IMPACT OF SIMULATED ACID RAIN (SAR) ON CATALASE, PEROXIDASE, PROLINE AND MALONDIALDEHYDE CONTENT OF SPINACH (*SPINACIA OLERACEA*)

Vimlesh Rawat & Ratna Katiyar ,

Department of Botany

Lucknow University,

Lucknow

ABSTRACT

Present study was carried out to investigate the impact of simulated acid rain (SAR) of different acids (HCl, H_2SO_4 and HNO_3) on green leafy vegetable spinach (Spinacia oleracea L.) belongs to the Family Chenopodiaceae. Two distinct pH-levels 5.0 and 3.0 were made by using HCl, H_2SO_4 and HNO_3. Plants treated with SAR containing HCl showed slight induction in catalase, peroxidase, proline and malondialdehyde content of spinach. The data revealed SAR treatment containing H_2SO_4 and HNO_3 acid showed more significant increase in all aforesaid parameters.

Keywords: *Simulated acid rain, Spinach, Chenopodiaceae, proline.*

INTRODUCTION

Air pollution is the worst outcome of the industrial revolution as it has become a major source of acid rain and posing a threat to the healthy existence of natural and cultivated ecosystems. Acid rain can be called as an unseen plague of the industrial age. The principal constituents responsible for acid rain are sulphur dioxide and nitrogen dioxides. These are emitted from chimneys of industrial plants and other sources causing profound deterioration of urban air quality resulting from industrialization, urbanization, economic growth associated with an increase in energy demands (Kabir, *et al.*, 2012). The reaction of these gases with water vapour present in clouds form acids. Sulphur dioxide, nitrogen dioxide, carbon dioxide are the most responsible pollutants causing damage to the material. The intensity of damage caused by sulphur dioxide is more compared to the other pollutants. Acid rain has adverse impacts on almost all living and non living things. It has been recognized that herbaceous plants are more sensitive to direct injury by acid rain than woody plants (Heck *et al.*, 1986). Acidity damages vegetation while susceptible microbial species are eliminated from the soil affecting processes such as decay and decomposition of organic debris and the capacity of a balanced regulation of nutrients. Soils in general have a greater buffering capacity than aquatic system. However excessive amount of acids introduced by acid rains may disturb the entire soil chemistry (Bunyard, 1985).

Keeping in view the importance of acid rain, the present study was aimed to assess the effect of different levels of simulated acid rain on catalase, peroxidase, proline and malondialdehyde content of Spinach (*Spinacia oleracea*)

MATERIALS AND METHODS

The experiment was performed in Plastic pots which were filled with 2 kg garden soil properly mixed with compost were used for the experiments. The experiment was performed under glass house conditions. Simulated acid rain was applied thirty days after germination and the simulated acid rain solutions were made by using hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and Nitric acid (HNO_3).Different pH were obtained by adding distilled water to each solution and it was maintained by using pH meter. Required pH solutions were prepared immediately before application. Spinach, a leafy vegetable was selected for the experiment as it has enough leaf area to get exposed. Plants were grown in ambient conditions for four weeks and then pots were exposed to simulated acid rains with pH 5 and pH 3. Spraying of SAR was performed by using domestic hand sprayer and allowed to fall on the foliage as well as the soil. Control plants were separated from treated plants to avoid any kind of disturbance. The treatment was properly sprayed as rainfalls on plants on alternate days for four weeks.

Proline content was estimated by the method of Bates *et al.,* (1973). Lipid peroxidation was measured in terms of thio-barbutric acid reactive substances (TBARS) formation by the method of Heath and Packer, (1968). Catalase was assayed by an adaptation method of Euler and Josephson (1927). Peroxidase was assayed by the modified method of Luck (1963).

Data were analyzed statistically. Average mean and standard error (Mean ± SE) was calculated. Least significant difference LSD (*) at P ≤ 0.05 and highly significant (**) P ≤ 0.01 levels to determine the significant differences and percentage decrease over control were also calculated.

RESULTS

Catalase and peroxidase activity was found to be increased with the increasing pH of SAR treatment. It was recorded as 424.6 µmol H_2O_2 decomposed mg^{-100} FW and 46.2 mg^{-100} FW respectively in control plants. The maximum significant increase in catalase and peroxidase was observed as 6.24, 10.55, 11.92 and 24.02, 38.31, 53.03 % respectively at pH 3 SAR treatments of HCl, H_2SO_4 and HNO_3 respectively over control.

proline content was found to be increased with the increasing pH of SAR treatment. It was recorded as 2.40 nmol g^{-1}FW in control plants. A significant induction was observed as 37.5, 83.33 and 116.67 % at pH3 SAR treatments of HCl, H_2SO_4 and respectively over control.

MDA content was found to be increased with the increasing pH of SAR treatment. It was recorded as 79.00 nmol g^{-1}FW in control plants. A significant induction was observed as 28.73, 44.18 and 48.73% at pH 3 SAR treatments of HCl, H_2SO_4 and HNO_3 respectively over control.

DISCUSSION

Enzymatic activity was found to be induced with the increase in acidic pH. Accumulation of ROS may be due to the disruption of the balance between their production and the antioxidative system activity which is composed of enzymatic antioxidants such as CAT and POD. It is well documented that abiotic stress can induce an imbalance of the redox homeostasis of cell. Activities of antioxidative enzymes (CAT, POD etc.) change with the intensity of the stress factor (Vuleta *et al.,* 2009). According to some authors various reactions of species to acid stress depends on difference in the ability of CAT and POD to remove free radicals. In particular, POD is regarded as more sensitive to acid stress than CAT (Lihong *et al.,* 2008). POD is known as poly-functional enzyme, taking active part in cell metabolism (Gazarian *et al.,* 2006). Its main function is protection against the harmful effect of hydrogen peroxide. Moreover, its activation may be connected with the realization of protective abilities of plant and retention of cell homeostasis.

Induction in proline and MDA content was observed in all the three acid treatments at pH 3. Accumulation of proline is also an indication to plant adaptation to unfavourable conditions. It regulates the osmotic equilibrium between the cytoplasm and vacuole. Positive co-relation between proline accumulation and stress intensity has been reported by Greenway and Munns, (1980). Extracellular and intracellular acidification due to strong acid treatment at a subzero temperature might cause damage due to conformational changes in macromolecules, phase transition of some membrane lipids (Cevc, 1987), decrease in various metabolic activities and generation of reactive oxygen species (Yu *et al.,* 2002) in some herbaceous plants sensitive to acid treatment. Tankha and Gupta, (1992) showed the increase in content of proline with increasing SO_2 concentration. According to existence of SO_2 and CO in the industrial area as the result of chemical activities, these results probably indicate that it has been clearly inconceivable to designate a harmless threshold toxic SO_2 concentration for level of particular species since other **environmental factors** during pollution profoundly affect the degree of damage (Seyyednejad *et al*., 2009). The deleterious effects of the pollutants are caused by the production of reactive oxygen species (ROS) in plants, which cause peroxidative destruction of cellular constituents. It has been reported that proline acts as a free radical scavenger to protect plants away from damage by oxidative stress (Wang *et al.,* 2009). Estimation of MDA amount, which is a secondary end product of polyunsaturated fatty acid oxidation, is widely used to measure the extent of lipid peroxidation as indicator of oxidative stress (Lin and Kao, 2000). Peroxidation of lipids in plant cells appears to be initiated by a number of multifarious roles in oil pollution including light harvesting and protection from oxidative damage caused by drought (Oke and Hamburger, 2002).

CONCLUSIONS

Present study concludes that acid rain adversely affects the growth of spinach plants. Induced defense mechanism of spinach is the clear indication of stressed conditions. All the studied parameters were found increased and it was more significant at pH 3. Damaging effects of sulphuric acid and nitric acid were more prominent in comparison to hydrochloric acid. Consequently, spinach which is a widely-grown vegetable is expected to be threatened over time if the anthropogenic sources of acid rain formation are not managed effectively. Acid deposition has the potential to reduce the yield and market value of important crops. In the wake of above study it is necessary to minimize the use of all such products which enhance the production of SO_2 and NO_2. The awareness among the public is also important to stimulate the concerned authorities to initiate control and remedial measures.

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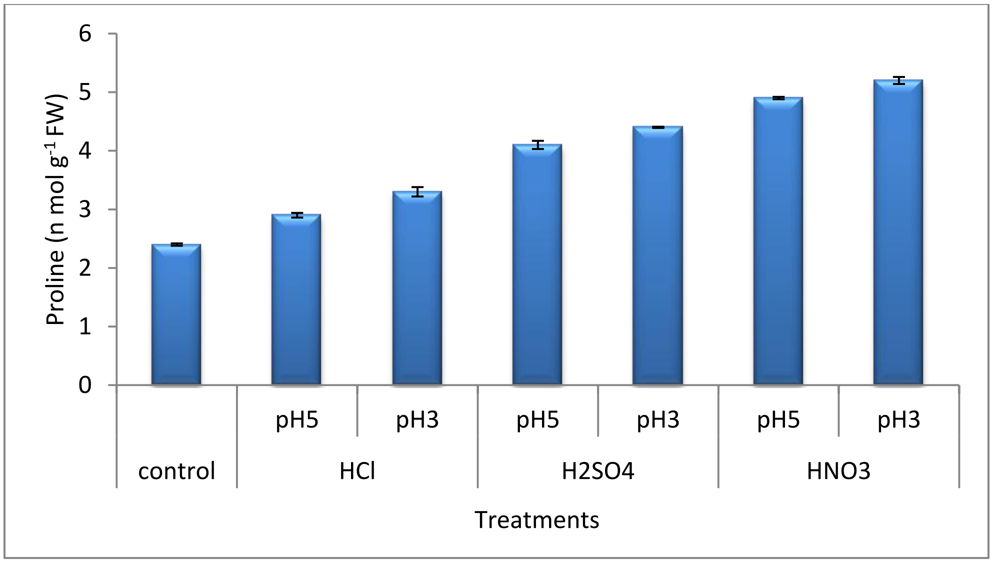

Fig1. Effect of simulated acid rain (SAR) of different pH on proline content of spinach (*Spinacia oleracea)*

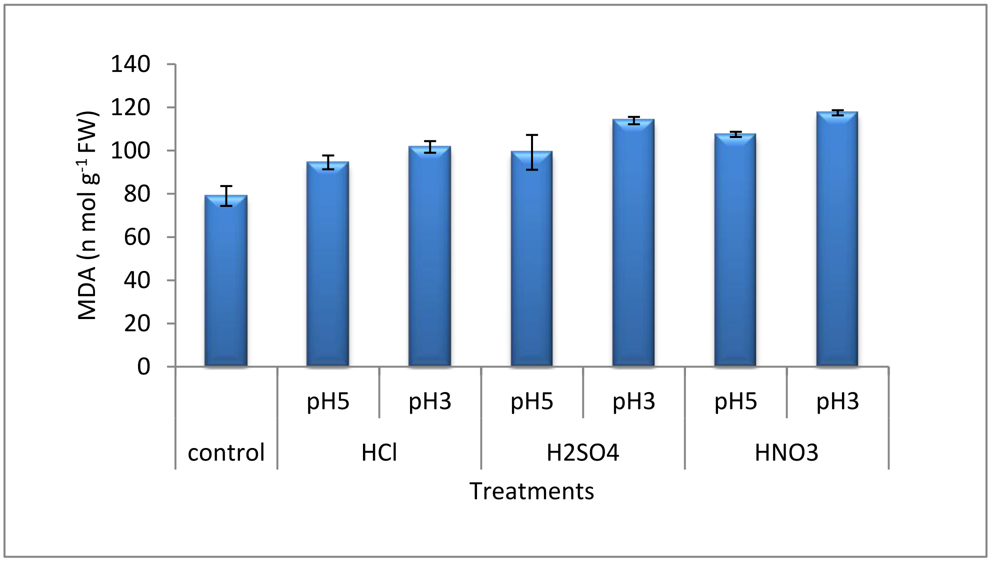

Fig2. Effect of simulated acid rain (SAR) of different pH on MDA content of spinach (*Spinacia oleracea)*

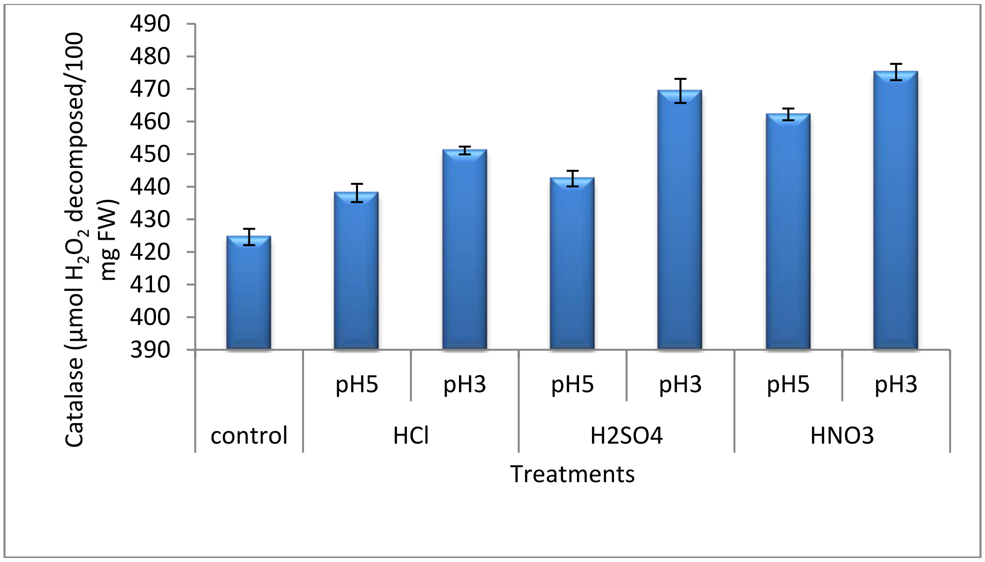

Fig3. Effect of simulated acid rain of different pH on catalase activity of spinach (*Spinacia oleracea)*

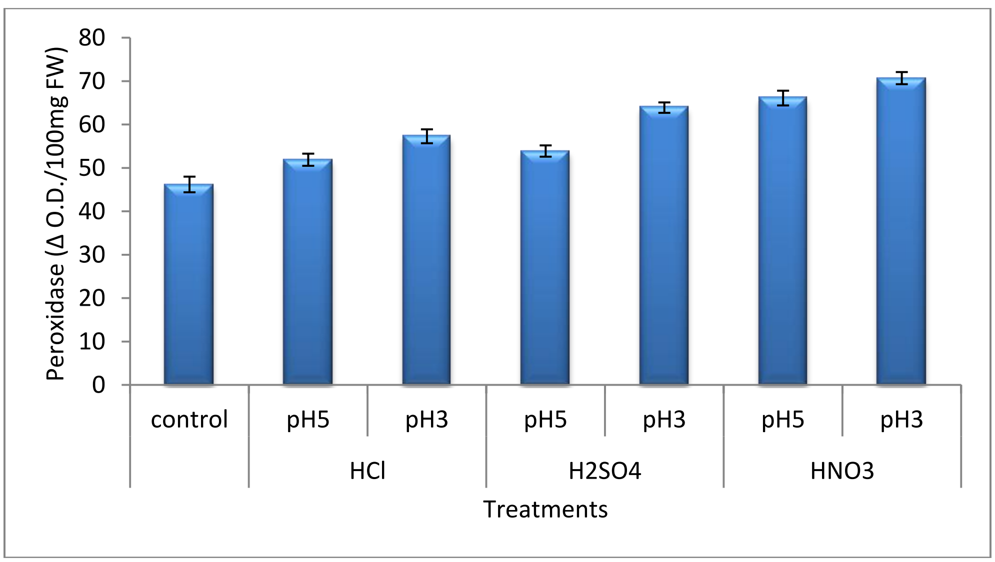

Fig4. Effect of simulated acid rain of different pH on peroxidase activity of spinach (*Spinacia oleracea)*