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DOSE RESPONSE STUDY OF N, N'-DIMETHYLUREA (DMU) ANTIDOTE TOTAL FOLIAR PROTEIN IN *Lycopersicon esculentum* AGAINST THE EFFECT OF O_3

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ABSTRACT

To overcome the problem of agriculture loss due to air pollution, an alternative method would involve the application of protective chemical components to plants in the field to prevent injury. The studies of the potential for protection of vegetation from the oxidative gases using chemical application have been performed over the last three to four decades. This would allow the plant to grow under completely natural conditions and to be exposed to fluctuating natural concentrations of ground level ozone. Such diverse groups of chemical compounds are known as antidotes. 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 O_3 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 O_3 gaseous exposure. This may because the treatment adds to the internal/ inherent tolerance capacity to the crop plants, which may help in the reduction of losses. 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 N, N'-Dimethylurea (DMU) 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 49.24% and 52.84% respectively. N, N'-Dimethylurea (DMU). was found best for the amelioration of ozone toxicity.

KEYWORDS : Amelioration, Antidotes, Environmental Pollution, Ozone, Lycopersicon esculentum, N, N'-Dimethylurea (DMU)

Atmospheric pollution is a major problem taking all nations of the world. Rapid urban and industrial growth has resulted in vast quantities of potentially harmful waste products being released into the atmosphere. The consequence has been that air pollution has caused widespread damaged to vegetations, crops, wildlife, building materials and climate. Air pollutants interact in variety of way with all living things. Since plants are dominant life forms in number and size over most of the earth surface, they interact with gaseous and particulate pollutants. Some plants absorb the pollutant with no concomitant effect on their growth and functioning while some were considered to be relatively more sensitive. Increasingly physiological, structural and biochemical effects or injuries are being used in the pollution studies. Depending upon the concentration and duration of gaseous exposure, plant response is arbitrarily classified in to three categories, acute injury, chronic injury and physiological injury. Injuries of plant finally result from the exhaustion of its repairing and compensating potential, which leads to faster alteration of biochemical pathways.

Ozone is a strong, gaseous oxidizing agent, which reacts rapidly with other atmospheric constituents. Photochemical oxidants result from atmospheric interactions of NO_2 and sunlight. Ozone is relatively unstable and releases its third oxygen atom readily so that it oxidizes or burns things more readily and at lower concentration than does normal oxygen. Chemically ozone is very active; it has a short average lifetime in the air because of the effect of sunlight on normal oxygen. Ozone forms a natural layer high in the atmosphere (Stratosphere) this ozone layer protects us from UV. The formation of ozone is the result of complex non-linear physical and chemical processes in the atmosphere including two classes of precursor pollutants NO_2 (Nitrogen dioxides) and VOC (Volatile Organic Compounds) Thus, in the lower atmosphere, ozone is a secondary pollutant formed from gaseous precursors. The effect of ozone on plants can be subtle. At very low concentration ozone can reduce growth rate while not producing visible injury. At high concentration ozone kills leaf tissue, killing entire leaves and if the pollutant level remains high, killing whole plant.

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Experiments with ozone protectant chemicals have also indicated that ozone can cause significant effects on the yield of *Lycopersicon esculentum* in and around New Delhi, on radish and turnip yields at a rural site in the Nile delta (Hassan et al., 1995), on yields of *Phaseolus vulgaris* in the Valley of Mexico (Ashmore and Marshall 1999; De Bauer 2003), and on soybean (*Glycine max*) in the Pakistan Punjab (Wahid et al. 2001). Recent data demonstrate that rural ozone levels are high enough to potentially affect yields of winter wheat (Chameides et al. 1999) and other crops (Zheng et al. 1998).

MATERIALS AND METHODS

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

O_3 (60µg/m^3) Gas treatment

Experimental Set

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.

Mode of Application of Antidotes

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.

Instruments Employed in Study

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 (O_3) Generation

Through UV- B exposure of air by Byers & Saltzman, 1958) - Micro amount of ozone liberate iodine when absorbed in a 1% solution of potassium iodide buffered at pH 6.80±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 Byers & Saltzman, 1958, 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.

Estimation of Protein Content (Lowry et.al., 1951)

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 in2% Na_2CO_3 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

Numerous techniques are available for identifying or measuring response of plants to oxidative stress such as physiological and biochemical changes. Many of these changes in stomatal conductance photosynthesis leaf pigments, proteins, and lipids or other macromolecules and metabolite pool can be used as indicator for assessment of pollutant effects on vegetation and crops (Heath et al., 1997).

Amelioration Study of Foliar Protein Content

Proteins are synthesized in plants with the help of amino acids. Plant absorbs nitrogen in the form of NO_3. This is subjected to reduction, so NO_3 is converted into NH_3. This NH_3 reacts with carbonic acid to form amino acids. Finally, amino acids condense, to form proteins.

Plant Age 51 Days

The effect of oxidative pollutant on plants occurs when they enters the plant leaves disrupting oxidative photosynthesis processes, decreases in stomatal conductance, reduction in chlorophyll content, reduced carboxylation efficiency, energy budgets of the plant, decrease in Rubisco activity, affecting soluble protein, reduces growth and yield response Many metabolic pathways are altered by O_3. Ozone can cause different effects on different protein pools (Friend et al., 1992). Studies of Rubisco chemistry from O_3 treated leaves suggest oxidative followed by rapid protease digestion (Junqua et al., 2000). Functional and structural breakdown of chloroplast leads photo inhibition, loss of ribulose and rubisco protein (Heath et al., 1997) in the apoplast and in the outer surface of the plasmalemma damaged through O3 exposure (Mudd et al, 1997a). 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., 1997b). 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 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

Table 1 : Effect of O_3 (60µg/m^3) on Foliar Protein (mg/g Fresh wt.) in Tomato Plant and Their Amelioration by N, N'-Dimethylurea (DMU) Antidote

Plant age (In days)	Dose	N, N'-Dimethylurea (DMU)Antidote (ppm)
30 days	Control	06.14± 0.15
51 days Gaseous treatment for 20 days	Control	09.36± 0.65
	Treatment	07.82± 0.48
Loss$_{-1}$ at 1st stage (%)		**16.45**
62 days Antidote treatment and 10 days for recovery	Control	13.81 ± 0.12
	20 ppm	18.44 ±0.73
	100 ppm	19.85 ±1.41
Recovery$_{-1}$ at 1ststage (%)	20 ppm	**49.24**
	100 ppm	**52.84**
83 days Gaseous treatment for 20 days	Control	14.31 ±0.08
	20 ppm	10.33 ±0.17
	100 ppm	13.45±0.56
Loss$_{-2}$ at 2nd stage (%)	20 ppm	**43.98**
	100 ppm	**33.24**
94 days Antidote treatment and 10 days for recovery	Control	17.83 ±3.87
	20 ppm	12.79 ±0.14
	100 ppm	15.33 ±0.61
Recovery$_{-2}$ at 2nd stage (%)	20 ppm	**19.23**
	100 ppm	**12.26**

±: Standard Deviations
DMU - N, N'-Dimethylurea

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). Some workers reported that applications of two recently developed fungicides (azoxystrobin and epoxiconazole) provided protection to spring barley to relatively high O_3 exposures and resulted in increases in leaf soluble protein content as well as the activity of several antioxidative enzymes (e.g., Superoxide dismutase, catalase, ascorbate-peroxidase, and glutathione reductase).

N, N'-Dimethylurea (DMU) 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 DMU brings recovery of 49.24% and 52.84% 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 O_3 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 O_3 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; De Caria et al., 2000; 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 at 20-ppm was 43.98 % and 100-ppm was 33.24%. (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 19.23% and 100-ppm the minimum recovery observed was 12.26 %. (Table-1) The use of antidotes and fertilizer may inhibit response of O_3 and protects crops from the damage (Haryani, K. 2002; Dubey, 2002).

REFERENCES

Ashmore M. R. & Marshall F.M., 1999. Ozone impacts on agriculture: an issue of global concern. Advances in Botanical Research 29, 3152.

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.

Byers D. H., and Saltzman B. E.., 1958. Determination of ozone in air by neutral and alkaline iodide procedures. J. Am. Ind. Hyg. Assoc.,19, 251-257.

Chameides W.L. and Xingsheng L., Xiaoyan, T. (1999); Is ozone pollution affecting crop yields in China? Geophysical Research Letters 26, 867870.

De Bauer, M.L., 2003. Air pollution impacts on vegetation in Mexico. In Air Pollution Impacts on Crops and Forests a Global Assessment (eds L.D. Emberson, M.R. Ashmore & F. Murray), pp. 263286. Imperial College Press, London, UK.

DeCaria A. J., Pickering K. E., Stenchikov G. L., Scala J. R., Stith J. L., Dye J. E., Ridley B. A., Laroche P., 2000. A cloud-scale model study of lightning-generate NOx in an individual thunderstorm during STERAO-A. J. Geophys. Res. [Atmos.] 105: 11601-11616.

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., & Taylor G. E., 1992. Biochemical composition of loblolly pine reflects pollutant exposure, Tree Phytol., 11, 35.

Hassan I. A., Ashmore M. R., and Bell J. N. B., 1995. Effect of ozone on radish and turnip under Egyptian field conditions. Environmental Pollution 89, 107-114.

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.

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.

Junqua M., Biolley J. P., Pie S. Kanoun M., Duran R. & 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.

Leitao L., Goulas P. & 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. & Santrock J., 1997a. Ozone does not react with human erythrocyte embrane lipids. Arch. Biochem. Biophys, **341**: 251-258.

Mudd J. B., Dawson P. J., Tseng S. &Liu F. P., 1997b. Reaction of ozone with protein tryptophans: and III, serum albumin, and cytochrome C. Arch. Biochem. Biophys. **338**: 143-149.

Overmyer K., Brosche M. & Kangasjarvi J., 2003. Reactive oxygen species and hormonal control of cell death. Trends Plant Sci. **8**: 335-342.

Overmyer K., Tuominen H., Kettunen R., Betz C., Langebartels C., Sandermann, H., Jr. & 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. & Arteca R. N.., 1997. Ozone-induced oxidative stress: mechanisms of action and reaction. Physiol. Plant. **100**: 264-273.

Rao M. V. & Davis K. R., 2001. 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.

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**:235245..

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.

Vahala J., Ruonala R., Keinanen M., Tuominen H. & Kangasjarvi J., 2003. Ethylene insensitivity modulates ozone-induced cell death in birch. Plant Physiol. **132**: 185-195.

Wahid A., Milne E., Shamsi S.R.A., Ashmore M. R. & Marshall F. M., 2001. Effects of oxidants on soybean growth and yield in the Pakistan Punjab. Environmental Pollution, **113** : 271-280.

Wellburn A., 1994. Air pollution and climate change: the biological impact. Harlow, Essex, United Kingdom: Longman Scientific & Technical, 268.

Zheng Y., Stevenson K. J., Barrowcliffe R., Chen S., Wang H. & Barnes J.D., 1998. Ozone levels in Chongging: a potential threat to crop plants commonly grown in the region? Environmental Pollution, **99**, 299-308.