EFFECTS OF SIMULATED ACID RAIN ON MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS OF SPINACH (*SPINACIA OLERACEA*)

Vimlesh Rawat,
Department of Botany
Lucknow University,
Lucknow

Ratna Katiyar,
Department of Botany
Lucknow University,
Lucknow

ABSTRACT

The study was carried to evaluate the impact of simulated acid rain (SAR) of different acids (HCl, H_2SO_4 and HNO_3) pH-levels 5.0 and 3.0 on green leafy vegetable spinach (Spinacia oleracea L.) of the Family Chenopodiaceae. Plants treated with HCl showed damaging effects at pH 3.0 which was least damage as compared to H_2SO_4 and HNO_3. Both acids (H_2SO_4 and HNO_3) showed remarkable significant damage in terms of decrease in fresh and dry weight, number of leaves/plant, chlorophyll, protein content.

Keywords: *Simulated acid rain, Spinach, Chlorophyll, Protein content etc.*

INTRODUCTION

Acid rain is an unseen plague of the industrial age. In Asia, India and China are mostly affected mainly because of the large numbers of factories. The term acid rain was first used by Robert Angus Smith, a scientist working in Manchester in the 1870s. Rain water in its natural state is slightly acidic with a pH value ranging from 5.6–7.0 due to the presence of carbon dioxide in the atmosphere which dissolves in the rain as it falls (Zabawi *et al.,* 2008). Acid rain is infact the mixture of H_2SO_4 and HNO_3, where the ratio of these two may vary according to the relative quantity of oxides of sulphur and nitrogen present in the atmosphere. Plant or any other material that is exposed to acid rain for a long time ends up damaged or changed in some way. Vegetation and soils are also affected by acid rain. As its direct interaction with vegetation affects its morphological, biochemical and physiological activities while indirect interaction through soil interferes with nutrient availability to plants. Plants need soil nutrients and minerals, such as calcium, magnesium and potassium, to grow. When sulphuric and nitric acids fall onto the earth they dissolve certain nutrients and minerals thus wash them out and make them unavailable to trees and plants. The leaf is the most sensitive organ to pollution and has been the target of many studies. The anatomical analysis of injuries caused by pollutants on the leaf blade of plant species have been used in various studies to assess the real damage caused by pollutants (Silva et al., 2000; Chaves *et al.,* 2002). Various authors showed the leaf responses to acid rain by means of anatomical studies of the injuries (Percy and Baker, 1987) Acid rain exposure of plants results in characteristic foliar injury symptoms, modified leaf anatomy, structural changes in the photosynthetic pigment apparatus and a decrease in chlorophyll a and b contents (Shaukat and Khan, 2008).

The present study was carried out to assess the impact of simulated acid rain (SAR) of different acids on fresh and dry weight of plants, number of leaves/plant, chlorophyll and protein content of green leafy vegetable spinach.

MATERIAL AND METHOD

For this experiment the simulated acid rain (SAR) was prepared in the laboratory by diluting acids (HCl, H_2SO_4 and HNO_3) to develop a different acidic level (pH 3.0 and 5.0) with the help of the pH meter. Each level was prepared freshly for each spray. 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. Spinach was selected for the experiment because it is a leafy vegetable having enough leaf area to get exposed. Plants were grown in ambient conditions for four weeks and then the pots were exposed to simulated acid rains with pH 5 and pH 3. Control plants were separated and required space was maintained between the different treatments to avoid any kind of disturbance. The treatment was properly sprayed as rainfalls on plants on alternate days for four weeks.

Fresh weight of plant was taken with the help of an electronic balance. For dry weight plant was dried in pre-heated oven set at 70^0C for 48 hours. After that it was cooled in a dry environment (desicator) and then weight was taken with the help of an electronic balance. It was expressed in grams (g). Number of leaves was counted after completion of the treatment period of four weeks. Chlorophyll (Chl) and carotenoids (Car) in leaves were extracted in 80% acetone by the method of Arnon (1949). Protein was assayed according to the method of Lowry *et al*., (1951).

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

RESULTS

Visible symptoms such as chlorosis, necrosis and inward curling of leaves were also observed during the treatment period. After four weeks the leaves were collected to analyze the toxic responses, morphological and biochemical changes in the plants.

Fresh and dry weight plant^{-1} was found to be decreased with the increasing pH (pH 5 and pH 3) of SAR. FW was recorded as 28.2 g in control plants. Maximum significant decrease in fresh weight of plants was observed by 23.40, 24.82 and 26.59 % at pH 3 SAR treatments of HCl, H_2SO_4 and HNO_3 respectively over control. Dry weight of plants was found to be decreased with the increasing pH of SAR treatments. It was recorded as 10.0 g in control plants. Maximum significant decrease in DW of plants was observed by 8.0, 23.0 and 28.0 % at pH 3 SAR treatments of HCl, H_2SO_4 and HNO_3 respectively over control.

Number of leaves plant^{-1} was also found to be decreased with the increasing pH of SAR treatment. It was recorded as 13.25 in control plants. The maximum significant reduction in number of leaves plant^{-1} was observed as 32.57, 45.45 and 47.73% at pH 3 SAR treatments of HCl, H_2SO_4 and HNO_3 respectively over control.

Photosynthetic pigments were found to be reduced when exposed to the SAR treatments of pH 5 and 3. Chl 'a' was recorded as 1.90 mg g^{-1}FW in control plants. The maximum significant reduction in chl 'a' was observed to be 8.42, 8.42 and 10.52 % at pH3 SAR treatments of HCl, H_2SO_4 and HNO_3 respectively over control. Chlorophyll 'b' was recorded as 1.78 mg g^{-1}FW in control plants. The maximum significant reduction in chlorophyll 'b' was observed as 24.72, 26.40 and 37.07 % at pH3 SAR treatments of HCl, H_2SO_4 and HNO_3 respectively over control. Total chlorophyll content was recorded as 3.77 mg g^{-1}FW in control plants. The maximum significant reduction in total chl content was observed as 28.38, 31.83 and 35.54 % at pH3 SAR treatments of HCl, $H_2SO_4$and HNO_3 respectively over control. Carotenoids content was recorded as 0.83 mg g^{-1}FW in control plants. The maximum significant reduction in carotenoids content was observed to be 20.48, 30.12 and 36.14 % at pH3 SAR treatments of HCl, H_2SO_4 and HNO_3 respectively over control.

Protein content under SAR exposure was found to be decreased with the increasing pH of SAR treatment. It was recorded as 2.68 mg g^{-1}FW in control plants. Significant decrease in protein content was recorded as 11.58, 11.94 and 13.80 % at pH 3 SAR treatments of HCl, H_2SO_4 and HNO_3 respectively over control.

DISCUSSION

Acidic pollutants lowered the leaf pH which declined the physiological balance of sensitive plant species and under stress conditions plants exhibited certain morphological and biochemical alterations. Acid rain exposure to plant results in a characteristic of foliar injury symptoms, modified leaf anatomy (Park and Yanai, 2009) and also, a reduction in plant growth and yield (Dursun *et al.,* 2002). Wang (2010) found that plants exposed to low pH rain (pH 3.0) are generally retarded with leaf chlorosis, necrotic spot coupled with dehydration of the plants. Simulated acid rain exposure caused chlorosis, necrotic lesions and leaf tip injuries at different pH levels of *V. unguiculata*. Present study showed remarkable reductions in all the growth parameters studied. Meerabai, *et al.,* (2012) reported that leaves were very susceptible to the gaseous pollutants and these pollutants entered into the leaves *via* stomata and reacted with the surface of mesophyll cells. The reduction in biomass accumulation due to SAR may also be a consequence of reduced photosynthesis (Singh and Agrawal, 1996). Fresh and dry weight of crop plants was reduced with SAR in case of lower pH if the acidity was more than pH-3.0 it exceeded damage to the crops (Jiro Harada, 1992).

Lower pH causes severe damage to the photosynthetic pigments as observed in the present study. The photosynthetic efficiency is strongly dependent on leaf pH (Liu and Ding, 2008). Photosynthetic pigments were also inhibited with respect to acidity levels. Decrease of photosynthetic rate with acid rain was mainly related to the decreased chlorophyll content of leaves. Chlorophyll 'a', chlorophyll 'b' and total chlorophyll showed a decreasing trend. The reduction might be due to the removal of Mg^{++} from the tetrapyrol ring of the chlorophyll molecules by H^+ (Foster, 1990) or due to the increase of transpiration by acid rain (Evans *et al.,* 1997). Carotenoid is being tougher than chlorophyll but much less efficient in light absorption helps the valuable but much fragile chlorophyll and protects it from photo-oxidative destruction (Siefermann-Harms, 1987). Carotenoids content decreased on exposure to sulfur dioxide (Irshad *et al.,* 2011) and short term treatment of SO_2 damaged pigment system-II, decreased the fluidity of thylakoid membrane and affected the process of electron transport (Liu *et al.,* 2007). Spraying with simulated acid solution reduced the amount of carotenoids which was also reported by Odiyi and Eniola, (2015). Decrease in protein content was observed in the present study. Reduction in total protein content under stress conditions might be due to damage caused by reactive oxygen species (ROS) to proteins. Reduction in protein content might be due to the enhanced rate of protein denaturation and breakdown of existing protein to amino acid is the main cause of reduction in protein content. Agarwal and Deepak (2003) determined that SO_2 enrichment results in diminished leaf protein levels by 13% and the decrease is attributed by breakdown of existing protein and reduction in synthesis. Meng He *et al.,* 2011 have also reported a decrease in soluble protein content after being sprayed with SAR.

CONCLUSIONS

Effects of simulated acid rain (SAR) were also observed on spinach **(***Spinacia oleracea*). Higher the acidity of the SAR more significant was the inhibitions. Under the treatment of weak or neutral acid rain, nutritional substance contents lead to an increase in level compared to that of the control. Study of SAR of different acids namely HCl, H_2SO_4 and HNO_3 of pH 3.0 and 5.0 reveals the damaging effects of acidic pH on green leafy vegetable spinach. Simulated acid rain of pH 3.0 of all three acids showed damaging effects on spinach but SAR treatment of H_2SO_4 and HNO_3 acid showed severe damages at pH 3.0. The study clearly indicates that there is an urgent need to monitor and reduce such kind of emissions that acidify the atmosphere and awareness among local and international communities on the negative impacts of burning fossil fuel must be created. Industries should devise a means of safely collecting the gases emitted from their plants so that it does not contaminate the atmosphere; vehicles that do not emit oxides of sulphur and nitrogen should be produced in commercial quantities and made relatively affordable.

REFERENCES

- A.G. Mohamad Zabawi, S. Moh Esa and C.P. Leong (2008). Effects of simulated acid rain on germination and growth of rice plant. J. Trop. Agric. and Fd. Sc. 36(2): 000–000.
- Agarwal, M., Deepak, S.S., (2003). Physiological and biochemical responses of two cultivars of wheat to elevated levels of CO_2 and SO_2, singly and in combination. *Environmental Pollution,* 121, 189-197.
- Arnon, D. T. (1949). Copper enzymes in isolated Chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.,* 24, 1-15.
- Bakalovic N, Passardi F, Ioannidis V, Cosio C, Penel C, Falquet L, Dunand C (2006) Peroxidase: A class III plant peroxidase database. Phytochemistry. 67(6), 534-539.
- Bates, R., Waldren, P. and Teare, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil,* 39, 205-207.
- Chaves, A. L. F.; Silva, E. A. M.; Azevedo, A. A.; Oliva, M. A. and Matsuoka, K. (2002), Ação do flúor dissolvido em chuva simulada sobre a estrutura foliar de Panicum maximum Jacq. (colonião) e Chloris gayana Kunth. (capim-Rhodes) – *Poaceae*. *Acta Botanica Brasilica,* 16, 395-406.
- Dursun, A., Kumlay, A. M., Yilderin, E. and Guvenc, I. (2002). Effects of simulated acid rain on plant growth and yield of tomato. *Acta Hort.,* 579, 245-248.
- Heath, R. L. and Packer, L. (1968). Photo oxidation in isolated chloroplast .I. Kinetics and Stoichiometry of fatty acid peroxidation. *Arch. Biochem. Bio. Physic.,* 125, 189-198.
- Irshad, A. H., Ahmad, S. F. and Sultan, P. (2011). Effect of sulphur dioxide on the biochemical parameters of spinach (*Spinacea oleracea*). *Trakia J. Sci.* 9(1), 24-27.
- Jiro Harada, (1992). Effects of acid rain on crops and agricultural environment. 296-299.
- Kacharava, N., Chkhubianishvili, E., Badridze, G., Chanishvili, S. and Mazanishvili, L. (2013). Antioxidant response of some Georgian wheat species to simulated acid rain. *AJCS*, 7(6), 770-776.
- Liu, Y. J. and Ding, H. (2008). Variation in air pollution tolerance index of plants near a steel factory: implication for landscape-plant species selection for industrial areas. *Wseas Trans. Environ. Dev.*, 4, 24-32.
- Lowry, O.H., Rosenbrough, N.J., Farr and Randal, A.L. (1951). Protein measurement with folin phenol reagent. *J. Biol. chem.,* 193, 265.
- Meerabai, G., Venkata, R.C. and Rasheed, M. (2012). Effects of industrial pollutants on physiology of *Cajanus cajan* (L.) *Fabaceae*. *Int. J. Environ. Sci.,* 2 (4), 1889-1894.
- Meng, H., Dong, D., Wang, J., Yang, K., Tian, L., Sun, W. and Fang C. (2011). Effects of Simulated Acid Rain on Main Nutritional

Indicators of Three Leafy Vegetables. *Chem. Res. Chinese Universities,* 27(3), 397-401.

- Odiyi, B. O. and Eniola, A. O. (2015). The Effect of Simulated Acid Rain on Plant Growth Component of Cowpea (*Vigna unguiculata*) L. Walps. *JJBS,* 8 (1), 51-54.
- Percy, K.E., Baker, E.A., 1987. Effects of simulated acid rain on production, morphology and composition of epicuticular wax and on cuticular membrane development. *New Phytol,.* 107, 577–589.
- Shaukat, S.S. and Khan, M.A. (2008). Growth and Physiological Responses of Tomato (*Lycopersicon esculentum* Mill.) to Simulated Acid Rain. *Pakistan Journal of Botany,* 40, 2427-2435.
- Siefermann-Harms D (1987) The light harvesting and protective function of carotenoids in photosynthetic membranes. Physiol Plant 69:561–568.
- Silva, L. C.; Azevedo, A. A.; Silva, E. A. M. and Oliva, M. A. (2000), Flúor em chuva simulada: sintomatologia e efeitos sobre a estrutura foliar e o crescimento de plantas arbóreas. Revista Brasileira de Botânica, 23, 383-391.
- Singh, A. and M. Agrawal, (1996). Responses of two cultivars of *Triticum aestivum* L. to simulated acid rain. *Environmental Pollution,* 91, 161- 167.
- Wang, C. Q. (2010). Exogenous calcium alters activities of antioxidant enzymes in *Trifolium repens* L. leaves under peginduced water deficit. *J. Plant Nutr.,* 33, 1874-1885.

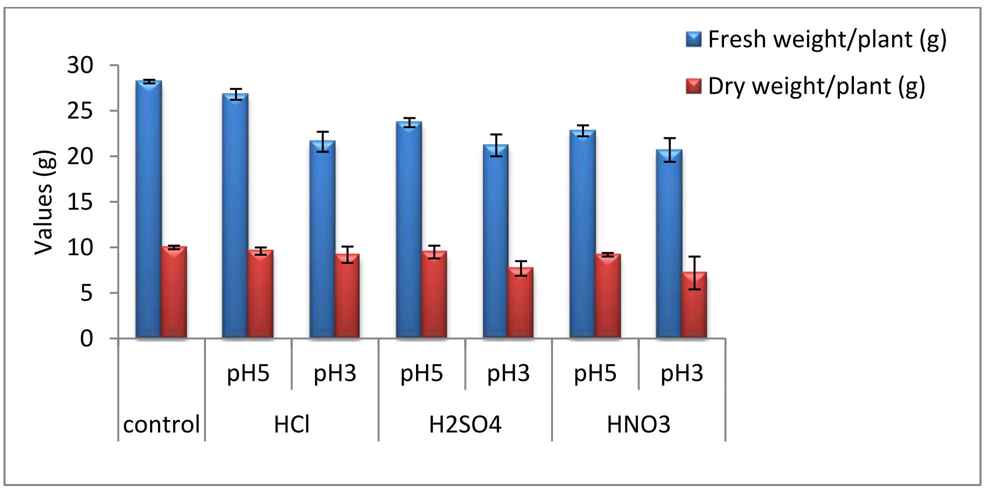

Fig1. Effect of simulated acid rain (SAR) of different pH on fresh & dry weight of spinach (*Spinacia oleracea)*

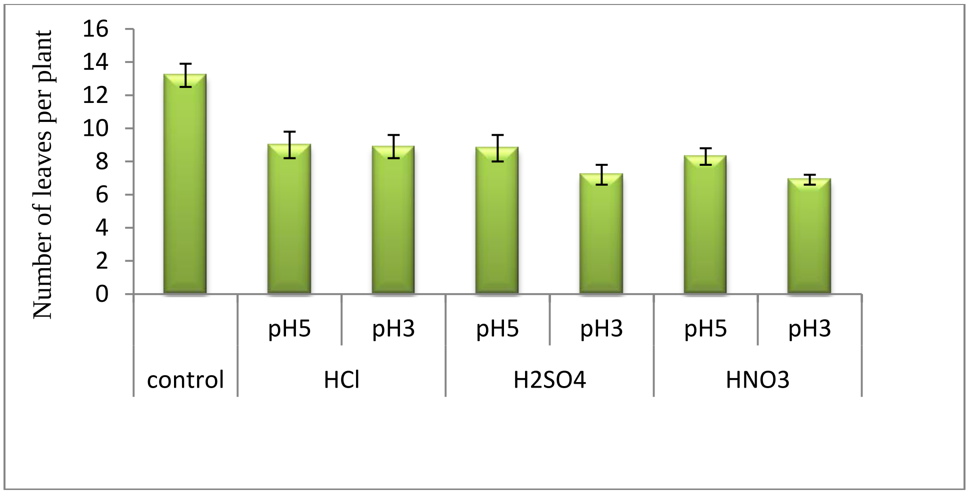

Fig2. Effect of simulated acid rain (SAR) of different pH on number of leaves per plant of spinach (*Spinacia oleracea)*

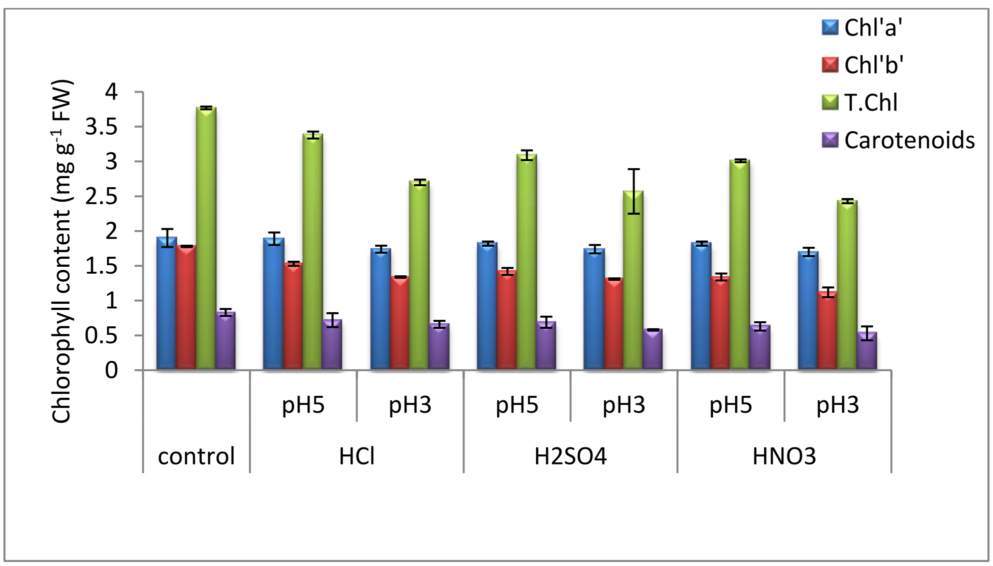

Fig3. Effect of simulated acid rain (SAR) of different pH on photosynthetic pigments of spinach (*Spinacia oleracea)*

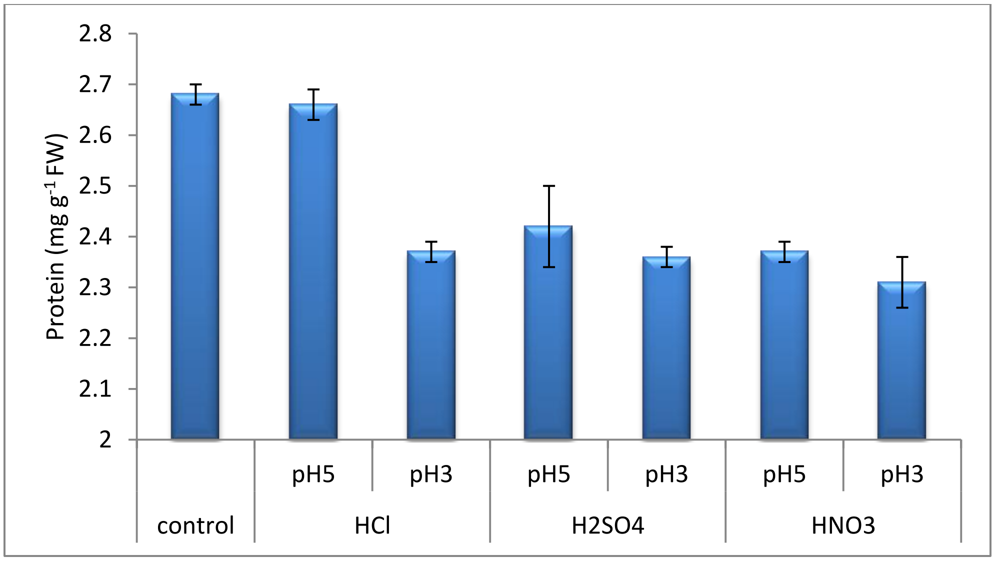

Fig4. Effect of simulated acid rain (SAR) of different pH on protein content of spinach (*Spinacia oleracea)*