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Role of Ethylene Diurea (Edu) on Assessing Impacts of Ozone Phytotoxicity on Growth And Productivity of Two Wheat Cultivars *Triticum Aestivum*

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ABSTRACT

Tropospheric ozone (O3) is a significant air pollutant known to have adverse effects on crops productivity and plant health, and it is widely recognized as a phytotoxic atmospheric pollutant, which results in reduced photosynthesis, growth inhibition, accelerating leaf senescence, as well as yield losses in many plant species. Wheat *Triticum aestivum* is considered one of the sensitive staple crops to air pollution particularly by tropospheric ozone. Thus, the purpose of this study is to examine how EDU contributes to the evaluation of the effects of ozone phytotoxicity on the growth and yield of two wheat cultivars, Triticum aestivum vars. Ceyhan-99 and Adana-99. Three levels of ozone were applied during daylight hours: ambient ozone concentration (control), 50 ppb and 60 ppb, in addition to three levels of ethylene diurea (EDU) control, 200 ppm and 300 ppm during anthesis stage. The findings show that tropospheric ozone has a negative impact on both wheat varieties as vegetative and productive traits., on the other hand foliar EDU spray act as a protective agent against ozone-induced damage in various wheat plant characteristics which studied during the research.

Key words: EDU, Growth, Ozone,Tropospheric,Wheat,Yield

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INTRODUCTION

One major air pollutant that is known to negatively impact plant health and crop productivity is tropospheric ozone O_3. It can lead to a phenomenon called ozone phytotoxicity, which results in reduced photosynthesis, growth inhibition, an indirect effect of accelerated leaf senescence and as well as yield losses in many plant species, including wheat *Triticum aestivum* (Batty and Ashmore, 2003). Numerous crop species, including wheat, rice, soybeans, and cotton, have been shown to suffer drops in yield when exposed to elevated ozone concentrations. Therefore, it is imperative to comprehend how ozone affects wheat cultivars in order to develop effective mitigation strategies, particularly in areas where ozone pollution is prevalent (Grantz & Wrochna, 2008). Ethylene Diurea (EDU), a chemical compound with the chemical formula (C5H8N2O2), has been widely used as a protective agent against ozone-induced damage in various plant species. EDU acts as an ozone scavenger and protects plants by reducing ozone uptake through stomata. This compound has been particularly useful in assessing the impact of ozone phytotoxicity on wheat, a staple crop with global significance (Li et al., 2015). Several studies have demonstrated the effectiveness of EDU in reducing ozone-induced damage in wheat by preserving photosynthetic efficiency, stomatal conductance, and overall plant health. The protective properties of EDU have been attributed to its ability to scavenge ozone in the leaf apoplast, thus reducing its detrimental effects on cellular processes (Agrawal et al., 2012). The increasing threat of elevated ozone (O3) concentration on agricultural productivity holds special significance in the world specially in Asian region, where around 60%

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of the world's undernourished population resides, as reported by the (MEA, 2005). Furthermore, this region is expected to experience substantial population growth by the year 2030, according to the (FAO, 2002). At the same time in the Mediterranean region, ozone (O3) levels often surpass the prescribed limits designed to safeguard both natural plant life and crops, as stated by (EEA, 2013). Moreover, projections related to climate change anticipate an increase in temperatures, which is expected to result in elevated background concentrations of O3, as highlighted in the (IPCC, 2007). Hence, there is an immediate requirement to develop the capacity to evaluate both the existing and anticipated dangers posed by (O3) exposure to crops around the world. The purpose of this study is to examine how EDU functions in evaluating the effects of ozone phytotoxicity on the growth and yield of two wheat cultivars, *Triticum aestivum*, and provide insights into the potential use of EDU as a practical tool for mitigating ozone-induced damage in wheat crops. By comparing the responses of these cultivars with and without EDU treatment, by using EDU in wheat farming, we can learn more about the defensive mechanisms and possible advantages, contributing to the development of sustainable agricultural practices in regions affected by ozone pollution.

MATERIALS AND METHODS

Study area: The experiment was carried out in the University of Duhok's College of Agriculture, Engineering Science research area in the Kurdistan Region during the 2022–2023 growing season. The purpose of the experiment was to evaluate the effect of ethylene diurea (EDU) in reducing the detrimental effects of tropospheric ozone on the productivity and growth of two wheat cultivars, Triticum aestivum vars. Ceyhan-99 and Adana-99. The field experiment was conducted in the rain-fed environment of the Duhok governorate, which is situated in the Kurdistan Region of northwest Iraq. The region is known for its borderline semi-arid and Mediterranean climate, which features extremely hot and dry summers with average temperatures above 40C and cold, wet winters with average temperatures below 10C.

Seeds preparation and cultivation: Prior to planting, the seeds of two different types of wheat were cleaned and sieved. 30 kg/dunum, or 13 g.m2, was the modified sowing rate, which was split across 5 rows/m2. The density was also modified to account for the actual purity and germination % of the seeds. One day prior to sowing, seeds were treated with fungicide (Vitavax 200 wp) at concentrations of 2g.kg-1. Manual seeding took place in late November of 2022.

Ozone and EDU treatment: The agronomic practices and inputs applied to all 54 plots were the same, with the notable exception of the treatments applied to the wheat varieties with the following ozone concentrations: ambient air concentrations (control 32–37 ppb), 50 ppb, and 60 ppb by using a specific portable ozone generator from (QJ-002, multi-functional ozone generator, China); at the anthesis stage, by using an open top chamber suggested by (Heagle et al., 1973); additionally, application of ethylene diurea (EDU) at concentrations of control, 200 ppm and 300 ppm two days prior to ozone fumigation for four weeks consequently.

Experimental design: Three factors were included in the experiment: two varieties, three O_3 gas concentrations, and three EDU concentrations. Each factor had three replications in the randomize complete block design (RCBD) method. The dimensions of the plot were 1 m2 with a 60 cm distance between each plot and 2 m between each block. Five rows, spaced 20 cm apart, were formed in each plot.

Studied characteristics

1. Relative water content (RWC %) of flag leaf: was taken in accordance with (Smart and Bingham, 1974) at the peak of the anthesis stage during the O_3 fumigation process.

2. Flag leaf area (cm)2: During the O3 fumigation process, flag leaf area was measured at the peak of the anthesis growth stage in accordance with (Kandic et al., 2009).

3. Total biomass (above ground biomass Kg/ dunum): Following harvesting, the whole above-ground biomass was air dried in accordance with (Cabrera et al., 2011).

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4. Harvest index (HI): Harvest index was determined according to (Williams et al., 1989; Stockle et al., 1994) after measuring the air-dried biomass and grain yield for each plot.

5. Grain weight (Kg/m2): Following threshing and harvesting at maturity, each plot's seeds were cleaned, and a digital balance was used to record the grain output (Meyer et al., 2000).

Statistical analysis: The study's data underwent statistical analysis using SAS 9.4 software developed by SAS Institute, Inc. in Cary, North Carolina. The analysis involved conducting one-way ANOVA, following the methodology outlined by **(**Duncan, 1955**).**

RESULTS AND DISCUSSION

Chlorophyll content in flag leaf (SPAD): The findings from Table 1 clearly demonstrate a significant difference (P ≤ 0.05) in chlorophyll content among different varieties, with Cehyan displaying the highest value at 49.96 and Adana registering the lowest at 36.63 SPAD. Reduced chlorophyll content in the flag leaves of both species was associated with elevated levels of ozone in the atmosphere. Interestingly, at 56.56, the control value was higher than at 50 and 60 ppb concentrations, which displayed values at 41.50 and 31.83 SPAD, respectively. In contrast, increasing concentrations of EDU led to a positive augmentation in chlorophyll content for both varieties. Remarkably, the control exhibited the lowest value at 37.11 in comparison to concentrations of 200 and 300 ppm, which demonstrated higher values at 44.56 and 48.22 SPAD, respectively.

Table 1: Effect of EDU on minimizing ozone stress on chlorophyll content (SPAD) in flag leaf of two wheat cultivars

Cultivars	EDU concentration (ppm)	O3 concentration (ppb)			Cultivar * EDU	Means
		Control	50	60		
Adana	Control	56.33 ab	33.00 c	21.00 de	36.78 b	
	200	58.67 a	34.67 c	22.00 de	38.44 b	36.63 b
	300	53.00 ab	31.00 cd	20.00 e	34.67 b	
Cehyan	Control	56.33 ab	34.00 c	22.00 de	37.44 b	
	200	56.33 ab	58.33 a	59.33 a	58.00 a	49.96 a
	300	58.67 a	58.00 a	46.67 b	54.44 a	
Means		56.56 a	41.50 b	31.83 c		
Cultivar * O3 conc.	Adana	56.00 a	32.89 d	21.00 e	Means	
	Cehyan	57.11 a	50.11 b	42.67 c		
EDU * O3 conc.	Control	56.33 a	33.50 c	21.50 d	Control	37.11 b
	200	55.83 a	44.50 b	33.33 c	200	44.56 a
	300	57.50 a	46.50 b	40.67 b	300	48.22 a

The means for each factor or their interaction that follow with the same letters do not differ significantly at the 0.05 level in the Duncan Multiple test.

Flag leaf area (cm): The findings from Table 2 highlight significant differences (P ≤ 0.05) in flag leaf area among varieties, with Cehyan displaying the highest value at 35.53 cm and Adana registering the lowest at 23.65 cm. Elevated ozone concentrations corresponded to a decrease in the flag leaf area for both varieties. Notably, the control exhibited the highest value at 42.63 cm, whereas the 50 and 60 ppb concentrations showed lower values at 25.46 cm and 20.68 cm, respectively. Conversely, increasing concentrations of EDU led to a positive augmentation in the flag leaf area for both varieties. Remarkably, the control displayed the lowest value at 23.89 cm compared to concentrations of 200 and 300 ppm, which demonstrated higher values at 32.67 cm and 32.22 cm, respectively.

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Table 2: Effect of EDU on minimizing ozone stress on flag leaf area (cm) of two wheat cultivars

Cultivars	EDU concentration (ppm)	O3 concentration (ppb)			Cultivar * EDU	Means
		Control	50	60		
Adana	Control	41.13 cde	18.00 f	12.04 g	23.72 c	23.65 b
	200	46.42 a	19.24 f	9.17 h	24.94 c	
	300	39.13 de	17.00 f	10.71 gh	22.28 d	
Cehyan	Control	43.08 bc	19.24 f	9.83 gh	24.05 c	35.53 a
	200	41.13 cde	38.67 e	41.37 cd	40.39 b	
	300	44.88 ab	40.64 cde	40.93 cde	42.15 a	
Means		42.63 a	25.46 b	20.68 c	Means	
Cultivar * O3 conc.	Adana	42.23 a	18.08 d	10.64 e		
	Cehyan	43.03 a	32.85 b	30.71 c		
EDU * O3 conc.	B1	42.11 b	18.62 e	10.94 f	Control	23.89 b
	B2	43.78 a	28.95 c	25.27 d	200	32.67 a
	B3	42.01 b	28.82 c	25.82 d	300	32.22 a

The means for each factor or their interaction that follow with the same letters do not differ significantly at the 0.05 level in the Duncan Multiple test.

Relative water content (%): The findings from Table 3 reveal significant differences ($P \leq 0.05$) in relative water content among varieties, with Cehyan displaying the highest value at 57.07%, while Adana exhibited the lowest at 56.27%. Increasing concentrations of ozone resulted in a decrease in the relative water content of both varieties. Notably, the control displayed the highest value at 71.13%, whereas the 50 and 60 ppb concentrations showed lower values at 54.14% and 44.74%, respectively. Conversely, higher concentrations of EDU led to a positive increase in the relative water content of both varieties. Remarkably, the control showcased the lowest value at 55.43% compared to concentrations of 200 and 300 ppm, which demonstrated higher values at 57.49% and 57.09%, respectively.

Table 3: Effect of EDU on minimizing ozone stress on relative water content (%) of two wheat cultivars

Cultivars	EDU concentration (ppm)	O3 concentration (ppb)			Cultivar * EDU	Means
		Control	50	60		
Adana	Control	70.97 b	56.22 d	44.34 gh	57.18 a	56.27 b
	200	73.59 a	54.23 e	45.58 g	57.80 a	
	300	67.16 c	51.32 f	43.05 h	53.84 b	
Cehyan	Control	72.03 ab	53.43 e	45.57 g	57.01 a	57.07 a
	200	70.97 b	56.22 d	44.34 gh	57.18 a	
	300	72.03 ab	53.43 e	45.57 g	57.01 a	
Means		71.13 a	54.14 b	44.74 c	Means	
Cultivar * O3 conc.	Adana	70.57 b	53.92 c	44.32 d		
	Cehyan	71.68 a	54.36 c	45.16 d		
EDU * O3 conc.	Control	69.60 b	52.38 d	44.31 e	Control	55.43 b
	200	72.28 a	55.22 c	44.96 e	200	57.49 a
	300	71.50 a	54.83 c	44.96 e	300	57.09 a

The means for each factor or their interaction that follow with the same letters do not differ significantly at the 0.05 level in the Duncan Multiple test.

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Total biomass (kg): The findings from Table 4 demonstrate significant differences ($P \leq 0.05$) in total biomass among varieties, with Cehyan displaying the highest value at 1.29 kg, while Adana exhibited the lowest at 1.02 kg. Increased concentrations of ozone corresponded to a decrease in the total biomass of both varieties. Notably, the control showcased the highest value at 1.43 kg, while the 50 and 60 ppb concentrations exhibited lower values at 1.10 kg and 0.93 kg, respectively. Conversely, higher concentrations of EDU led to a positive increase in the total biomass of both varieties. Remarkably, the control depicted the lowest value at 1.03 kg compared to concentrations of 200 and 300 ppm, which demonstrated higher values at 1.09 kg and 1.34 kg, respectively.

Table 4: Effect of EDU on minimizing ozone stress on total biomass (kg) of two wheat cultivars

Cultivars	EDU concentration (ppm)	O3 concentration (ppb) Control	50	60	Cultivar * EDU	Means
Adana	Control	1.47 bc	1.10 ef	0.60 hi	1.06 d	1.02 b
	200	1.60 a	1.13 e	0.70 h	1.14 c	
	300	1.27 d	0.83 g	0.50 i	0.87 e	
Cehyan	Control	1.40 c	1.00 f	0.60 hi	1.00 d	1.29 a
	200	1.47 bc	1.53 ab	1.60 a	1.53 a	
	300	1.40 c	1.00 f	1.57 ab	1.32 b	
Means		1.43 a	1.10 b	0.93 c	Means	
Cultivar * O3 conc.	Adana	1.44 a	1.02 d	0.60 e		
	Cehyan	1.42 a	1.18 c	1.26 b		
EDU * O3 conc.	Control	1.43 b	1.05 e	0.60 g	Control	1.03 c
	200	1.33 c	0.92 f	1.03 e	200	1.09 b
	300	1.53 a	1.33 c	1.15 d	300	1.34 a

The means for each factor or their interaction that follow with the same letters do not differ significantly at the 0.05 level in the Duncan Multiple test

Harvest index (HI): The outcomes from Table 5 underscore significant differences ($P \leq 0.05$) in harvest index among varieties, with Cehyan demonstrating the highest value at 0.42, while Adana showcased the lowest at 0.39. Elevated ozone concentrations correlated with a decrease in the harvest index of both varieties. Notably, the control depicted the lowest value at 0.36, whereas the 50 and 60 ppb concentrations displayed higher values at 0.41 and 0.45, respectively. Conversely, increasing concentrations of EDU led to a positive increase in the harvest index for both varieties. Remarkably, the control showed the lowest value at 0.37 compared to concentrations of 200 and 300 ppm, which exhibited higher values at 0.41 and 0.47, respectively.

Table 5: Effect of EDU on minimizing ozone stress on Harvest index (HI) of two wheat cultivars

Cultivars	EDU concentration (ppm)	O3 concentration (ppb) Control	50	60	Cultivar * EDU	Means
Adana	Control	0.33 fgh	0.41 de	0.47 bc	0.40 c	0.39 b
	200	0.28 h	0.30 h	0.34 fgh	0.31 e	
	300	0.38 efg	0.47 bc	0.55 a	0.47 ab	
Cehyan	Control	0.32 h	0.33 gh	0.38 def	0.34 d	0.42 a
	200	0.43 cd	0.41 de	0.47 bc	0.44 b	
	300	0.43 cd	0.53 ab	0.47 c	0.48 a	
Means		0.36 c	0.41 b	0.45 a	Means	
Cultivar * O3 conc.	Adana	0.33 d	0.39 c	0.45 a		
	Cehyan	0.39 c	0.42 bc	0.44 ab		
EDU * O3 conc.	Control	0.33 d	0.37 c	0.43 b	Control	0.37 c
	200	0.36 cd	0.36 cd	0.44 b	200	0.41 b
	300	0.41 b	0.50 a	0.51 a	300	0.47 a

The means for each factor or their interaction that follow with the same letters do not differ significantly at the 0.05 level in the Duncan Multiple test

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Grain yield (kg): The findings from Table 6 highlight significant differences ($P \leq 0.05$) in grain yield among varieties, with Cehyan showing the highest value at 0.41 kg, while Adana displayed the lowest at 0.37 kg. Elevated ozone concentrations correlated with a decrease in the grain yield of both varieties. Notably, the control exhibited the highest value at 0.46 kg, whereas the 50 and 60 ppb concentrations showed lower values at 0.39 kg and 0.32 kg, respectively. Conversely, increasing concentrations of EDU led to a positive increase in the grain yield for both varieties. Remarkably, the control showed the lowest value at 0.37 kg compared to concentrations of 200 and 300 ppm, which demonstrated higher values at 0.40 kg and 0.41 kg, respectively.

Table 6: Effect of EDU on minimizing ozone stress on grain yield (kg) of two wheat cultivars

Cultivars	EDU concentration (ppm)	O3 concentration (ppb) Control	C2	C3	Cultivar * EDU	Means
Adana	Control	0.49 a	0.42 e	0.29 h	0.40 c	0.37 b
	200	0.45 bcd	0.35 g	0.23 j	0.34 e	
	300	0.47 b	0.40 f	0.27 i	0.38 d	
Cehyan	Control	0.44 cde	0.34 g	0.22 j	0.33 e	0.41 a
	200	0.49 a	0.42 e	0.47 b	0.46 a	
	300	0.44 cde	0.44 de	0.46 bc	0.45 b	
Means		0.46 a	0.39 b	0.32 c	Means	
Cultivar * O3 conc.	Adana	0.47 a	0.39 cd	0.26 e		
	Cehyan	0.46 b	0.40 c	0.38 d		
EDU * O3 conc.	Control	0.47	0.38	0.25	Control	0.37 c
	200	0.47	0.38	0.35	200	0.40 b
	300	0.46	0.42	0.36	300	0.41 a

The means for each factor or their interaction that follow with the same letters do not differ significantly at the 0.05 level in the Duncan Multiple test

Ethylene diurea (EDU) is a chemical compound used to mitigate the harmful effects of ozone on plants. When plants are exposed to high levels of ozone, it can lead to oxidative stress and damage, affecting their growth and productivity. EDU is believed to counteract these effects by scavenging reactive oxygen species (ROS) and reducing ozone uptake through the stomata (Li et al., 2019). In the context of assessing ozone phytotoxicity on wheat cultivars like *Triticum aestivum*, EDU has been utilized in studies to understand its impact on plant growth and productivity. Manning et al. (2019) found that applying EDU can alleviate some of the detrimental effects of ozone exposure, for instance they examined the effects of ozone on two wheat cultivars, one being more sensitive to ozone than the other. They found that the application of EDU helped mitigate the negative impacts of ozone on both cultivars, particularly in terms of reducing visible leaf injury, maintaining higher photosynthetic rates with notable positive effects on leaf area, plant height and above ground biomass. Another study by Pleijel et al. (2012) investigated the use of EDU in assessing the impacts of ozone on wheat yield. Their findings indicated that while ozone negatively affected the grain yield of wheat, the application of EDU helped in preserving the yield by protecting the plants from ozone-induced damage with higher harvest index. Another study by Li et al., (2019) investigated the impact of EDU on wheat yield parameters under ozone stress. They observed that EDU application led to a significant increase in grain yield and improved grain quality in ozone-exposed wheat plants compared to untreated plants. Research conducted by Sharma et al. (2019) they investigated the effects of ozone on two wheat cultivars, potentially using EDU as a protective agent. Their study explored the positive impact of EDU on many growth parameters, photosynthetic activity, chlorophyll content and productivity in different wheat cultivars. Similarly, Vahdati et al. (2017), conducted a study evaluating the role of EDU in protecting wheat plants against ozone-induced damage. They found the protective role of EDU on physiological and biochemical parameters to elucidate the

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protective mechanism of EDU against ozone stress in wheat. Another study conducted by Li, et al. (2021). Mitigation of ozone stress on wheat plants by Ethylene Diurea (EDU) Treatment: they observed a positive role of EDU in maintaining Photosynthesis rate, increasing antioxidant defense, and preserving chloroplast and chlorophyll content and structure. On the other hand, EDU has effective role in protecting wheat plants from ozone-induced oxidative stress under various soil moisture conditions (Hassan et al., 2020). However, despite the promising results observed in many studies, the effectiveness of EDU in mitigating ozone-induced stress can vary depending on several factors such as concentration, application method, environmental conditions, and the specific cultivar being studied. Therefore, while EDU shows potential in reducing ozone-induced damage in wheat, further research is needed to fully understand its mechanisms and optimize its application for different cultivars and environmental scenarios (Feng et al., 2018).

CONCLUSION

In summary, the results of the research indicates that EDU application can alleviate ozone-induced phytotoxicity in different wheat cultivars *Triticum aestivum* by reducing oxidative stress and improving plant growth and productivity. EDU, being an antioxidant compound, has been proposed as a protective agent against ozone-induced damage by scavenging reactive oxygen species (ROS). In the context of wheat cultivars, the application of EDU has shown varying degrees of effectiveness in alleviating ozone-induced phytotoxicity. EDU treatment can lead to improved growth parameters, such as increased photosynthetic rates, enhanced chlorophyll content, reduced visible injury symptoms, consequently improving grain yield and quality indicating its potential role in minimizing the negative impacts of ozone on *Triticum aestivum*. However, while EDU demonstrates promise in reducing ozone-induced stress, its efficacy seems to depend on various factors, including concentration, application timing, environmental conditions, and the specific cultivar of wheat. Different wheat varieties may respond differently to EDU treatment, and its effectiveness may vary based on the severity and duration of ozone exposure.

CONFLICT OF INTEREST

The author declares that they have no conflicts of interest.

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The authors declare that they have not received a fund.

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دور الإيثيلين ثنائي يوريا في تقييم آثار السمية النباتية للأوزون على نمو وإنتاجية صنفين من القمح

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المستخلص

الأوزون التروبوسفيري هو ملوث هواء معروف أن له آثار ضارة على إنتاجية المحاصيل وصحة النباتات، ومن المعروف على نطاق واسع أنه ملوث جوي سام للنباتات مما يؤدي إلى انخفاض عملية التمثيل الضوئي، وتثبيط النمو، وتسريع شيخوخة الأوراق، وكذلك يؤثر سلبيا علئ انتاجية المحاصيل الزراعية. اصناف الحنطة الناعمة تعتبر من النباتات الاساسية الحساسة للتلوث الهوائي و خاصة ب الأوزون التروبوسفيري لذلك، تم إجراء هذا البحث لمعرفة دور الإيثيلين ثنائي يوريا في تقييم آثار السمية النباتية للأوزون على نمو وإنتاجية صنفين من القمح، جيهان-99 و ادنة-99. تم تطبيق ثلاثة مستويات من الأوزون خلال ساعات النهار: تركيز الأوزون المحيط (التحكم)، 50 جزء في البليون و60 جزء في البليون، بالإضافة إلى ثلاثة مستويات من الإيثيلين ثنائي يوريا (التحكم) عامل المقارنة. 200 جزء من المليون و 300 جزء من المليون. أشارت النتائج إلى وجود تأثير ضار للأوزون التروبوسفيري على الصفات الخضرية والإنتاجية لكلا صنفي القمح. ومن ناحية أخرى فإن الرش الورقي ب الإيثيلين ثنائي يوريا يعمل كعامل وقائي ضد الأضرار الناجمة عن الأوزون في مختلف خصائص نبات القمح التي تمت دراستها خلال البحث.

الكلمات المفتاحية: الأوزون التروبوسفيري، الإيثيلين ثنائي يوريا، الحنطة، النمو، الانتاجية.