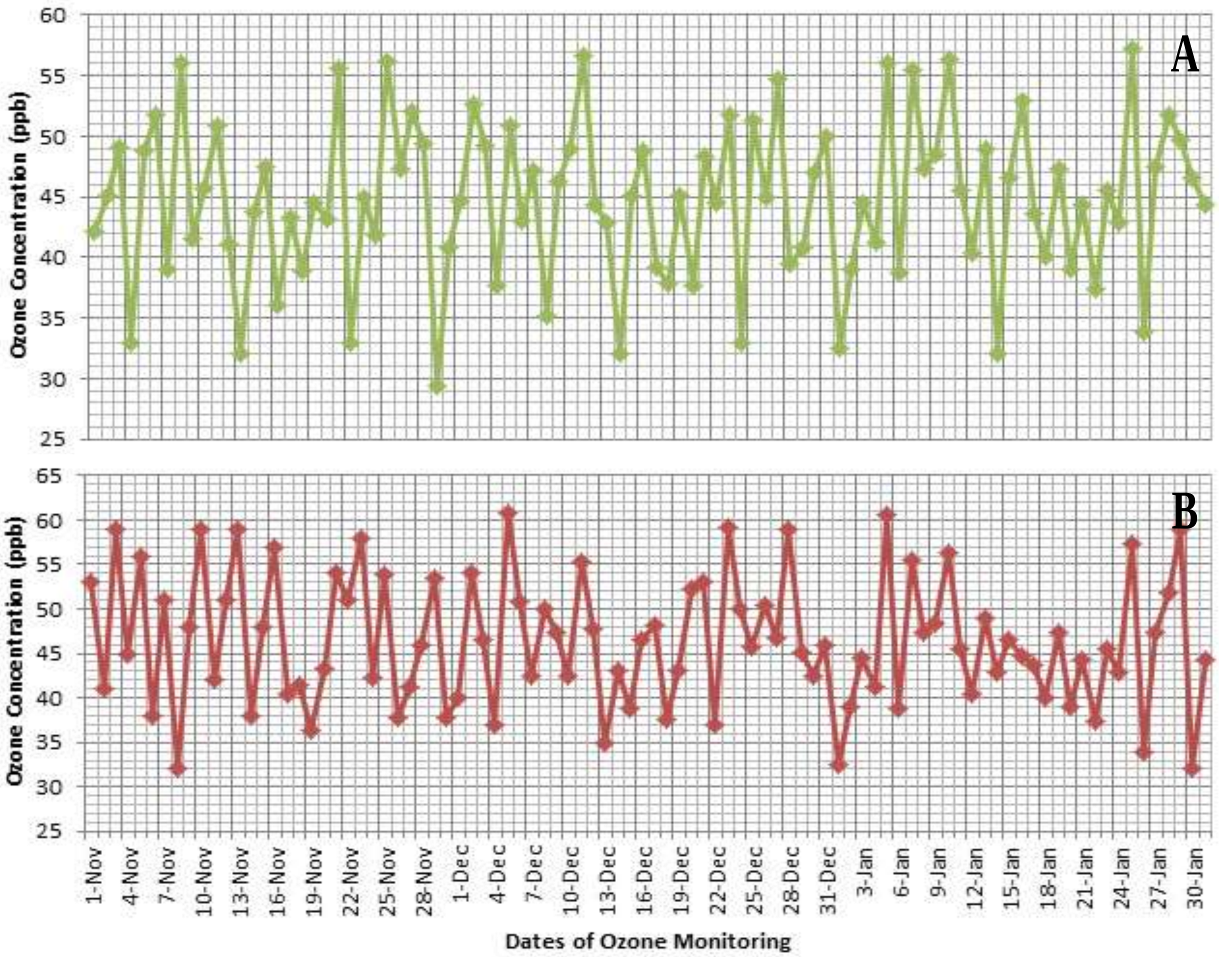

Fig. II. Variations in ozone concentrations during the experiment years (A) 2017-18 (B) 2018-19

recorded as 78 ppb at the harvest of crop during the January.

Yield attributes

In the recent experimental study, various concentrations of EDU (150, 300, 450, and 500 ppm) were applied on two cultivars of pea (Meteor and Sprinter) by using two different methods of application, i.e. soil drench and foliar spray, to take into account the ameliorative results of EDU on the yield of plants against the ambient ozone stress. The yield and yield components effects were highly elevated due to the application of EDU, when compared with control in both cultivars of pea (Meteor and Sprinter) in the two continuous seasons of growth. The components of yield showed significant increase in both cultivars provided with various EDU concentrations applied as soil drench and foliar spray. The maximum increase in the number of pods/plant was observed in EDU-450 applied as soil drench (86.14% and 78.64%) in Meteor and Sprinter, respectively during growth season 2017-18 (Fig. III). Similar trend in the percentage of increase in the number of pods/plant (95.36 % and 78.64 %) was observed in Meteor and Sprinter, respectively during the second growth season 2018-19. Average pod size and pod weight per plant also increased significantly in Meteor cultivar with maximum increase in 450 ppm treatment of EDU applied as soil drench (25.19% and 41.29%) while in foliar spray application (23.37% and 36.83%) increases were observed during 2017-18. Similarly, in Sprinter cultivar, the maximum increases were found in 450 ppm treatment, i.e. 12.66% and 53.13% in soil drench application while the values were 8.85% and 45.35% in foliar spray application. Same trend of increase was followed in pea plants during growth season 2018-19 (Fig. IV).

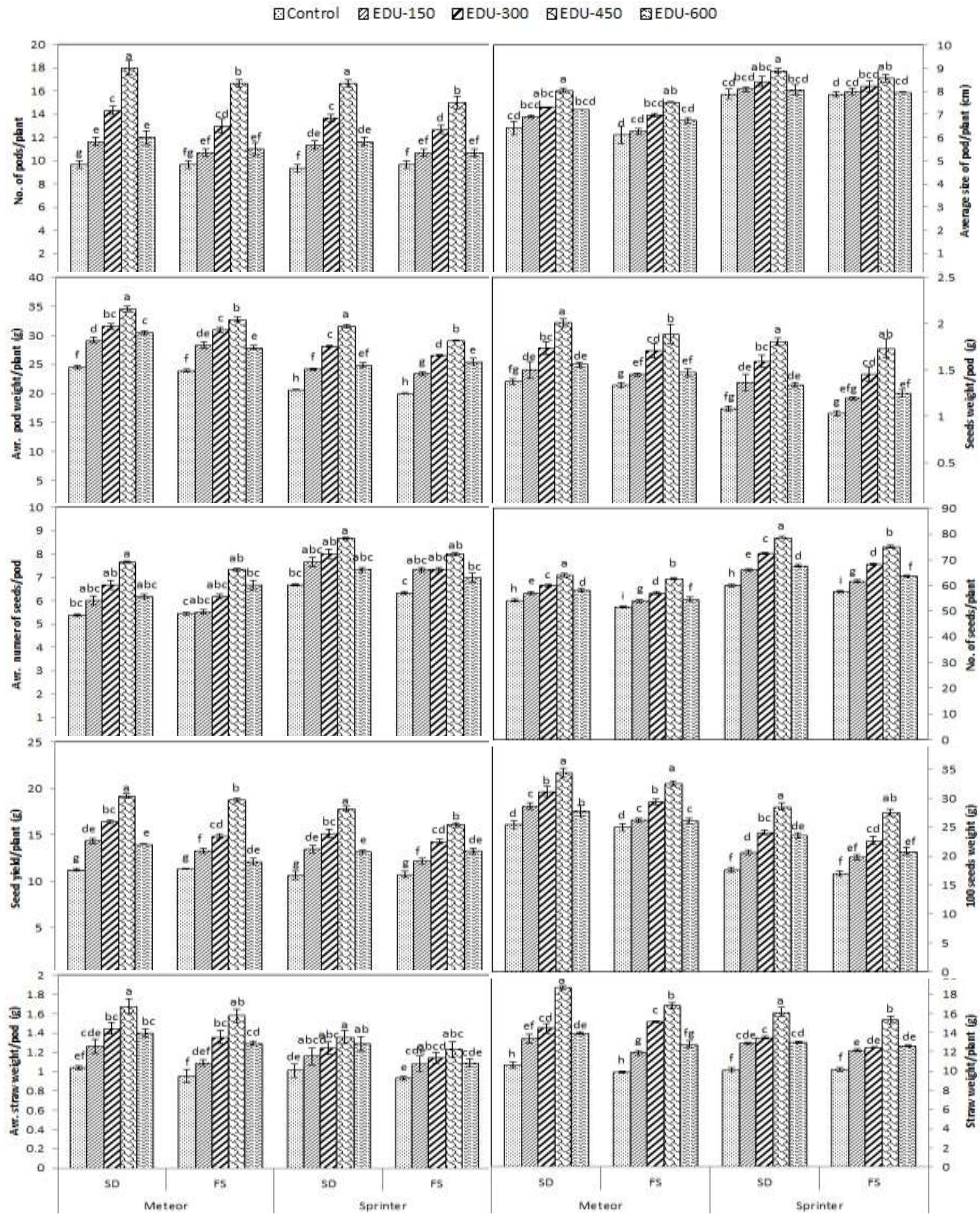

Fig. III. Impact of ambient ozone pollution on yield and yield components of two pea cultivars at 80 DAS during growth seasons 2017-18

The weight of seeds in Meteor cultivar increased significantly (8.66%, 26.03%, 45.61%, and 12.95%) when applied with 150, 300, 450, and 600 ppm treatments of EDU as soil drench, respectively. A similar trend was followed in foliar spray application of EDU 150, 300, 450, and 600 ppm treatments with the magnitude 8.26%, 27.64%, 40.95% and 9.74%, respectively as compared with

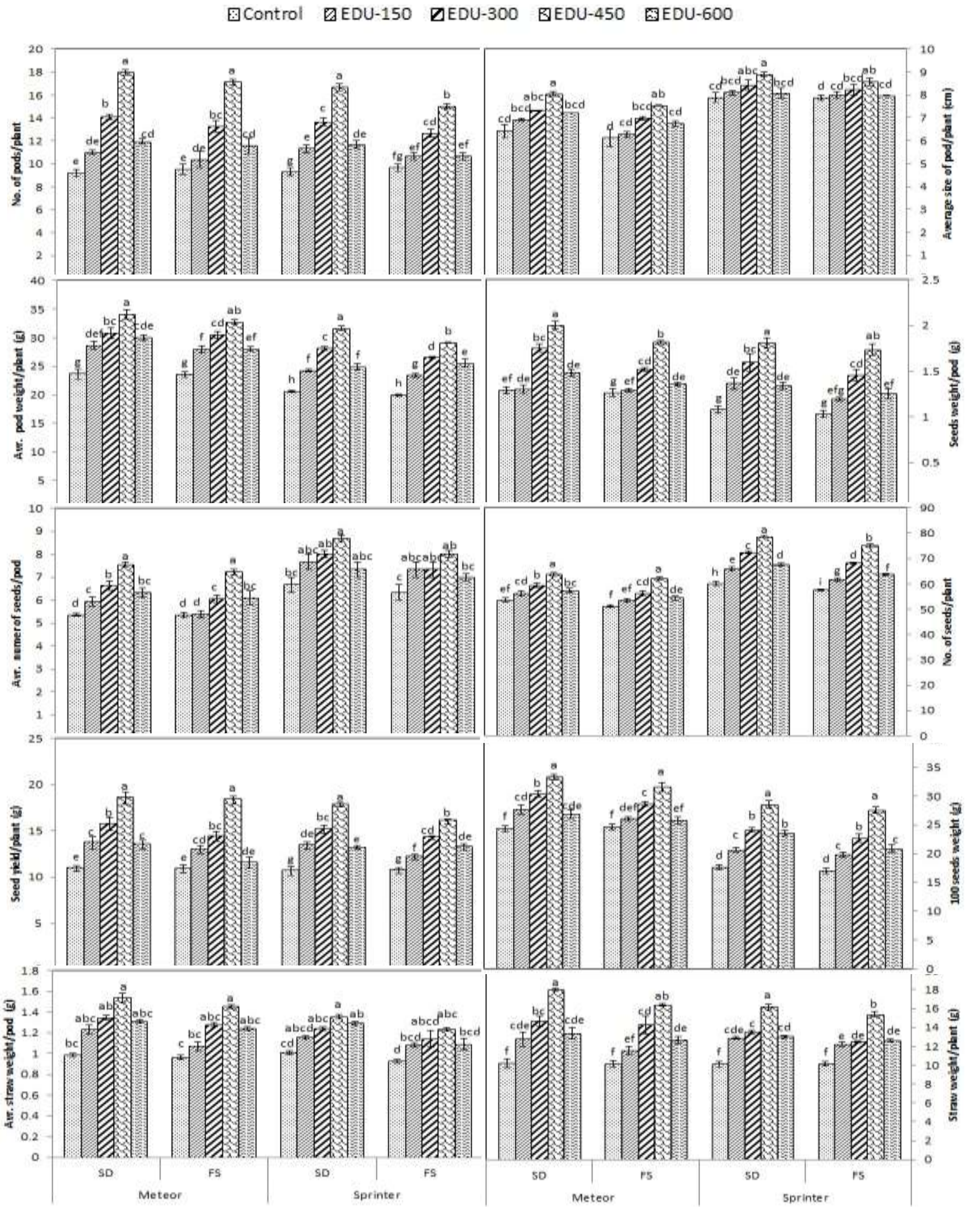

Fig. IV. impact of ambient ozone pollution on yield and yield components of two pea cultivars at 80 DAS during growth seasons 2018-19.

control. In cultivar Sprinter, the maximum increase in the weight of seeds was observed under EDU-450 treatment in both soil drench and foliar spray applications, i.e. 67.28% and 68.16%, respectively followed by EDU-300 (47.84% and 41.08% in soil drench and foliar spray, respectively). The minimum increase was recorded in EDU-150 plants in case of both methods of application in both pea cultivars.

There was a direct relation among yield parameters of Meteor and Sprinter varieties with EDU concentrations producing increased yield at increasing EDU concentrations. The best yield was obtained by treating plants with 450 ppm EDU concentrations. On the other hand, 600 ppm EDU concentrations produced decreased results of yield in both growth seasons. Varying concentrations of EDU allowed the sesame cultivars' increased pod weight, seed number and seed weight/ plant.

Maximum increment was observed for average pod straw weight and weight of straw per plant in EDU-450 treatment in foliar spray (66.21%) and soil drench method (76.15%), respectively during the first growth season 2017-18 while minimum increment was found in soil drench treatment of EDU-150 ppm (14.59 %) in Sprinter for average straw weight of pod and EDU-150 ppm of foliar spray method (20.19 %) for straw weight/plant.

Harvest Index

EDU applications significantly increased the harvest index of Meteor and Sprinter cultivars of pea and by increasing the EDU concentration, there was an increase in harvest index as well. The increase was more pronounced in 450 ppm concentration of EDU applied as soil drench in both cultivars during both growth seasons. Harvest index was significantly high in Meteor cultivar as compared with Sprinter during growth season 2017-18 and 2018-19. The harvest index in Meteor cultivar increased significantly (7.4, 25.2, 54.2, and 14.6%) when applied with 150, 300, 450, and 600 ppm treatments of EDU as soil drench, respectively. A similar trend was followed in Sprinter cultivar with soil drench application of EDU 150, 300, 450, and 600 ppm treatments with the magnitude 3.1, 9.6, 31.8, and 2.1% respectively (Fig. V). During season 2018-19, in cultivar Meteor the maximum percentage of increase in harvest index was observed in EDU-450 treatment (59.1%) followed by EDU-300 (28 %), EDU-600 (11.6%), and EDU-150 (6%). The minimum harvest index was recorded for plants treated with EDU-150 in case of both cultivars of

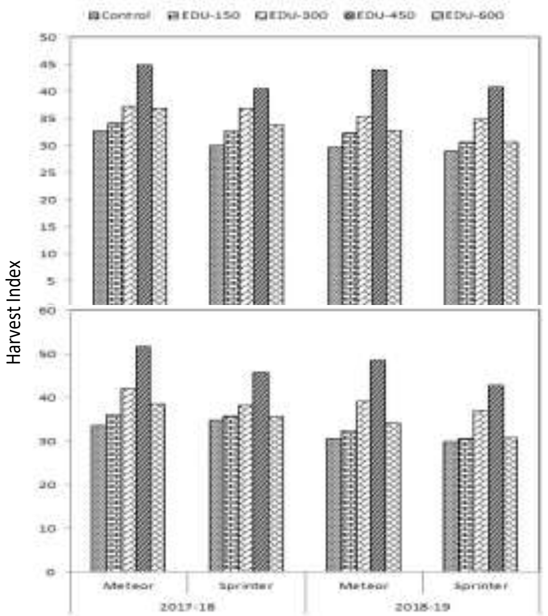

Fig. V. Impact of ambient ozone pollution on harvest index (%) of two pea cultivars (viz., Meteor and Sprinter) with EDU treatments applied as Soil Drench (A) and Foliar Spray (B) during two growth seasons

pea in both growing seasons. The same trend was followed in foliar spray application of various EDU concentrations on two pea cultivars during both growth seasons.

Discussion

The present study demonstrated clear effects of EDU on seeds weight per pod during the growth season 2017-18. Wahid et al. (2001)evaluated the soybean plants for O_3 effects by application of EDU through conducting an experiment at three different sites as rural, urban, and roadside rural areas, finding an increment in the number of pods (38-109%), seed number/ plant (9%), and seed weight/ plant (47-170) along with a small increment in 1000 seed weight during 1996 experimentation on two different sites while 15% increment was found in case of deep rural site. The results of growth season 1996 with experiment conditions of O_3 (40-48 ppb), NO_2 (14-27 ppb) demonstrated an increased yield due to increased pod number per plant with the results of high seed number per pod by 400 ppm-EDU treatments in comparison with the non-treated plants.

A direct relation was found in the study between yield parameters of Meteor and Sprinter varieties and EDU concentrations, producing increased yield at increasing EDU concentrations. According to Wahid et al.(2012) , the best concentrations of EDU in the land conditions of Pakistan were 375 ppm and 500 ppm. In comparison with NEDU, the pod weight increment by EDU treatments for *Phaseolus vulgaris* was recorded as 31-65% (Brunschön-Harti et al., 1995), 14% (Vandermeiren et al., 1995), 20% (Tonneijck and Van Dijk, 1997), and 52% (Astorino et al., 1995). Results for yield parameters of different crops by application of EDU concentrations were also recorded by Feng et al. (2010). EDU treatments allowed 50% increment in grain number and about 82% increment in weight of grain showing positive results of EDU (Baqasi et al., 2018).

Significant maximum increment in 100 seeds weight was observed in Sprinter cultivar in EDU-450 treatment of foliar spray method (62.82%) followed by EDU-450 treatment of soil drench method (62.06%) while minimum increase was found in EDU-600 treatment of foliar spray method (4.40 %) in Meteor. In the experiment of Ashrafuzzaman et al.(2017)the positive effects of foliar EDU treatments enhanced 8% of panicle number and 14% of grain yield in rice plants along with various increments in morphological parameters. Singh et al. (2015)explained about the increased yield effects on various crop plants due to the EDU effects, more specifically rice crop. EDU-450 ppm allowed a significant increment in the weight of pod/ plant of *Phaseolus vulgaris* in genotypes of S156 and DDW by 55% and 46%, respectively over control. Moreover, 56% of seed weight increased in S156 genotype (Yuan et al., 2015). About 33-43% of more increase in seed yield was recorded for various sesame varieties at higher concentration effects of EDU as compared with the un-treated plants. Hence, nominating the EDU for decreasing environmental pollution induced yield loses (Wahid et al., 2012).

Ozone has been highlighted as the major contributor in air pollution. It has also been recognized as the most damaging air pollutant due to its damaging effects upon vegetation. With respect to the issues of air quality, its estimated concentration in a year is about 0.1 to 1 ppb (Coyle et al., 2003). Ozone production is highly promoted during the summer and spring by photochemical reactions under the sun. So, in this regard, the higher concentrations of ozone were highly promoted in the presence of long days and higher temperatures. The precursors of ozone have been added up in the environment form the emissions of vehicle and industrial exhausts. The mean values of ozone for the Northern Hemisphere upon mid-latitudes were estimated to be increased by 20-45 ppb which is twice the concentration estimated from the previous century (Vingarzan, 2004). The future predictions about the concentrations of ozone are a global rise about 20 to 25% by the year 2050 and the same predictions have been more alarming for India and South Asia as even higher in the year 2020 (Van Dingenen et al., 2009).

Tiwari et al.(2005)compared plants treated with EDU and NEDU, demonstrating that the wheat cultivars M-533 and M-234 had increased yield of 19-21% and 25-70%, respectively. In the present investigation, seed yield was also enhanced significantly in both cultivars with maximum increase of 70.70 % being observed in Meteor cultivar in EDU-450 treatment of soil drench method while minimum increase of 6.85 % was found in EDU-600 treatment of foliar spray method. Agrawal et al.(2005)reported increases in the number of pods, dry weight of pods, and yield by 52%, 26%, and 32%, respectively because of EDU in case of *Vigna radiate* (mung bean) plants under effect of >60 ppb concentration of ozone (O_3). Feng et al. (2010) conducted a meta-analysis of EDU, showing increased effects of EDU with 10% increase in the yield of beans and 3.8% increase in the weight of seeds. The comparison between the effects of sensitive and tolerant genotypes (S156 and DDW) of snap beans demonstrated that only 50% increase in the yield of seed was observed for sensitive genotypes (Yuan et al., 2015).

Wahid et al.(2012)reported the increment in several parameters of yield due to the shielding effect on three varieties of sesame under EDU treatments. As compared to NEDU, the application of EDU (375 and 500 ppm EDU) allowed the pod number/ plant to increase in all cultivars of sesame. Green mature pods of *Phaseolus vulgaris* were enhanced by the application of EDU in studies of Tonneijck and Van Dijk (2002).

Conclusion

Ozone stress reduced yield parameters of *P. sativum*. The current research revealed that 45 ppm of EDU application in soil proved significant in alleviation of O_3 stress in two varieties of *P. sativum*. Current research can be taken as reference to enhance the yield of other horticultural crops.

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