AMELIORATION STUDY OF TOTAL FOLIAR PROTEIN IN Lycopersicon esculentum. MILL (TOMATO) BY EXPLORING SODIUM ERYTHROBATE ANTIDOTE AGAINST THE EFFECT OF O₃

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ABSTRACT

There will always be a need to evaluate the quantitative and qualitative loss in various crops and to mitigate it by inducing scavenging potential (tolerance) in the crop plants against air pollution stress through the application of certain plant protectants. The protein level in plant is more important due to its participation in enzyme structure that plays key role in various metabolic activities of plant growth, development and yield Protein synthesis a building up process in plants is also prone to O3 toxicity. Considerable evidence indicates that the free radicals can inactivate proteins by modifying amino acid residues. Loss in Protein content was found in Tomato crop against the gaseous concentration simultaneously significant recovery appeared against O3 gaseous exposure. At the juncture of the study it appears that atmospheric stress responsible for significant crop loss including all physiological and biochemical process. Antioxidant treatment can induce remarkable tolerance for various stress in crops studied. This may because the treatment adds to the internal/ inherent tolerance capacity to the crop plants, which may help in the reduction of losses in all the parameters studied. The present study, decrease in protein content in 51 days Tomato plant age was recorded. The loss in Tomato plant was 16.45 %. After 62 days Plant age Sodium Erythrobate (SE) used during the study was found effective and could reduce the deleterious effect of ozone in Tomato crops. The recovery observed after loss in Tomato plant low dose and high dose of SE brings recovery of 52.55 % and 57.20 % respectively. Sodium Erythorbate (SE) was found best for the amelioration of ozone toxicity.

KEYWORDS: Amelioration, Antidotes, Environmental Pollution, Ozone, Lycopersicon esculentum, Sodium Erythrobate

Everything, which surrounds us, may collectively be termed as the Environment. Living and non-living things around the environment, has influenced and shaped our lives since time immemorial. It is from the environment that we get food to eat, water to drink, air to breathe, land to stand and all necessities of day-to-day life. Human activity generates a tremendous amount of waste materials. These are discharged in various components of the environment in which they bring about undesirable damages. The phenomenon of such damages is termed as Environmental Pollution which has been defined as An undesirable change in physical, chemical or biological characteristics of air, water or land that will be or may be harmful to human and other life.

In the recent past the problem of air pollution is rapidly growing in urban areas throughout the world. Air pollutants may be conveniently divided into two categories. Primary Pollutants, such as sulphur dioxide, carbon monoxide, nitrogen oxides, particulates and hydrocarbons, are emitted directly into the atmosphere. Secondary Pollutants includes troposheric (ground level) ozone and are formed by, subsequent series of chemical reaction between primary pollutants (nitrogen oxides and reactive

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hydrocarbons) and normal atmospheric compounds.

In India first time O_3 injury was reported by Bambewala in (1986) on potato from Punjab. Crop (pulse crop, oil crops and cash crop) loss due to SO_2 alone and along with O_3 has been reported by Dubey (1997, 2002).

MATERIALSAND METHODS

Experiments were conducted at the Institute of Environment Management and Plant Sciences, Vikram University Ujjain (M.P.). Sodium Erythrobate (SE) was selected as Antidotes and *Lycopersicon esculentum* Mill (Tomato) (cv. Pusa samrat, Family Solanaceae) crop was selected to Experiments.

Ten healthy seeds of Tomato crop were sown in earthen pots separately containing 3 kg. Black Cotton Soil. After 10 days of germination and plant growth, thinning was carried out and 4-5 plantlets were allowed to grow further in each pot. Total 32 numbers of pots for Tomato crop was sown for spray treatment. After one month of normal growth plants were subjected to different combination and concentration of gaseous pollutant at the rate of 6hrs/ day. A control set was also run simultaneously. Each set was run with three replicates.

Solution of concentration of 20 ppm and 100 ppm referred as low dose and high dose respectively was prepared of Sodium Erythrobate antidotes (SE) with acetone and distilled water. 100 ml of each solution was sprayed with the calibrated spray gun on plants at the age of 51 and 83 days.

Open Top Chamber (OTC) type of study has been carried out for treatment of O_3 of gaseous pollutant in this investigation. The crop plants were exposed to gaseous pollutant in chamber. It was covered with transparent polythene. The top of the chamber was left open. All the crop plants to be exposed and considered to be control were placed in the chamber to obtain similar environmental condition with or without fumigation. The gas generated pass in the open top chamber through inner side Teflon tube in which holes of 1mm at 30 cm apart done.

Ozone (O₃) Generation

Through UV- B exposure of air Micro amount of ozone liberate iodine when absorbed in a 1% solution of potassium iodide buffered at pH 6.80±0.2. The iodine is determined spectrophotometrically by measuring the absorption of triodide ion at 352nm. The stoichiometry is approximated by the following reaction:

$$O_3 + 3KI + H_2O = KI_3 + 3KOH + O_2$$

Ozone was generated by flowing the air through UV-Tubes at fixed rate of flow rate. The amount of gas generated was monitored by standard method with the help of Portable gas sampler by using the 1% potassium buffer solution and absorbance was read at 352nm at UV-VIS spectrophotometer. Concentration of ozone in the air was calculated with the help of a standard graph prepared by diluting a stock solution of 0.025 M iodine.

Protein content in the leaves was estimated by taking the 1mg sample, crushed with NaOH and centrifuge at3000 rpm for 10 min. After this 0.2 ml supernatant was collected in centrifuge tube and distilled water and trichloro acetic acid were added and again centrifuge at same rpm. Now sediment was dissolved in 2% Na₂Co₃ solution and folin reagent was added and blue colour appears and

intensity was to be read at 750 nm and concentration was calculated with standard graph. Standard graph was prepared by dissolving crystalline bovine albumin.

RESULTS AND DISCUSSION

Amelioration Study of Foliar Protein Content

Life without protein is impossible as they are required for the formation of cell, cell components and tissues so proteins are the building blocks of all organisms. Biological specificity between various organisms depends on their protein consumption. Protein constitutes many metabolic enzymes and any alteration in these enzymes may bring about a dramatic/ drastic change in the plant metabolism. The normal synthesis of protein can be distributed by any environmental stress (like pollution) either by inhibition of some amino acid synthesis or by causing some damage in amino acid sequences (Jain, 2002; Kumar, 2002).

Plant Age 51 Days

Many metabolic pathways are altered by O₃. Ozone induced changes in protein pattern can be mediated by altered gene expression (Heath and Taylor, 1997). Ozone can cause different effects on different protein pools (Friend et al., 1992). For instance, it is known to decrease the concentration of Rubisco. Studies of Rubisco chemistry from O₃ treated leaves suggest oxidative followed by rapid protease digestion (Junqua et al., 2000). While the synthesis of enzymes involved in the antioxidation may increase. Primary impact of ozone is cell walls and adjacent plasmalemma (Sandermann, 1997). Functional and structural breakdown of chloroplast leads photo inhibition, loss of ribulose and rubisco protein in the apoplast and in the outer surface of the plasmalemma damaged through O₃ exposure. Three amino acid residues are particularly sensitive to ozone. Ozonolysis will open up the pyrrol ring of tryptophan and oxidize the sulphydryl group (- SH) of cysteine and methionine to form disulphide bridges (-S-S) Sulphoxides (Wellburn, 1994; Mudd et al, 1997). Similarly in the present study decrease in protein content in Tomato plant was recorded. The loss in Tomato plant was 16.45 %. (Table-1).

Plant age (In days)	Dose	Sodium Erythrobate
30 days	Control	06.14± 0.15
51 days Gaseous treatment for	Control	09.36 ± 0.65
20 days	Treatment	07.82 ± 0.48
Loss ₋₁ at 1^{st} stage (%)		16.45
62 days Antidote treatment and 10 days for recovery	Control	13.81 ± 0.12
	20 ppm	19.73 ±0.48
	100 ppm	21.87±0.69
Recovery ₋₁ at 1^{st} stage (%)	20 ppm	52.55
	100 ppm	57.20
83 days Gaseous treatment for 20 days	Control	$14.31{\pm}~0.08$
	20 ppm	12.91 ± 0.09
	100 ppm	$15.98{\pm}~0.07$
Loss ₋₂ at 2 nd stage (%)	20 ppm	34.56
	100 ppm	26.93
94 days Antidote treatment asnd 10 days for recovery	Control	17.83 ± 3.87
	20 ppm	$15.63{\pm}0.58$
	100 ppm	18.44 ± 0.22
Recovery ₋₂ at 2 nd stage (%)	20 ppm	17.40
	100 ppm	13.34

TABLE 1 : Effect of O³ (60 μg/m³) on Foliar Protein (mg/g Fresh wt.) in Tomato Plant andTheir Amelioration by Sodium Erythrobate (SE) Antidote

±: Standard Deviations

SE: Sodium Erythrobate

Plant Age 62 Days

After considerable reduction of protein content under the O_3 of gaseous exposure, significant recovery observed on this plant age which suggests that Sodium Erythrobate antidote treatment mitigate the adverse effect of air pollution. Plant antioxidant system, which scavenges naturally, occurring ROS (Reactive Oxygen Species) compounds, could function as primary mechanism to alleviate the oxidative burden resulting from ozone exposure. Reactive oxygen species may also act as a signal to initiate or coordinate other processes such as ethylene production, which induces senescence (Pell et al., 1997; Schraudner, et al., 1997). Reactive oxygen species are detoxified by antioxidant systems in the apoplasm, cytoplasm, and chloroplast

Sodium Erythrobate (SE) used during the study was found effective and could reduce the deleterious effect of ozone in Tomato crops. The recovery observed after loss in Tomato plant low dose and high dose of SE brings

recovery of 52.55% and 57.20% respectively. (Table-1).

Plant Age 83 Days

Plant responses depend not only on the inherent characters of plant species but also on the stage of development, age and nutritional status (Kovacs, 1992). On this age O₃ shows considerable loss in the protein content in Tomato crops. Tropospheric ozone has profound negative impacts on the growth, development and productivity of plants. Many metabolic pathways are also altered by ozone exposure. A toxic product of O₃ may migrate through the cytoplast to react with photosynthetic processes, or a spurious signal generated at the membrane may affect some control process or signal transduction pathway, which ultimately alters the carbon assimilation and protein synthesis. (Schraudner et al., 1998; Overmyer et al., 2000, 2003; Rao et al., 2002; Booker et al., 2004; Leitao et al., 2003; Rao and Davis, 2001; Sandermann, 2000; Vahala et al., 2003). The maximum loss in protein in Tomato plant on this plant age was 59.09%. (Table-1)

Plant Age 92 Days

Significant recovery in protein content after antidote treatment in O_3 of gaseous exposure was observed which suggest that antidotes treatment must be promising in ameliorating the effect of air pollutants and allows the plants grow under natural conditions. With the application of different antidotes significant recovery was observed in the studied crops. In Tomato plant at 20- ppm the minimum recovery observed was 17.40% and 100-ppm the minimum recovery observed was 13.34 %. (Table-1) The use of antidotes and fertilizer may inhibit response of O_3 and protects crops from the damage (Haryani, K. 2002).

REFERENCES

- Bambawale O. M., 1986. Evidence of ozone injury to a crop plant in India. Atmospheric Environment 20, 1501-1503.
- Booker F. L., Fiscus E. L., Miller J. E., 2004. Combined effects of elevated atmospheric carbon dioxide and ozone on soybean whole-plant water use. Environ. Manage. 33: S355-S362.
- De Kok L. J. and Tausz M., 2001. The role of glutathione in plant reaction and adaptation to air pollutants. In: Grill, D.; Tausz, M.; De Kok, L. J., eds. Significance of glutathione in plant adaptation to the environment. Amsterdam, The Netherlands: Kluwer Publishers : 185-208.
- Dubey P. S., 1997. Pulses, Oil and Fiber Crop loss due to air pollution in India. Project Report submitted to Imperial College. Centre for Environmental Technology, London.
- Dubey P. S., 2002. Assessment of Agricultural and Fruit Crop Loss due to air pollution in South West M.P. Project Report submitted to ICAR, Ministry of Agriculture and Forestry, Govt. of India, New Delhi.
- Friend A. L., Tomlinson P. T., Dickson R. E., O'Neill E. G., Edwarda N. T., and Taylor G. E., 1992.
 Biochemical composition of loblolly pine reflects pollutant exposure, Tree Phytol., 11, 35.

- Haryani K., 2002. Evaluation of responses of certain crops against air pollutant in field and laboratory conditions. Ph. D. Thesis, Vikram university, Ujjain (M.P.) India.
- Heath R. L., Taylor G. E., Jr., 1997. Physiological processes and plant responses to ozone exposure. In: Sandermann, H.; Wellburn, A. R.; Heath, R. L., eds. Forest Decline and Ozone: a comparison of controlled chamber and field experiments. a comparison of controlled chamber and field experiments. New York, NY: Springer-Verlag; pp. 317-368.
- Jain N. K., 2002. Studies on induction of Scavenging Potential against Sulphur dioxide toxicity in lentil and Mung bean. Ph. D. Thesis, Vikram University, Ujjain (M.P.) India.
- Junqua M., Biolley J. P., Pie S. Kanoun M., Duran R., and Goulas P., 2000. In *vivo* occurrence of carbonyl residues in residues in Phaseolus vulgaris proteins as a direct consequence of a chronic ozone stress, Plant physiology and Biochemistry, **38**: 853-861.
- Kovacs M., 1992. Herbaceous flowering plants. In: Biological indicators in environmental protection. (eds. Kovacs, M.) Ellis Horewood, New York.
- Kumar D., 2002. Qualitative and Quantitative Loss Assessment in a few crops due to Air Pollution. Ph. D. Thesis, Vikram university, Ujjain (M.P.) India.
- Leitao L., Goulas P. and Biolley J. P., 2003. Time-course of Rubisco oxidation in beans (*Phaseolus vulgaris* L) subjected to a long-term ozone stress. Plant Sci. 165: 613-620.
- Lowry O. H., Rosebrough N. J., Farr A.C. and Randali R.J.,, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., **193**, 265-275.
- Mudd J. B., Dawson P. J. and Santrock J., 1997. Ozone does not react with human erythrocyte embrane lipids. Arch. Biochem. Biophys. **34**1: 251-258
- Overmyer K., Brosche M. and Kangasjarvi J., 2003. Reactive oxygen species and hormonal control of cell death. Trends Plant Sci. 8: 335-342.

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- Overmyer K., Tuominen H., Kettunen R., Betz C., Langebartels C., Sandermann, H., Jr. and Kangasjarvi, J., 2000. Ozone-sensitive Arabidopsis rcd1 mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell **12**: 1849-1862..
- Pell E. J., Schlagnhaufer C. D. and Arteca R. N., 1997. Ozone-induced oxidative stress: mechanisms of action and reaction. Physiol. Plant. 100: 264-273.
- Rao M. V., Davis K. R., 2002. The physiology of ozone induced cell death. Planta, **213**: 682-690.
- Rao M. V., Lee H. I., Davis K. R., 2002. Ozone-induced ethylene production is dependent on salicylic acid, and both salicylic acid and ethylene act in concert to regulate ozone-induced cell death. Plant J. 32: 447-456.
- Schenone G., Botteschi G., Fumagalli I. and Montinandi F., 1992. Effects of ambient air pollution in open top chambers on bean (*Phaseolus vulgaries* L): effects on growth and yield. New phytologist, **122**: 689-697.

- Schraudner M., Moeder W., Wiese C., Van Camp W., Inze D., Langebartels C., Sandermann H. Jr., 1998. Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco Bel W3. Plant J., 16:235-245.
- Schraudner M., Langebartels C., Sandermann H., 1997. Changes in the biochemical status of plant cells induced by the environmental pollutant ozone. Physiol. Plant. 100: 274-280.
- Sandermann H. Jr., 2000. Ozone/biotic disease interactions: molecular biomarkers as a new experimental tool. Environ. Pollut. 108: 327-332.
- Sandermann H., Jr., Wellburn A., Heath R. L. (1997); Forest decline and ozone: synopsis, in Forest Decline and Ozone, Sandermann, H., Jr., Wellburn, A., Heath, R. L., Eds., Springer- Verlag, Berlin, 369.
- Vahala J., Ruonala R., Keinanen M., Tuominen H. and Kangasjarvi J., 2003. Ethylene insensitivity modulates ozone-induced cell death in birch. Plant Physiol., 132: 185-195.
- Wellburn A., 1994. Air pollution and climate change: the biological impact. Harlow, Essex, United Kingdom: Longman Scientific & Technical, 268.