Effect of Ozone on the Quality of Two Legume Crops (Vicia faba, and Pisum sativum) around Riyadh City

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Abstract: Ozone (O_3) is considered as one of the most phytotoxic pollutants with deleterious effects on living and non-living components of ecosystems. In the present investigation effects of different levels of ambient O_3 at different locations was perform on the two genus *Vicia faba* L. and *Pisum sativum* L. It was observed that O_3 had a significant negative effect on all the studied parameters, but they differ in magnitude and nature of growth with different plant species. The results also revealed that *Vicia faba* L. was more sensitive to ozone than *Pisum sativum* L.

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Introduction

Ozone gas is considered as one of the hazardous pollutants of the ecosystem. It is produced in large quantities as a result of complex interactions between ultraviolet radiation and lightning and the primarily this pollutants is produced in factories, power plants as well as fuels used in automobiles (IPCC, 2001). The gas plays a secondary role in the warming the Earth, where the 7% of it is used in heating the earth (Krupa, et al., 1998). Naturally the gas ranges in between 20-30 nl/L (Sandermann, 1998). Recently it has been reported that its level increased four times in urban areas (Findley, et al., 1997) which has caused adverse effect on the whole ecosystem including the plant. The development and productivity of the crop is severely affected due to the increased level of ozone in the atmosphere which might be due to the disruption in the ionic imbalance caused by damage to the cell membranes and leads to the decrease in the rate of photosynthesis. This loss is directly correlated with the decrease in the growth and dry matter production.

The intensity of the damage to the crop varies from species to species depending on the susceptibility, age, period of exposure and the concentrations of the gas (Krupa and Jäger, 1996). It was reported earlier that symptoms appear faster on susceptible as compared to non-susceptible plant species (Butler and Tibbitts, 1979; Reich and Amundson, 1985).

At present the city of Riyadh is experiencing a high degree of ozone gas pollution which is resulted mostly due to the industrial and agricultural activities. The level of ozone pollution in city is increasing day by day and it reached at a level of 60-148 ppb (Al-Muehi, 2010; Muhaisin, 2011). Therefore, the present experiment was planned with an objective to explore the effect of different level of O3 present on the different site of the city on the quality of the two winter crops i.e. *Vicia faba* and *Pisum sativum*.

Materials and methods

Study site:

The city of Riyadh, Saudi Arabia lies between a latitudes 19° 30′ and 27° 30′ N, longitudes 42° and 48° east, and at an altitude of about 600 m above sea level. The total area of Riyadh is 4.900 km^2. The city of Riyadh had dry desert climate which is extremely hot in summer (45° C) and moderately cold in winter (7°C) and the highest average rainfall reached 20.1 mm during the winter.

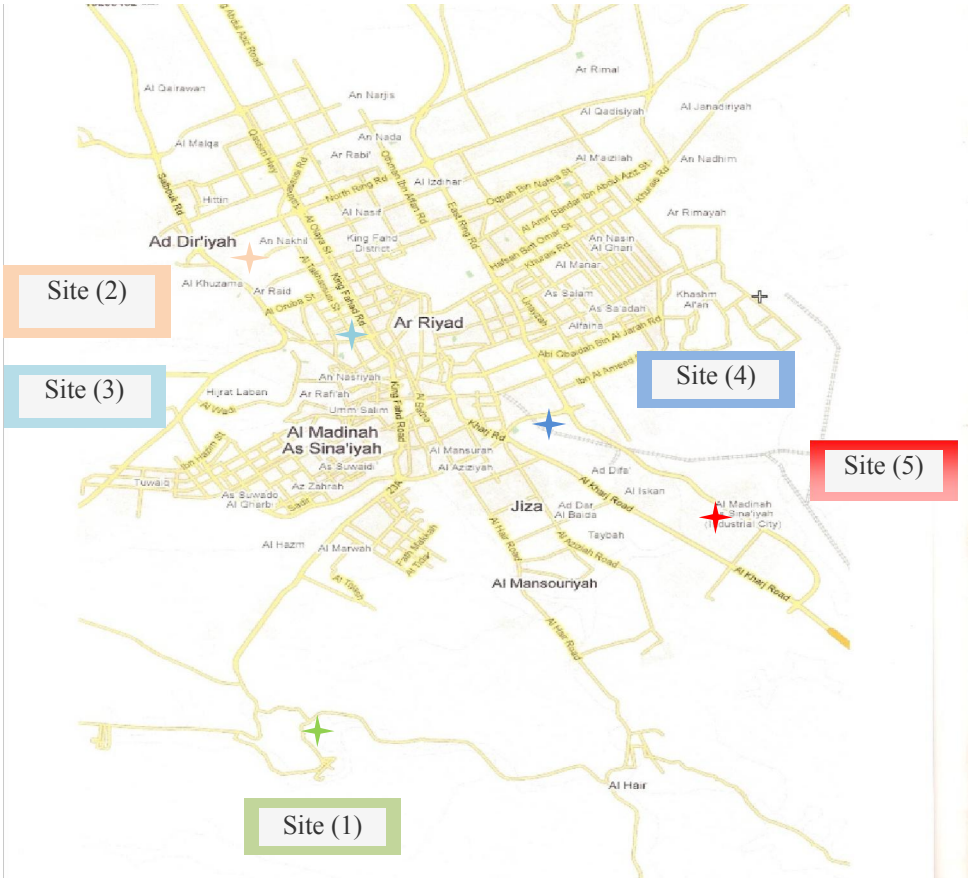
Methods

The study was conducted at four different sites within the city of Riyadh, representing different levels of pollution (Fig. 1), as well as under the controlled conditions (17 ppb) using a field growth chamber (Mandl, et al., 1973) The levels of gas in the different sites in the city are as follows: King Saud University (61), King Fahad Road (71), Climate Garden (77), and the second industrial city (112) which was analyzed by an ozone analyzer (Model UV-100, Serial #111 EcoSensors Inc).

The seeds of faba bean and pea were sown in the plastic pots (40 cm^2) filled with sand and clay, mixed in a ratio of 1:1 on 14/11/2007. The plants were moved to various sites on 28/11/2007 (after emergence of the initial leaves). Each treatment consists of 24 pots and each pot is represented by a five plants. The samplings were done at 30, 60 and 90 days after the exposure of the plants to the ozone. Plant samples were divided into different parts and dried in an oven at a

temperature of 80°C for 48 hours in the laboratory of Department of Botany & Microbiology. Total carbohydrates and reduced carbohydrate was estimated using the method of Hodge and Hofreiter (1962). The non-reducing sugar was calculated by subtracting the value of reducing sugars from total sugars.

For estimating the fat contents, the Saudi Standards (Saudi Arabian Standards and Specifications, Method No. 547.1982 M) was used; whereas, soluble protein was estimated according to the method of Bradford (1976). Total protein was determined on the basis of the ratio of nitrogen sample, which was analyzed by Micro Kjeldahl Method and multiplied it by 6.25. Data were analyzed statistically by the analysis of variance (3Way ANOVA) using a statistical package (SAS-Institute, 2000). Means were compared using the least significant difference test (LSD) (Steel and Torrie, 1982).



Map 1. Sites in Riyadh city organized depending on ozone level: 1- Derab area 2- King Saud University 3- King Fahad Road 4- Climate Garden 4- Second Industrial area

Results

Leaves of both the crops show typical visible injury symptoms such as chlorosis, necrosis and mottling upon exposure of O_3.

Sugars

The results presented in Table (1 A) that the mean concentration of reducing sugars in the leaves of faba bean and peas were 57.8 and 46.9 mg / g, respectively. Significant increasing shows differences exist between ozone levels (1 and 5) where significant reductions were observed for both plants.

The results presented in Table (1 A) also show that the mean concentration of non- reducing sugars in the leaves of faba bean and pea plants were 200.8 and 203.8 mg/g, respectively, and the results indicated the existence of significant differences between the effects of ozone levels (1 and 5) for both plants.

The results presented in Table (1 A) indicated that the mean concentration of total sugars in the leaves of faba bean and pea plants reached 258.6 and 249.9 mg/g, respectively, and the results show the existence of significant differences between the values as a result of ozone exposure as the concentration of gas increased for both plants. Ozone injury symptoms were evident for both plants at the high concentration

of all types of sugars. This reduction increased significantly as the ozone level increased.

Fats :

Table (1 a) shows change in fat that content in the leaves of faba bean and pea plants which, were 3.96% and 4.1% respectively, the two genotypes included in the study for comparative purposes all showed a significant decline in fat content in response to ozone effect.

Table 1: Effect of ozone gas on (A) sugars contents of faba bean and pea plants (B) Mean of decline in the ozone levels (2-5) compared to the control%. Means followed by the same letters in each column were not significantly different using LSD test at 0.05 probability level.

Plant / Ozone levels	(A)					
	Reducing sugars		Non-reducing sugars		Total sugars	
	Faba bean	Pea	Faba bean	Pea	Faba bean	Pea
1	85.4a±12.8	75.2±12.4	216.9a±98.1	224.6a±75.3	301.7±107.6	299.9a±65.5
2	60.3b±16.5	47.8b±7.4	230.9a±87.6	206.7b±68.0	291.2a±97.9	254.6±74.4
3	53.6c±16.4	46.9b±7.03	234.1a±79.8	200.1b±41.6	287.7±94.9	247.0bc±47.0
4	45.8b±26.0	34.1c±12.7	164.8c±35.4	208.4b±35.2	210.6±57.5	239.6c±40.1
5	43.6d±24.9	30.3c±5.01	158.2c±35.4	179.1c±32.01	201.8c±46.0	208.6d±35.6
LSD0.05	4.7	4.7	11.9	13.9	10.7	13.2
Mean	57.7a±24.4	46.9b±18.3	200.8a±76.1	203.8a±53.1	258.6a±91.4	249.9b±60.0

(B)									
	Reducing sugars			Non-reducing sugars			Total sugars		
LSD0.05	Faba bean	Pea	LSD 0.05	Faba bean	Pea	LSD 0.05	Faba bean	Pea	LSD 0.05
	42.2a	45.3a	4.4	1.3b	6.9a	3.9	15.7b	20.1a	2.4

Proteins:

ANOVA revealed significant variation in the effects of ozone on protein content of the leaves of both faba bean and pea plants (Table 2A) at the 0.05 level. The mean percentage of total protein in the leaves of a plant faba bean and pea were 8.3% and 10.9%, respectively with the reduction ranging from 1-5 for faba bean and pea plants as compared to the control.

Measurements of soluble proteins in both faba and pea leaves showed exposure to ozone resulted in a significant decline ($P<0.05$) as the ozone concentration increased. The mean concentration of soluble protein in the faba bean leaves, peas reached 8.9 and 9.7 mg/g respectively (Table 2A). The impact of ozone on protein, fat and sugars, varied between species and resulted in declines in soluble proteins of 8.6% and 2.1% in faba bean and pea respectively.

Table 2: Effect of ozone gas on (A) fats, total protein and dissolved protein contents in faba bean and pea plants (B) Mean of decline in the ozone levels (2-5) compared to the control%. Means followed by the same letters in each column were not significantly different using LSD test at 0.05 probability level.

Plant / Ozone levels	(A)					
	Fats		Total protein		Dissolved protein	
	Faba bean	Pea	Faba bean	Pea	Faba bean	Pea
1	5.36a±1.05	5.65a±1.08	11.2a±2.0	16.0a±1.77	9.1a±0.22	9.9a±0.23
2	4.51b±0.33	4.44b±0.66	7.9c±2.62	11.1b±3.28	8.0b±0.14	9.9a±2.2
3	3.69c±0.40	4.10b±1.17	8.9b±2.68	10.7bc±3.00	9.0b±0.14	9.7b ±0.19
4	3.74c±1.00	3.56c±0.23	6.8d±3.09	9.8c±2.50	8.8c±0.29	9.7c±0.29
5	2.50d±0.41	2.73±0.44	6.5d±2.43	7.2d±2.50	8.7d±0.10	9.5d±0.33
LSD0.05	0.40	0.36	0.99	1.1	0.07	0.07
Mean	3.96a±1.18	4.10a±1.24	8.3b±3.00	10.9a±3.84	8.9b±0.23	9.7a±0.28

(B)									
	Reducing sugars			Non-reducing sugars			Total sugars		
LSD0.05	Faba bean	Pea	LSD 0.05	Faba bean	Pea	LSD 0.05	Faba bean	Pea	LSD 0.05
	30.7a	32.2a	4.3	30.6b	40.2a	3.9	8.6a	2.1b	0.11

Discussion

This is first study of ozone concentrations in Riyadh, Central region, Kingdom of Saudi Arabia. A number of researchers pointed out the mechanism of the effect of ozone gas on organic content of the plant after the uptake of plant of ozone through the stomata where it dissolves in water in the cell wall and then interacts directly with the outer membrane (Plasmalemma) through the process of Ozonolysis creating active oxygen and super oxide), these active oxygen radicals interact with the plasma membrane and the amino acids targeting proteins in the cell membrane or with the enzymes as well as metabolites in the cell wall, these interactions changes the organic components of the cell, leading to accelerated aging and cell death. These interactions depend on the concentration of ozone, plant growth stage and plant species as well as environmental conditions (Long and Naidu, 2002; Fiscus, et al., 2005).

Ashmore (2005); Biswas, et al. (2008) and Morgan, et al. (2003) also noted that high ozone results in a decrease in photosynthesis and carbohydrates distribution and theses effects of differ from one plant species to another. Yang and Poovaiah (2000) reported that ozone lead to changes in metabolic processes during plant growth and that includes loss of certain proteins faster than the rate of their synthesis, which leads to premature aging.

Some researchers have also cited several hypotheses for the effects of ozone (Sandermann, 1998; Heath and Taylor Jr, 1997; Pell, et al., 1997; Rao and Davis, 2001) stating that ozone damages the cell membrane, leading to imbalance of ion and functional as well as lower the activity of Rubisco protein and lead to the loss of the photosynthetic activity.

In the current study the exposure to high levels ozone continuously resulted in significant effects on the organic content of plant leaves and led to a reduction in the content of sugars (reducing, non-reducing, total), proteins and fats.

Sanmartin, et al. (2003) and Drogoudi and Ashmore (2002) indicated that ozone affects the synthesis of carbohydrates through its impact on the process of photosynthesis or by influencing the distribution of carbohydrates and, indirectly, on the process of gas exchange to avoid toxicity. Köllner and Krause (2000) interpreted the decrease in carbohydrates in the leaves of potato plants at a concentration of 60 ppb as a result of the damage caused by the high level of ozone on the outer membrane of the cell.

Heath and Taylor Jr (1997), Pell, et al. (1997), Sandermann (1998) and Rao and Davis (2001) mentioned that among the assumptions of the impact of ozone on plants in the lack the ability to distribute carbohydrates to the parts of the plant. Andersen (2003) also attributed the decline to be due to the imbalances between the source and sink of metabolites on the contrary. Plessl, et al. (2007) found an increase in the potato leaf content of soluble sugars, and attributed that to the role of ozone in the accumulation of carbohydrates in plant parts.

Bender and Weigel (1995) explained in their study on some plant species and at the same ozone concentration that the decrease in carbohydrates when plants are exposed to ozone is due to the increased cellular respiration, and this increase represents an increase in the cost associated with the prevention of toxicity by enzyme activity, as well as the maintenance of the damage resulting from pollution, leading to consumption of carbohydrates.

The present study also showed a significant decrease in the concentration of sugars (reducing, non reducing, total) in the leaves of plant species which increases the severity of pollution, gas pollution.

Reducing sugars decreased with the increase in the concentration of ozone in the different sites. Rates of decline in the leaves of plant faba bean, pea plants didn't show the significant differences, and the influence of the ozone on the plants appeared at 61 ppb (the university site). The decrease in reducing sugars in this study agree with the results with wheat (Meyer, et al., 2000), and potatoes (Köllner and Krause, 2000) and alfalfa (Keutgen, et al., 2005) and sugar beet (Iglesias, et al., 2006) plants.

For the non-reducing sugars, pea plant demonstrated higher sensitivity to ozone compared to faba bean plants, where the influence of the gas on the leaves of faba bean was noted at the level of 77 ppb during the winter (climate garden) while on the leaves of peas plants at the level of 61 ppb (the University). These results agree with those reported by Keutgen, et al. (2005), Meyer, et al. (2000) and Köllner and Krause (2000) in potato and spring wheat plants.

As for the concentration of total sugars the pea plant showed the highest sensitivity to ozone as compared to faba bean plants. The influence of the ozone on the leaves of plants was noted significant at 61 ppb during winter (the university). These findings are consistent with those found by Keutgen, et al. (2005) in strawberries and Meyer, et al. (2000) and Köllner and Krause (2000) in the leaves of potatoes and spring wheat.

A number of researchers (Janero, 1990; Alaiz, et al., 1999) interpreted the low protein content of plant parts resulting from the stress of the ozone on the oxidative ability of gas which destroy many of the compounds that of vital importance such as amino acids and proteins as a result of the production of a the monodialdehyde compound during the oxidation process. Cross, et al. (1998) noted that the stress of the

ozone is due to damage proteins and lipids in the cell membrane. Moldau (1999) claimed that the accelerated aging results from the breakdown of fats due to increased the active oxygen, and as another explanation, Moldau (1999) and Pell, et al. (1997) represented that active oxygen resulting from the interaction of ozone with compounds within the cell is responsible for the stimulation of other compounds such as ethylene and this compound works to speed up aging through its impact on reducing the fat and protein content. Singh, et al. (2010) linked the low protein content due to the low content of potassium because the production of Rubisco protein is reduced at low potassium or when potassium is limiting. On the contrary, Plessl, et al. (2007) found an increase in protein and fat content in the leaves of potatoes plants under high levels of ozone (40-120 ppb), and attributed this to the role of gas in the distribution of protein and fat as a result of the accumulation of such materials. In the current study the exposure to high levels continuously ozone to resulted in significant decreases in protein content (total and soluble) and with the increase in the levels of ozone.

Leaf contents of total and soluble protein decreased with the increase in the severity of pollution with ozone, where faba bean plant showed a higher sensitivity to the gas as compared to pea for soluble protein concentration. The opposite happened where pea plants showed higher sensitivity for ozone compared to faba bean for the concentration of total protein and the significant influence of ozone on faba bean was noted at 61ppb (the university), and for pea at 71 ppb (King Fahd Road). These findings are consistent with both Keutgen, et al. (2005) for strawberries where they found a decline of 14.6% at a level of 78 ppb. Also higher than what Agrawal, et al. (2005) had found in *Vigna radiata* where they reported a decline of 9.8% in the leaves of the plant.

Conclusion

This quantitative analysis evaluated the effects of ambient ozone in the open-air on the growth of faba bean and pea plants in Riyadh, Saudi Arabia ozone caused visible injuries in the leaves of the two plant species as well as significant reduction in the leaves sugars, proteins and fat contents, suggesting that ozone is causing losses in the growth of plants under current ambient conditions.

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