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Short Note

Ozone and Ultraviolet Injury to Tobacco Plant in Anand District, Gujarat, India

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Abstract: Tropospheric ozone and ultraviolet radiation cause more damage to plants than any other air pollutants finally affecting their productivity. The present study was an attempt to understand the toxic effect of ozone and ultraviolet radiation on tobacco plant (*Nicotiana tabacum*) in Anand district, Gujarat, India. The area is well known for tobacco production and maximum of them are of export quality. Recently, it was reported that the quality of the tobacco is getting affected because of some anonymous atmospheric pollutant. In the quest for same the following study was carried out and the results revealed that when plants were exposed to ozone and ultraviolet radiation it showed marked alterations affecting their overall growth and gross morphology.

Key words: Tropospheric ozone, ultraviolet radiation, tobacco plant, glycine betaine.

Introduction

Environmental stress affects plants in number of ways and is a major focused area of research in modern agricultural biology. More changes in environmental ecology lead to additional deterioration in biodiversity. Adaptive changes driven by ecological stress should be studied at various hierarchical levels from molecules to metabolism and relativeness. Out of many atmospheric pollutants, ozone and ultrviolet radiation are believed to be two important environmental toxicants. Ozone is normally found in stratosphere and the main function is to trap lethal ultraviolet radiation and cosmic rays coming from Sun (Gupta, 2007). Volatile organic compounds are emitted from different sources like fuel combustion, natural emission, industrial by-products and forest fire. They interact with NO radicals to produce ground level ozone also known as tropospheric ozone or bad ozone. Increased concentration of this ozone leads to severe damages to human beings, animals and plants (Heagle, 1989). Abnormal distribution of ozone in troposphere and stratosphere also affects the absorption of ultraviolet radiation in Earth's atmosphere. All these lead to serious challenges.

High concentration of ozone and ultraviolet radiation affect plants in several ways. Particularly ozone causes plants to close their stomata, thus slowing down photosynthesis and plant growth (Heggestad and Middleton, 1959). Several other symptoms are also associated with ozone toxicity like chlorosis, necrosis, purpling of leaves, flecks formation, stipples, bronzing and reddening (Booker et al., 2009). On the other hand regular exposure of ultraviolet radiation to plants decreases plant height, fresh mass of leaves, shoots and roots.

Plants sometimes act as an important stress indicator of an individual area in response to atmospheric

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pollutant. In the present study we have selected tobacco (*Nicotiana tabacum*) as a model plant and have studied alteration in various biochemical parameters with respect to elevated ozone and ultraviolet radiation.

Materials and Methodology

All the chemicals used in the present study are of analytical grade procured from Sigma, S. D Fine chemicals and Hi-media, Mumbai, India.

Plant material: Small plantlets of tobacco plant (*Nicotiana tabacum*) was purchased from the local farm of Anand state, Gujarat. It was grown in pesticide-free soil, where temperature, pH and salt concentration were maintained for 120 days. Plants were divided into three groups: Control plant (G1), plant treated with ozone (G2) and ultraviolet light (G3).

Detection of ozone: Ozone in the air was detected by Schoenbein strips, specially prepared for the same (UCAR, 2004). Four different areas of Anand district were selected for the study namely Mota Bazar, GIDC, Gana and ADIT campus. The plants were grown in these selected areas for 60 days.

Ultraviolet irradiation: Plants were irradiated directly with ultraviolet radiation (long wavelength) for 30 minutes daily for 60 days.

After 60 days, plant leaves from each group were collected during each fortnight and following parameters were studied: Size and area of the leaf, degree of greenness, presence of patch, wet and dry weight, water content, chlorosis, purpling, nodal and internodal distance, and swelling in midrib. Later, completely dried leaves were processed for further investigation of different biochemical parameters.

Leaf extract: One gm of dried leaf powder was grinded in 50 ml of chloroform. The mixture was kept in magnetic stirrer for 2 hrs. The mixture was filtered and was stored at 4°C.

Estimation of total amino acids by Ninhydrin method: Two ml of diluted extract solution was treated with 2 ml of ninhydrin reagent. Tubes were incubated in boiling water bath for 15 mins, cooled at room temperature and further 3 ml of 50% ethanol was added. The absorbance was measured at 570 nm against blank (McGrath, 1972).

Estimation of total chlorophyll content: Chlorophyll was extracted in ethanol and the content was measured at 663 nm and 645 nm (Knudson et al., 1977).

Estimation of glycine betaine: Glycine betaine is reported to accumulate in plants in various stress condition. The estimation was done by dried leaf powder as per the method of Grieve and Grattan (1983). The leaves of selected tobacco plants were dried at room temperature for five days in aseptic condition and further dried in oven at 50°C for 48 hrs. This content was ground to coarse powder and stored in dark. 2.5 g of each sample was taken in 50 ml of ethanol and macerated. Then it was put on shaker for 72 hrs. The extracts were filtered with wattman filter paper no. 1 and concentrated by vacuum evaporation. The obtained powder was further processed for TLC to ensure the presence of betaine in leaves. Further, the betaine spots obtained on TLC plates was scrapped and was re-dissolved in chilled methanol and was processed for HPLC. Betaine was estimated by high performance liquid chromatography (HPLC) using C18 reverse column and mobile phase as methanol : phosphate buffer (50:50) with flow rate of 0.8 ml/min. The injection volume was 20 µl and elute was analyzed at 215 nm.

Result and Discussion

Schoenbein paper strips for the detection of ozone were placed in four different localities in and out of Anand city. The result showed that the area rich in ozone changes the colour of paper strips from white to dark brown and red. As per the U.S. EPA Air Quality Guide for ozone, API in these areas is between 151 to 200, which is unhealthy for living beings. This study significantly explained the presence of tropospheric ozone in the atmosphere (Figure 1a-e). Maximum ozone was found in GIDC area and ADIT campus. Both the places are surrounded by small scale industries. It is believed that the ozone concentration was considerably high because of the release of secondary pollutant by the industry.

The effect of ozone and ultraviolet radiation was studied on various morphological and physical parameters in leaf of tobacco plant. Table 1 explains the difference between control group (G1), leaf grown in ozone rich area (G2) and leaf irradiated with ultraviolet radiation (G3). The size of the treated leaf was found to be significantly smaller than that of control group. Decrease in leaf area along with weight, dry weight and nodal-internodal distance was also observed in leaf treated with ozone and UV radiation, significantly. Marked purpling, chlorosis and patches were also observed in the treated leaf (Figure 2a-e).

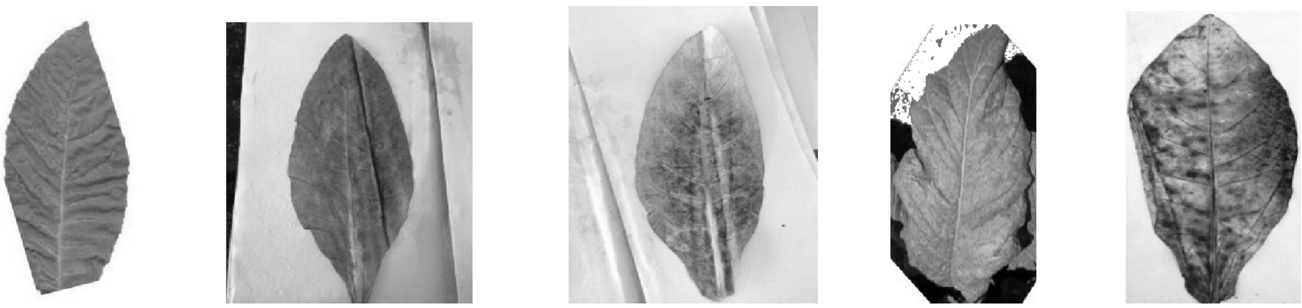
1a. Control 1b. Mota Bazaar 1c. GIDC 1d. Gana 1e. ADIT Campus

Figure 1a-e: Schoenbein paper strips for the detection of ozone in four different localities.

Table 1: Alteration in various parameters of leaves

Sr. No	*Parameters*	*Control leaves (G1)*	*Leaf grown in ozone rich area (G2)*	*Leaf irradiated with ultraviolet radiation (G3)*
1.	Size of leaf	Normal	Smaller than normal	Smaller than normal
2.	Area of leaf (cm^2)	387± 0.5	328± 0.2[a]	314 ± 0.2[a]
3.	Degree of greenness	Leaves were green	Lesser green	Lesser green
4.	Observed patches	Nil	Brown patches	Small brown dots
5.	Weight (gm)	21.83 ± 0.7	20.840 ± 0.56[a]	16.34 ± 0.12[a]
6.	Dry weight (gm)	3.9 ± 0.8	2.36 ± 0.14[a]	3.08 ± 0.25[a]
7.	Chlorosis	Nil	Was observed	Was observed
8.	Purpling	Nil	Was observed	Was observed
9.	Nodal and internodal distance	5.2 ± 0.66	4.6 ± 0.72[a]	4.2 ± 0.69[a]
10.	Swelling in midrib	Nil	Was observed	Was observed

Values are means ± S.E.M.; $n = 10$
[a]As compared to group 1: $p < 0.05$



2a. Control 2b. Gana (O_3 exposure) 2c. GIDC (O_3 exposure) purpling 2d. GIDC (O_3 exposure) chlorosis 2e. Leaf Irradiated by UV brown spots

Figure 2a-e: Alteration in leaf morphology due to ozone and ultraviolet injury.

Size of the Leaf

The leaves of the control plant were normal in size, whereas the treated leaves (G2 and G3), which were exposed to the ozone and UV, were smaller in size as compared to control. Studies have shown that plants affected by ozone are smaller, and may produce fewer healthy seeds (Kline et al., 2009). The leaf size decreases due to shrinkage in the plant cell. This may be because of deregulation in the Na^+- K^+.

Area of the Leaf (cm^2)

Plant exposed to ozone and UV showed lesser area than control leaf. This was certainly because of the smaller size of leaves. The leaf area was calculated by making squares of 4 cm^2 on the leaf surface and calculated on the basis of number of square.

Weight and Dry Weight (gm)

Decrease in overall weight and dry weight was found in leaf treated with ozone (G2) and ultraviolet radiation (G3) than that of control (G1).

Degree of Greenness

Degree of greenness directly correlates with the amount of chlorophyll present in the leaf. The controlled leaves (G1) were found to be more green in colour than that of treated ones (G2 and G3). This may be because of the degradation of the chlorophyll in the leaf. Purpling, chlorosis and brown spots were observed in the leaves due to ozone injury (Figures 2c, 2d and 2e) (Graph 1).

Chlorosis and yellowing of the leaf was found in plant exposed to ozone and ultraviolet light than that of control. Earlier it has been reported that chlorosis decreases the rate of photosynthesis finally affecting the productivity of the plant. These all lead to the overall less biomass of the plant, affecting the crop yield. Plants treated with ozone and ultraviolet light also showed brown dots. These occurrences of patches may be because of alteration in the metabolic pathway of the leaf. With continuing daily ozone exposure, classical symptoms (stippling, flecking, bronzing and reddening) are gradually obscured by chlorosis and necrosis has been reported (Krupa et al., 2001). Many plant species that are sensitive to ozone will show visible injury on the upper leaf surfaces. Discolouration will vary among species, ranging from red to purple to brown. These plants may also shed their leaves early.

Nodal and Intermodal Distance

In control leaf (G1), the nodal and internodal difference was appeared to be the normal one, whereas in the samples collected (G2 and G3), had lesser intermodal difference than the control had. This is due to the overall retardation in the growth of the plant finally affecting the overall biomass.

Swelling in the Midrib

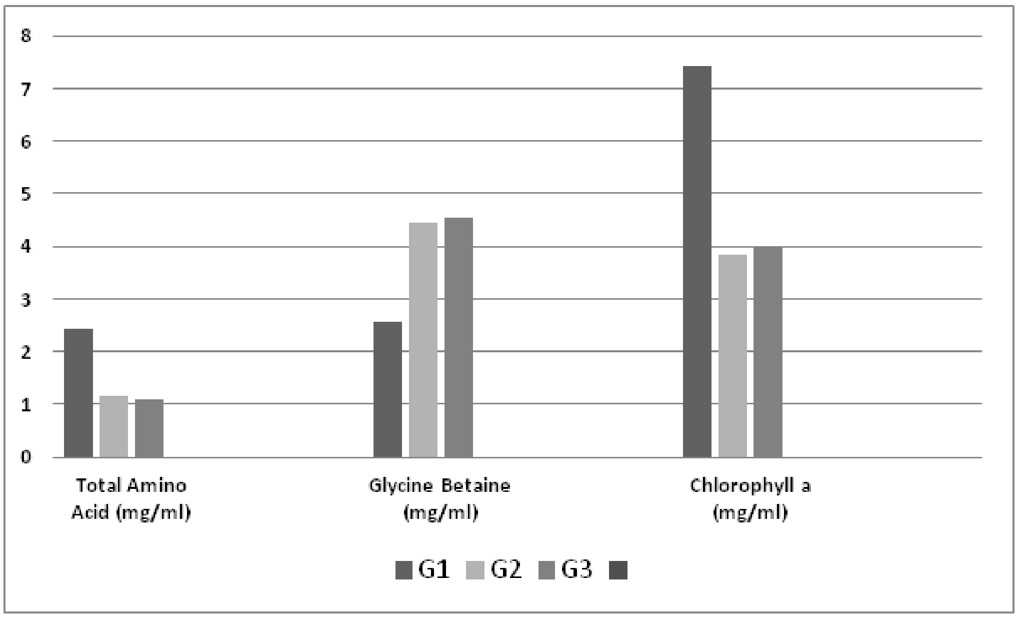
Generally, the ozone injury symptoms can be seen in between the vein or midrib, on the upper surface of the leaves. Here also swelling was observed in plant treated with ozone (G2) and ultraviolet light (G3).

Total Amino Acids and Glycine Betaine

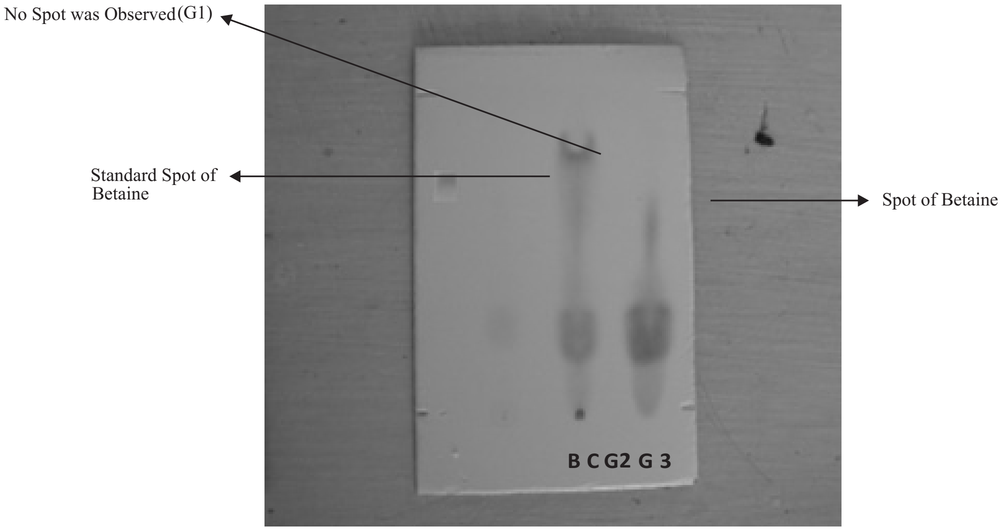
The measurement of amino acids concentration can exhibit the health status of the plants. During stressed condition the quality and the quantity of the amino acid alters. Significant decrease in the total amino acid was found in the plants treated with ozone (G2) and ultraviolet (G3) than that of control plants (G1) whereas increase in the concentration of glycine betaine was found in plants treated with ozone (G2) and ultraviolet (G3) than that of control (G1) (Graph 1).

TLC of processed leaves revealed the presence of Betaine in both ozone (G2) and ultraviolet (G3) treated plants than that of control (G1) (Figure 3). Figure 4 explains the HPLC of the isolated betaine from the leaves treated with ozone.

From the above study it was concluded that tobacco plant shows typical distinguishable features in response to tropospheric ozone pollution and ultraviolet radiation.



Graph 1: Alteration in chlorophyll, amino acid and glycine betaine content in control plant (G1), plant treated with ozone (G2) and ultraviolet light (G3).



(where *B* = Standard Betaine, *C* = Control leaf (G1), G2 = Leaf treated with ozone, G3 = Leaf treated with ultrviolet radiation (G3))

Figure 3: Isolation of betaine; TLC.

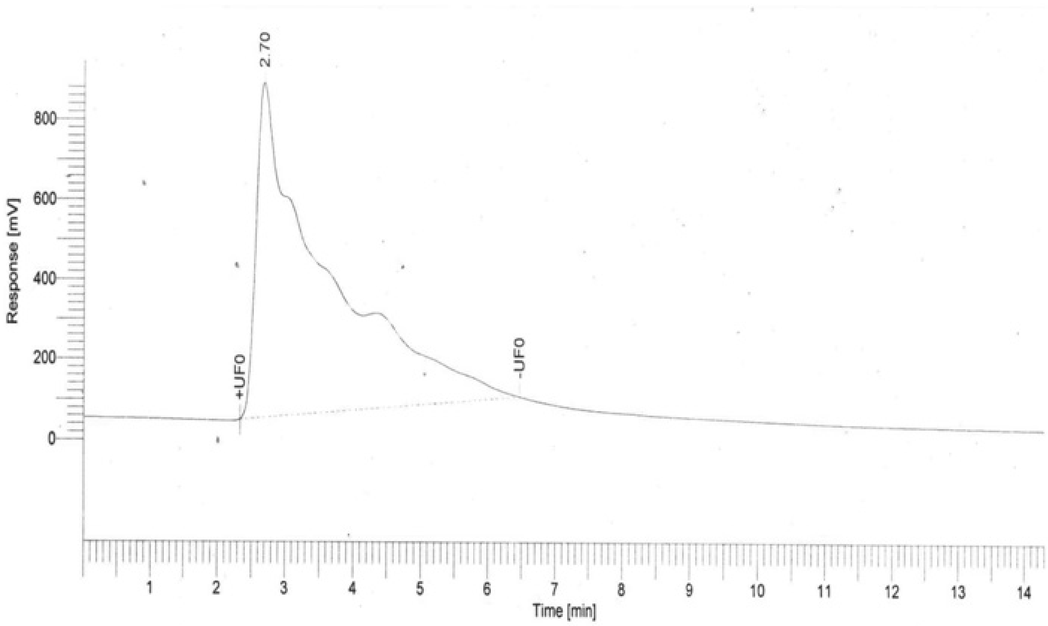


Figure 4: HPLC profile of leaf exposed to ozone; the peak obtained corresponds to glycine betaine.

TLC and HPLC profile further explained the presence of glycine betaine in the plant exposed to ozone and ultraviolet radiation. Betaine, also known as glycine betaine or N,N,N-trimethyl glycine, is a quarternary ammonium compound, derived from glycine. It is reported to accumulate in many plant species under drought, tropospheric ozone, salinity and temperature (high and low) stresses (Fiscus et al., 2005). Report suggests that the endogenous glycine betaine level can be induced by water stress and other stresses produced in the environment. Glycine betaine is a very efficient osmolyte found in a wide range of bacteria and plants. Very high concentrations of cytoplasmic glycine betaine have been reported in response to osmotic stress, which act as an osmoprotectant. Hence glycine betaine can be considered as an ideal biomarker for studying the toxic effect for ozone and ultraviolet toxicity in the plants.

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