Original Research Article

IMPACT OF ELEVATED OZONE ON CABBAGE

Abstract

Tropospheric ozone (O_3) is a pervasive greenhouse gas and air pollutant known for its detrimental effects on human health and vegetation. In the recent years, this tropospheric ozone has been rising steadily on the account of rapid urbanization and globalization. Hence,this study investigates the impact of elevated ozone levels on cabbage cultivars, Tekila and Primero, which are extensively grown in the high-altitude region of the Western Ghats, India, where ozone levels are a growing concern.The study employed a comprehensive experimental design, encompassing ozone concentration levels (ambient and elevated), cabbage varieties (Tekila and Primero), and different growth stages of the cabbage plants. Ozone fumigation at 200 ppb was used to simulate elevated ozone conditions, reflecting potential future scenarios. To assess the extent of impact, both physiological and biochemical parameters were extensively analyzed.The results revealed that elevated ozone concentrations had a significant negative impact on both cabbage cultivars. Photosynthetic rates, stomatal conductance, and chlorophyll content declined progressively as ozone exposure continued, leading to maximum reductions of 71.2%, 81.03% 32.98% respectively. However, protective mechanisms were activated in response to ozone stress, including increased proline by 32.24%, ascorbic acid by 64.75%, catalase by 3.58%, and peroxidase activities by 56%, suggesting the cabbage plants' efforts to mitigate oxidative damage.Overall, this study highlights the vulnerability of cabbage cultivars to elevated ozone levels and emphasizes the need for effective mitigation strategies to safeguard crop productivity and ensure sustainable agriculture in regions facing escalating ozone pollution. Further research is essential to develop and implement solutions that can protect vital crops like cabbage from the adverse effects of tropospheric ozone.

Keywords : Tropospheric ozone, Cabbage, physiological parameters, biochemical parameters

1. Introduction

Tropospheric ozone (O_3) is a significant greenhouse gas and air pollutant that poses a threat to both human health and vegetation worldwide. Over the latter half of the 20^{th} century, ground-level O_3 concentrations have seen substantial increases, primarily due to elevated levels of nitrogen oxides (NOx), volatile organic compounds (VOCs), and radical precursors (1-3). These increases have been particularly pronounced in rural areas of the Northern Hemisphere, raising concerns about O_3's impact on biodiversity (1, 2).

Despite efforts to reduce precursor emissions, O_3 levels are projected to remain high throughout the 21^{st} century (4), highlighting the need for effective mitigation strategies. Ozone has been shown to have detrimental effects on various plant species, including forbs, shrubs, and trees, with potential risks extending across different trophic levels and ecosystem processes(5). The formation of O_3 in the troposphere depends on the presence of NOx and reactive carbon molecules, with varying efficiency depending on location and emissions (6, 7). In less polluted areas, O_3 formation is limited by VOCs, whereas, urban centres~~ers~~ with high NOx emissions experience locally suppressed O_3 concentrations. Transport of NOx away from cities can lead to increased O_3 levels in suburban and high-altitude regions(7).

Furthermore, O_3 production is influenced by sunlight and temperature, with peak concentrations occurring during warm, sunny conditions, coinciding with the temperategrowing season (8, 9). This has significant implications for crops, with economic losses due to O_3 anticipated to increase substantially for staple crops like maize, soybean, rice, and wheat (10-13). In regions such as India, where O_3 concentrations in major cities are expected to rise significantly as a result the agricultural sector faces considerable challenges (14)

Amid these concerns, cabbage (*Brassica oleracea* var. *capitata*)stands as a globally important vegetable rich in nutrients and bioactive compounds (15). It is extensively cultivated, including in the high-altitude region of western ghats, with India ranking as one of the top producers. However, the increasing presence of tropospheric O_3, even at high altitudes, necessitates a deeper understanding of its impact on cabbage.Hence this study focuses on the impact of elevated ozone levels on cabbage cultivars' physiological and biochemical parameters.

2. MATERIALS AND METHODS

2.1. Experimental Site

A field study was carried out at the Horticultural Research Station, Ooty, situated at 11º42'47.02" N latitude and 76º72'40.07" E longitude, with an altitude of 2520 meters above mean sea level.

The soil type at the experimental site was identified as black soil with a clay loam texture, classified under the Ultisols order. Soil analysis revealed an electrical conductivity of 0.18, a pH level of 5.1, an organic carbon content of approximately 1.65, and nutrient values for NPK (Nitrogen, Phosphorus, and Potassium) at 298.2, 26.2, and 247.5 kg ha^{-1}, respectively. The field study was conducted between February and July 2023. Throughout this period, average maximum temperatures ranged from 23.3 to 35.6°C in the summer and 13.4 to 23.3°C in the winter. Relative humidity levels varied between 42.4% and 93.9%.

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2.2. Plant Materials

For the experiment, two cabbage cultivars namely Tekila and Primero which were extensively grown by farmers in Ooty were selected. These cultivars, ~~Tekila and Primero,~~ were chosen based on a preliminary survey conducted among local farmers. Seeds for both cultivars were obtained from the Horticulture Research Station, TNAU, Ooty, Tamil Nadu. Primero is a compact cabbage variety with a dark red color and a growth duration of 80 – 90 days, while, Tekila is a compact green cabbage with a growth duration of 90 – 100 days. Cabbage plant seedlings were cultivated in the nursery using protrays. These protrays were filled with a mixture of sterilized cocopeat, neem cake, and biofertilizers, with each protray requiring approximately one kilogram of cocopeat. One cabbage seed was sown per cell of the protray, and the seedlings were attentively nurtured for 15 days within a controlled polyhouse environment located at the Horticultural Research Station in Ooty.

2.3. Experimental Design

The study incorporated a comprehensive experimental design, covering the entire growth period of the cabbage plants, and adopted a Factorial Random Block Design. Three pivotal factors were taken into account: Factor 1 comprised two ozone concentration levels - ambient ozone concentration and elevated ozone concentration at 200 ppb. Factor 2 revolved around the distinct cabbage varieties under scrutiny. Factor 3 addressed the specific growth stages of the cabbage plants, recognizing their evolving developmental phases. Furthermore, plants were grown in replicates of three. This intricate and well-structured design ~~(Figure.)~~ allowed for a systematic and controlled exploration of how ozone concentration, cabbage varieties, treatment methods, and the growth stages of the plants interacted and influenced the overall growth and development of the cabbage crops. The study spanned several months, conducted from February through July 2023.

2.4. Ozone Fumigation

After four weeks in the nursery, the seedlings were transplanted to the Horticultural Research Station (HRS) in Ooty, India. The study employed a 3 x 3-meter Open Top Chamber (OTC) to simulate elevated ozone levels at 200 ppb, alongside an adjacent 3 x 3-meter area representing ambient ozone conditions. This concentration choice was based on observed levels of up-to 150 ppb, with expectations of future escalation, particularly at higher altitudes. Approximately one-week post-transplantation, the cabbage plants were subjected to daily ozone fumigation at 200 ppb from 09:00 AM to 4:00 PM, totaling 7 hours daily, aiming to replicate realistic climatic conditions and induce genuine ozone stress. Throughout the study, ambient ozone concentration in the research area fluctuated between 100 and 158 ppb, monitored by a handheld air quality monitor. An ozone generator (A4G, Faraday, India) employing high-frequency corona discharge technology produced ozone, conveyed through Teflon tubes positioned approximately 30 cm above the plant canopy at four points within the chamber. Precise control of emitted ozone levels, in accordance with OTC measurements, was achieved through adjustment of the feed gas flow rate (10–12 lpm). This was facilitated by a handheld air quality monitor, ensuring precise control and monitoring of the ozone environment.

2.5. Parameters

Following ozone exposure, a comprehensive assessment of physiological and biochemical attributes was conducted to gauge the impact on the cabbage cultivars across three distinct growth stages, namely the vegetative stage, head-forming stage, and maturity stage. Key physiological parameters such as the photosynthetic rate (A), stomatal conductance (g_s), and chlorophyll content were meticulously analyzed. Additionally, various biochemical attributes including catalase, peroxidase, malondialdehyde (MDA), proline (PRO), ascorbic acid (AsA), and IAAwere assayed to provide insights into the plants' responses to ozone exposure.Finally, growth and yield attributes of the crop were also assessed to find the impact that has been caused by elevated levels of ozone.

3. Results and Discussion

3.1 Photosynthetic Rate

The influence of varying ozone concentrations on cabbage cultivars Tekila and Primero is evident from the data in Table 1. Under ambient conditions, Tekila exhibited photosynthetic rates ranging from 23.14 to 25.78 µmol CO_2 m^{-2} s^{-1}, while, Primero's range was slightly higher, from 24.28 to 28.71 µmol CO_2 m^{-2} s^{-1}. Elevated ozone levels led to a significant decline in both cultivars' photosynthetic rates, with Tekila's rates ranging from 10.34 to 16.54 µmol CO_2 m^{-2} s-1 and Primero's ranging from 13.98 to 21.19 µmol CO_2 m^{-2} s^{-1}. This decline was pronounced from the vegetative stage and worsened as exposure continued through the Head Forming and Maturity Stages, resulting in maximum reductions of 71.2% for Tekila and 69.51% for Primero during the Maturity Stage. These reductions may be attributed to ozone-induced damage to electron transport, Calvin cycle enzymes, and PS II activity (16), as well as decreased nitrogen allocation for photosynthesis (17).

3.2. Stomatal Conductance

The changes in the stomatal conductance of cabbage cultivars at different crop growth stages with the application of protectants are presented in Table1. During the vegetative, head forming and maturity stages under ambient condition, the stomatal conductance ranged from 5.33 to 6.01H_2O m^{-2} $s^{-1}$and 6.12 to 6.52 H_2O m^{-2} $s^{-1}$and under elevated ozone level it ranged from 1.14 to 2.49 mol H_2O m^{-2} s^{-1} and 2.43 to 3.70 mol H_2O m^{-2} s^{-1}in cabbage cultivars Tekila and Primero, respectively.Like photosynthetic rate the stomatal conductance was also found to decline from the beginning of the crop growth i.e. vegetative stage. As the growth of crop progressed there was a drastic decrease in the stomatal conductance under elevated ozone stress. The maximum reduction was found in the cultivar Tekila and Primero during the maturity stage with about 81.03% and 62.73% reduction, respectively. This reduction in stomatal conductance might be the result of stomatal closure which enables the inhibition of influxing gases eventually leading to reduced carbon allocation ultimately leading to reduced photosynthetic activity (18), or a delay in the response of stomata by means of stomatal sluggishness to ozone stress(19). The reduction in stomatal conductance have also been reported in several other studies(20-23)

3.3. Chlorophyll content

The exposure to elevated levels of ozone substantially decreased the chlorophyll content with rising period of exposure. The reduction was higher i.e 32.98% in the cultivar Tekila during the head forming stage while, in the case of Primero it was about 32.1% at the maturity stage as shown in Table 1. The reduced chlorophyll content under the influence of elevated ozone levels might possibly be due to the chlorophyllase activity which stimulates chlorophyll degradation (24, 25) and also by means of vesiculation of chlorophyll (26). The reduction in chlorophyll content as a result of ozone stress has also been reported in (23, 27).

3.5. Carotenoid content

The carotenoid content of the Tekila cultivar ranged from 0.22 to 0.56 mg g^{-1} FW, whereas, the Primero cultivar exhibited a range of 0.19 to 0.46 mg g^{-1} FW (Table 1.). During the various crop growth stages of both cultivars under ambient and elevated ozone conditions, there was a slight increase in carotenoid content. The tTekila cultivar exhibited the highest carotenoid content, reaching 0.59 mg g^{-1} FW in ambient conditions and 0.48 mg g^{-1} FW in elevated ozone conditions, The decrease in in carotenoid levels can be attributed to several factors, including the detoxification of reactive oxygen species (ROS) by protective mechanismssuch protective mechanisms likely sustain carbon allocation, thereby maintaining photosynthesis, or they may reduce the diffusion of ROS molecules, as suggested in (28)

3.5. Malondiadehyde

The Malondialdehyde (MDA) content, measured at different stages of cabbage growth, in both ambient and elevated ozone conditions (eO_3 at 200 ppb) is shown in the Figure 1..The MDA content tends to be slightly higher under elevated ozone conditions compared to ambient conditions along all the crop growth stages and MDA ranged from 0.21 to 0.44 µmol g^{-1} FW for Tekila and from 0.22 to 0.43 µmol g^{-1} FW for Primerounder ambient conditions.Whereas, the values ranged from 0.57 to 0.71 µmol g^{-1} FW and 0.56 to 0.68 µmol g^{-1} FW under elevated conditions. The MDA content that was found in the current study, portrayed an approximate 50 % increase in both the cultivars under ozone stress and the cultivar had a maximum percentage of increase compared to the cultivar primero.Increased malonaldehyde content in O_3-exposed plants is a result of lipid peroxidation, which suggests a disruption in plasma membrane permeability leading to an unbalance in disrupted cellular homeostasis(29). Enhanced lipid peroxidation is attributed to the stimulated production of

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reactive oxygen species (ROS) such as O_2^{-0} and H_2O_2(30). Similar increase in MDA contents under ozone stress have been shown in blackgram cultivars (31)and in Grapevines (32).

3.6. Proline

Exposure to elevated levels of ozone concentration had a notable effect on the Proline content of the cabbage cultivars (Figure.1). The lowest proline content was observed in the cultivar Tekila, 0.21 µmol g^{-1} FW during the vegetative stage under ambient conditions while the highest was noticed at the maturity stage under elevated conditions, 0.71 µmol g^{-1} FW. Similar conditions were observed in the cultivar pPrimero as well i.e. decreased content under ambient conditions and elevated levels under ozone stress. The increase of the concentration may signify a defense mechanism exhibited by the plants as proline helps in stabilization of structures that are subcellular such as membranes, proteins and it also helps in buffering the redox potential of cells and scavenging of free radicals (33, 34). The findings under this study are also in line with the studies (29, 35)who noticed increased proline content on tropical trees and *Sida cordifolia*, respectively.

3.7. Ascorbic acid

The increase in ozone concentration exhibited a significant impact on ascorbic acid content at all crop growth stages (Figure.3). At vegetative stage, Tekila exhibited an Aascorbic Aacid content of 1.13 mg g^{-1} FW under ambient conditions and 1.52 mg g^{-1} FW under elevated ozone conditions. On the other hand, Primero had an Aascorbic Aacid content of 1.24 mg g^{-1} FW under ambient conditions and 1.67 mg g-^{1} FW under elevated ozone conditions during the same stage of growth. The highest value was seen during the maturity stage of both cultivars under elevated ozone levels. The plants ability to increase ascorbic acid might be attributed to the ROS that are produced by ozone(36).This increase in ascorbic acid content can also be a result of the reproduction and usage of ascorbic acid for the ROS detoxification. The same effect has also been demonstrated in studies (23)in rice and in mung bean (31, 37).

3.11. Electrolyte leakage

The highest levels of electrolyte leakage were consistently observed ~~in~~ for both cultivars across in all growth stages under elevated levels (Figure.4). For the Tekila cultivar, there was a 4 foldincrease in electrolyte leakage under elevated ozone conditions compared to ambient conditions across all stages of crop growth. Similarly, for the Primero cultivar, under elevated ozone conditions, the control exhibited an increase in electrolyte leakage by 3, 4, and 5 fold during the vegetative, head forming, and maturity stages, respectively, when compared to the same stages under ambient ozone conditions.This increase in electrolyte leakage may be due to loss of K^+ from cells which then destabilizes the structure of cell membrane (38)which are also in line with studies (20, 38)

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3.8. Catalase activity

The catalase activity showed an increase with rising ozone concentrations, as indicated in the Figure 5. Both cultivars showed increasing trends of catalase activity throughout the growth stages of cabbage. Comparing the two cultivars Primero showed highest catalase activity when compared with Tekila under both ambient conditions and elevated ozone levels. The increased catalase activity in Primero might be due to fact that it being a red cabbage, it has more scavenging ability than green cabbages (39). Furthermore, the increase in catalase activity might be attributed to the scavenging effect of catalase towards H_2O_2 and enabling the plants in maintaining its normal condition by preserving the equilibrium of ROS under stress. The findings are similar to the stuy(40).

3.9. Peroxidase activity

Exposure to elevated ozone exhibited a significant increase in peroxidase activity (Figure.5). Under both ambient and elevated ozone concentrations, the peroxidase activity exhibited a range of values. Specifically, for Tekila, peroxidase activity ranged from 0.77 to 5.41 mM purpurogallin formed min^{-1} mg^{-1} FW under ambient conditions and from 2.72 to 8.44 mM purpurogallin formed min^{-1} mg^{-1} FW under elevated ozone conditions. In the case of Primero, peroxidase activity ranged from 0.51 to 6.73 mM purpurogallin formed min^{-1} mg^{-1} FW under ambient conditions and from 3.01 to 8.3 mM purpurogallin formed min^{-1} mg^{-1} FW under elevated ozone conditions. The increased activity was due to the activation of defensive response against ozone exposure. This spike in the activity of peroxidase will enable the detoxification of H_2O_2 into H_2O (41). The current findings are on par with the findings (42, 43).

3.10. IAA

The Figure.7 illustrates changes in the IAA (Indole-3-acetic acid) content in both cabbage cultivars during different crop growth stages as a result of increased ozone levels and the application of protectants. In the vegetative stage, under elevated ozone conditions, the control of both Tekila and Primero cultivars experienced substantial reductions in IAA content, with Tekila decreasing by 63% and Primero by 56%, as compared to ambient conditions. This decrease in IAA content may be owing to the fact that plants require more energy and substrate to prevent or repair the injuries caused by elevated ozone levels (44). The decrease in IAA levels due to elevated ozone levels are in accordance with the studies conducted (45, 46).

4. CONCLUSION

In conclusion, elevated ozone concentrations have a profound and detrimental impact on the physiological and biochemical processes of cabbage cultivars Tekila and Primero across various growth stages. This study has demonstrated significant reductions in photosynthetic rates, stomatal conductance, chlorophyll content, and an increase in oxidative stress markers like malondialdehyde (MDA) under elevated ozone stress. Conversely, protective mechanisms such as increased proline, ascorbic acid, catalase, and peroxidase activities were observed, indicating the plants' efforts to combat ozone-induced oxidative stress. Additionally, carotenoid content showed a slight increase, potentially aiding in the maintenance of photosynthesis. These findings potray ~~protray~~ the vulnerability of cabbage cultivars to ozone pollution and highlight the need for strategies to mitigate these adverse effects. Further research is crucial for developing solutions that can safeguard crop productivity and ensure sustainable agriculture in the face of escalating environmental challenges.

Table 1. Effect of elevated ozone stress (200 ppb) and protectants on photosynthetic rate, stomatal conductance and chlorophyll content of cabbage cultivars Tekila and Primero

Stages	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})				Stomatal conductance (mol H_2O m^{-2} s^{-1})				Chlorophyll Content				Carotenoid content (mg g^{-1} FW)			
	Ambient		eO3 (200 ppb)		Ambient		eO3 (200 ppb)		Ambient		eO3 (200 ppb)		Ambient		eO3 (200 ppb)	
	Tekila	Primero	Tekila	Primero	Tekila	Primero	Tekila	Primero	Tekila	Primero	Tekila	Primero	Tekila	Primero	Tekila	Primero
Vegetative Stage	23.14 ± 0.09	24.28 ± 0.25	16.54 ± 0.23	15.39 ± 0.27	5.33 ± 0.07	6.12 ± 0.1	2.49 ± 0.01	3.70 ± 0.05	52.36 ± 0.85	51.62 ± 0.09	46.64 ± 0.28	48.40 ± 0.48	0.28 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.19 ± 0.01
Head forming Stage	25.63 ± 0.04	26.56 ± 0.56	21.66 ± 0.3	21.19 ± 0.2	5.75 ± 0.02	6.35 ± 0.11	1.59 ± 0.04	2.69 ± 0.01	58.21 ± 1.04	62.51 ± 0.59	39.01 ± 0.55	59.82 ± 0.18	0.35 ± 0.01	0.30 ± 0.01	0.29 ± 0.01	0.22 ± 0.01
Maturity Stage	25.78 ± 0.42	28.71 ± 0.26	10.34 ± 0.04	13.98 ± 0.19	6.01 ±0.09	6.52 ± 0.09	1.14 ± 0.02	2.43 ± 0.03	101.29 ± 1	117.86 ± 0.21	76.47 ± 0.56	80.02 ± 1.6	0.56 ± 0.01	0.46 ± 0.01	0.45 ± 0.01	0.29 ± 0.01
SED	0.15063				0.04115				0.47309				0.00805			
CD(0.05)	0.33154				0.09058				1.047126				0.1772			

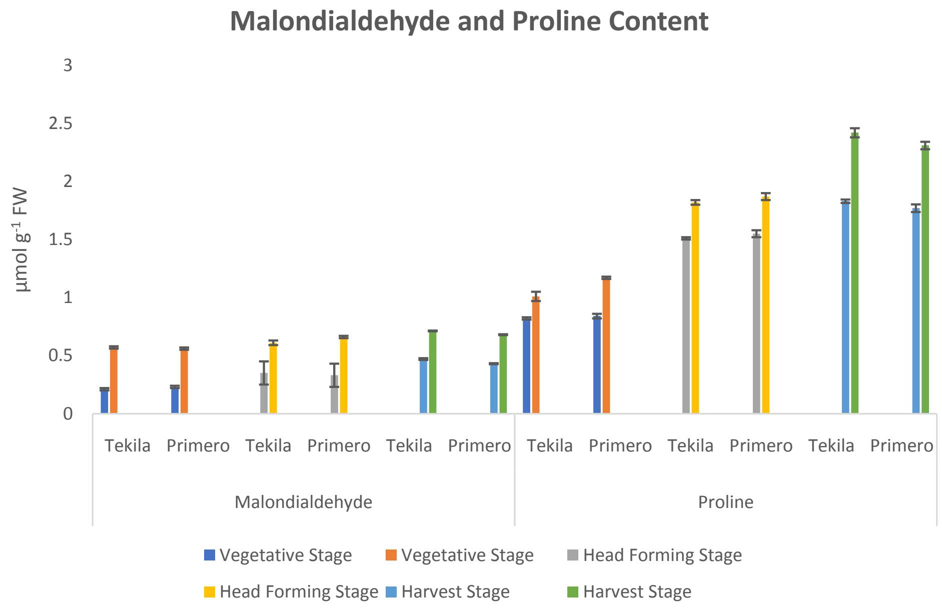

Figure1. Effect of elevated ozone stress (200 ppb) on malondialdehyde and Proline content of cabbage cultivars Tekila and Primero

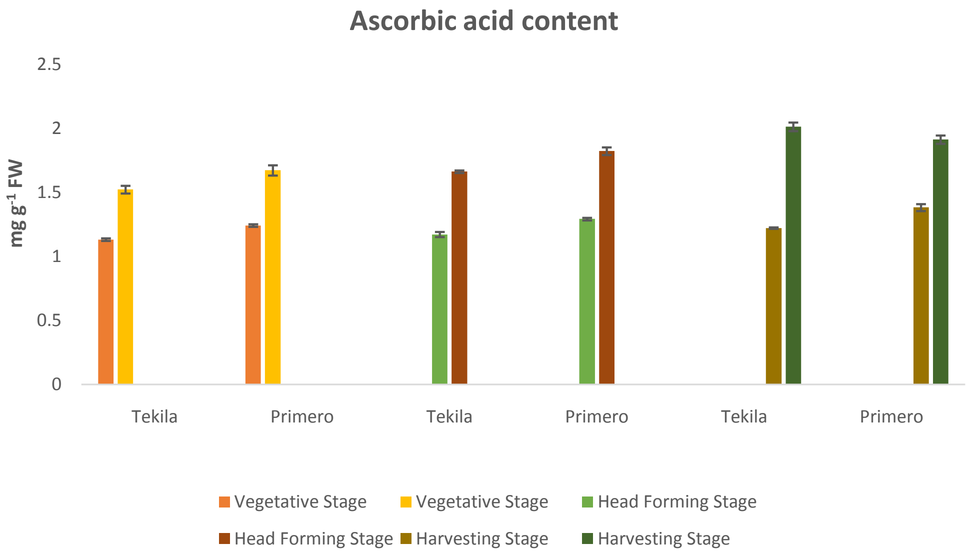

Figure2. Effect of elevated ozone stress (200 ppb) on ascorbic acid content of cabbage cultivars Tekila and Primero

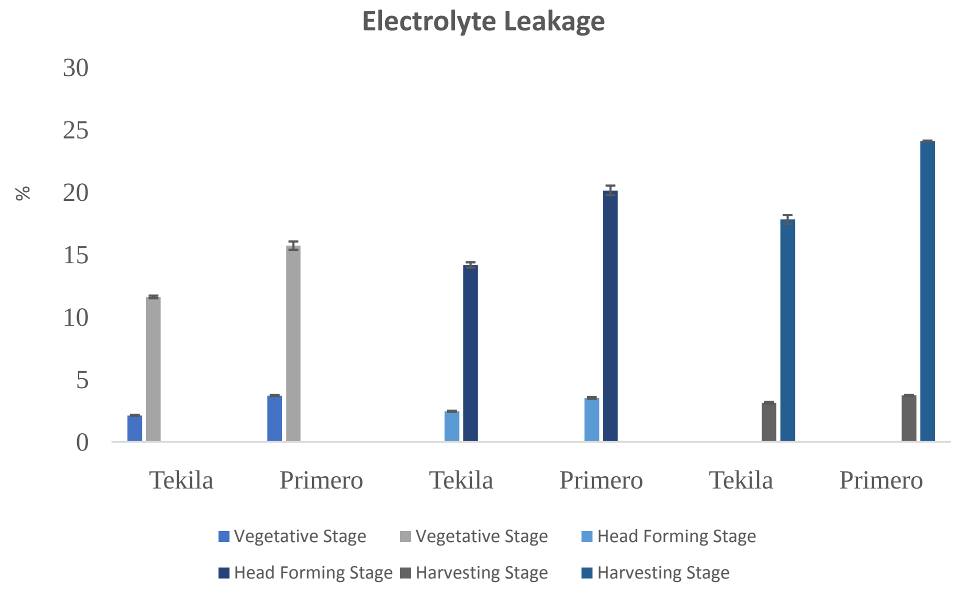

Figure4. Effect of elevated ozone stress (200 ppb) on electrolyte leakage of cabbage cultivars Tekila and Primero

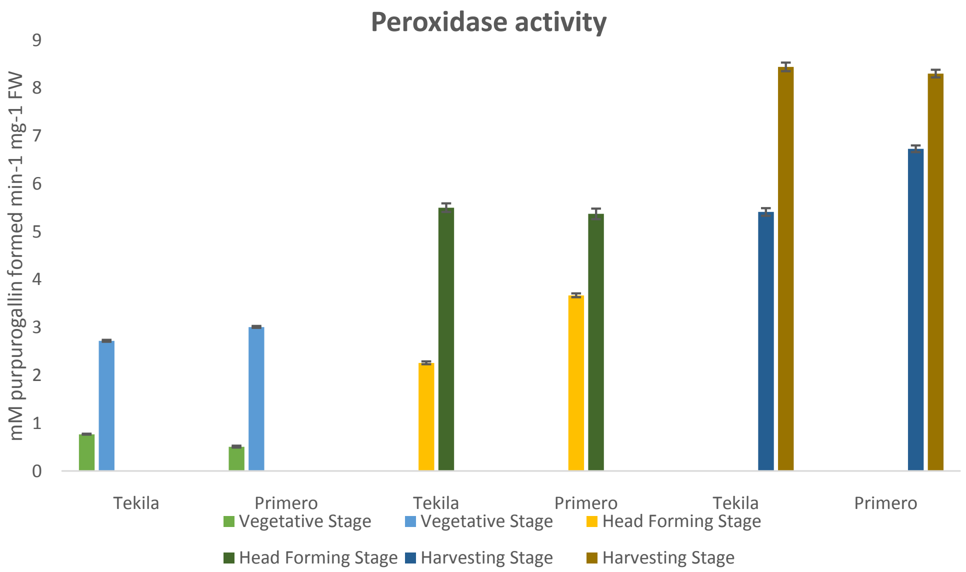

Figure 5. Effect of elevated ozone stress (200 ppb) on Peroxidase activity of cabbage cultivars Tekila and Primero

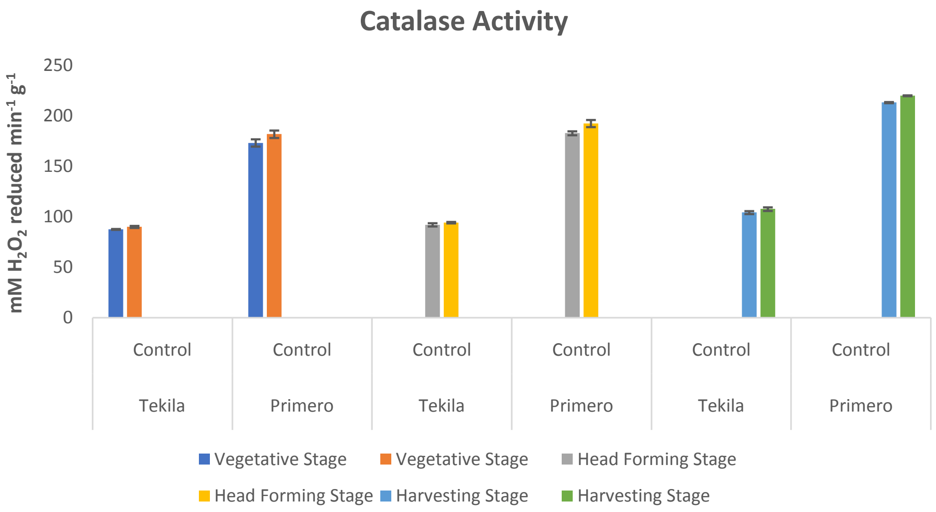

Figure 6. Effect of elevated ozone stress (200 ppb) on Catalaseactivity of cabbage cultivars Tekila and Primero

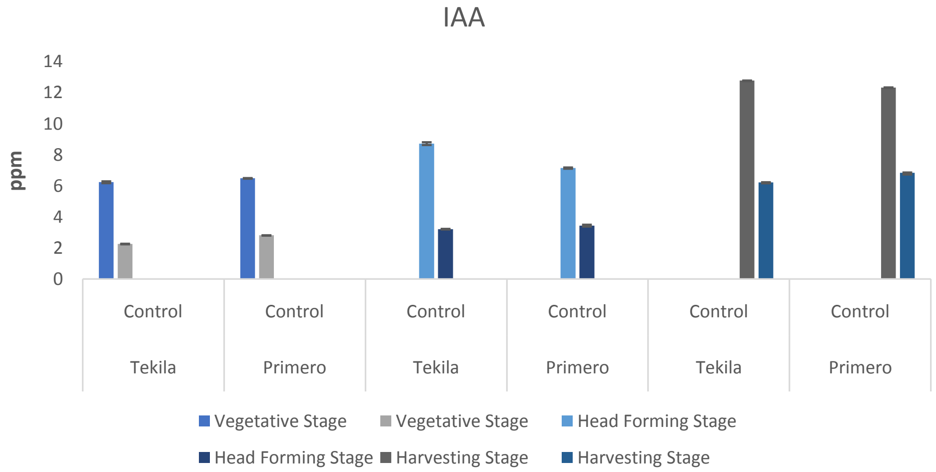

Figure 7. Effect of elevated ozone stress (200 ppb) on IAA of cabbage cultivars Tekila and Primero

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