Pakistan Journal of Nutrition 4 (6): 402-406, 2005
ISSN 1680-5194

Effects of Simulated Acid Rain on Vitamins A, E, and C in Strawberry (*Fragaria vesca*)

Ömer Munzuroglu[1], Erdal Öbek[2]*, Fikret Karatas[3] and Sule Yüksel Tatar[2]
[1]Department of Biology, Faculty of Science, Firat University, Elazig 23119, Turkey
[2]Department of Environmental Engineering, Firat University, Elazig 23119, Turkey
[3]Department of Chemistry, Faculty of Science, Firat University, Elazig 23119, Turkey

Abstract: In this study, the effect of acid rains implementation including pH 2-5 was determined on the matured strawberry fruits' levels of A, E, and C vitamins. The acid rains were implemented on the crops in two ways: spraying on upper soil parts and to roots. Vitamin levels of all strawberries were determined by High Performance Liquid Chromatography (HPLC). It was determined that vitamin levels of plants sprayed with simulated acid rains decreased in respect of pH and time when compared with control. Especially, sprayed roots were more affected in respect of vitamin levels than untreated plants.

Key words: Acid rain, strawberry, A, E and C vitamins, HPLC

Introduction

The quality of air has significant importance for all livings. Human, plant and animal health depend on unpolluted atmosphere. The polluting elements such as SO_2, NO_x, CO_2 and HF, which are emitted to the air by the burn of fossil veins, lead acidic deposition (or acid rains) as a result of complex physical and chemical reactions. Sunlight accelerates most of these reactions. The transportation of compounds, which convey acid rains through the prevailing winds for thousands of miles raises the pollution to very high rates. The plants, the primary producers, are affected much from the pollution (Munzuroglu *et al*., 2003).

Various studies have showed that acid rains had serious effects on plants' vegetative organs and generative structure [Munzuroglu *et al*., 2003; Creasy and Swartz, 1981; Forsline *et al*., 1983; Denis, 1988; Kohno and Kobayashi, 1989; Rinallo, 1992a,b; Rinallo *et al*., 1993; Sverdrap and De Vires, 1994; Kohno *et al*., 1995; Rinallo and Modi, 1995; Rinallo and Mori, 1996; Shan *et al*., 1996).

Acid rain (AR) induces changes in the cellular biochemistry and physiology of whole plant. Biological effects of acid deposition on plants are numerous and complex, and include visible symptoms of injury (chlorosis and or necrosis), and invisible effects such as reduced photosynthesis, nutrient loss from leaves, altered water balance, variation of several enzyme activities (Ferenbaugh, 1976; Evans, 1982).

The ability of plant to overcome the effect of the AR stress and to sustain its productivity may be related to the scavenging of stress-induced toxic oxygen species such as H_2O_2 (hydrogen peroxide), $OH^{!}$ (hydroxyl radical) and $O_2^{-!}$ (superoxide radical). Catalases and peroxidases are two major systems for the enzymatic removal of H_2O_2 in plants (Willekens *et al* 1995; Douglas, 1996; Whetten and Sederoff, 1995).

Scarce data, however, are available on the production of active oxygen species and antioxidant systems in plants subjected to AR. In normal conditions aerobic organisms are protected against oxidative damage by a variety of antioxidant systems. The antioxidant system is divided into two groups as enzymatic and nonenzymatic. Nonenzymatic system constitutes antioxidant vitamins such as vitamin A, E, C and Se. And they have been shown to react with organic free radicals and to protect biomembranes from damage induced by these free radicals (Laila *et al*., 1991; Halliwell, 1994).

Considerable levels of acid rains have been determined in different parts of Turkey. Effects of these rains on the livings have not been investigated sufficiently.

The determination of the vitamin contents of plants, which are natural sources, the especially the effects of environmental pollution on vitamin levels is quite important. This is of vital importance for future plans and for the measures to be taken. In this study, we aimed at determining the effects of simulated acid rain on the levels of A, E and C vitamin in strawberries in the agricultural area conditions.

Materials and Methods

Plant material and chemicals: As the crop material, matured strawberry (*Fragaria vesca*) crop fruits grown in the field condition were used. The AR was prepared according to Seufert *et al*. (1990) and contained the following components: NH_4NO_3 (1.3 g l^{-1}), $MgSO_4•7H_2O$ (3.1 g l^{-1}), Na_2SO_4 (2.5 g l^{-1}), $KHCO_3$ (1.3 g l^{-1}), $CaCl_2•2H_2O$ (3.1 g l^{-1}). After dilution of initial solution 1:100, pH value was adjusted to 1.8 with 1 N H_3PO_4 and 1 N H_2SO_4. pH values of solutions were as 5.0, 4.5, 4.0, 3.5, 3.0, 2.5 and 2.0. Acid rain is often defined as precipitation with a pH of less than 5.6 (Rinallo, 1992a). Simulated acid rains were prepared by deionized water. Deionized water (pH 6.5) has been used as control. pH measurements were done with a digital pH meter (WTW 330).

Acid rain implementation on crop: Acid rain was sprayed to the crop's vegetative and generative parts and poured on the soil of the plant's root. 10.0 ml acid rain was implemented onto the crop in both ways. This 10.0 ml acid rain was implemented onto the crop four times at 15 minutes intervals and each time 2.5 ml successively. Instead of pH solutions, deionized water implemented onto the control crops. After 24 and 48 hours the implementations A, E and C analyses were made onto the picked fruits. Ten crops (totally 80 crops) were used for every pH solution and control group. Vitamin content of each crop fruits was determined one by one.

Randomly chosen 5 fruits, which have the same appearance in terms of maturity level, from every crop were used.

Vitamin analysis: Both fresh strawberry samples (20 g) were mashed in a homogenizator and 2 g homogenate paste per sample was taken for extraction of vitamins A, E and 1 g per sample was taken for extraction of vitamin C. 4 ml of ethanol was added homogenates, vortexed and the mixture was centrifuged (Mistral® 2000) at 3500 rpm for 3 min at 4°C. The supernatant was also filtered through a Whatman No.1 paper, and to the filtrate 0.15 ml n-hexane was added and mixed. Vitamins A and E were extracted twice in hexane phase and the collected extract was dried under stream of liquid nitrogen. Dried extract was solubolized in 0.1 ml methanol for HPLC. Injections were made in duplicate for each sample. The quantification was made according to Catignani, (Catignani, 1983) and Miller *et al*. (1984) utilizing absorption spectra of 326 and 296 nm for vitamin A and E respectively. Techsphere ODS-2 packed (5 µm particle and 80 °A pore size) column (250 x 4.6 ID) with a methanol: acetonitril: chloroform (47: 42: 11, v/v) mobile phase at 1 ml min^{-1} flow rate.

The extraction of vitamin C was performed according to the method of Cerhata *et al*., 1994. To 1 g homogenized strawberry paste 1 ml of 0.5 M perchloric acid was added, vortexed and the volume was adjusted to 4 ml by adding doubly distilled deionized water (ddH_2O). The mixture was centrifuged at 4500 rpm for 10 min at 4°C. The supernatant was filtered as above and the vitamin C level was determined using the method of Tavazzi *et al*. (1992) by HPLC utilizing absorption spectra of 246 nm and a column (250 x 3.9 ID) packed with Tecopak C18 reversed-phase material (10 µm particle size) with mobile phase (3.7 mM phosphate buffer, pH 4.0) at 1 ml min-1 flow rate.

HPLC separations were accomplished at room temperature with a Cecil liquid chromatograph system (Series 1100) consisting of a sample injection valve (Cotati 7125) with a 20 µl sample loop, an ultra-violet (UV) spectrophotometric detector (Cecil 68174), integrator (HP 3395)

All chemicals and reagents used were of analytical grade and were purchased from Merck Chemical Co. (Darmstadt, Germany). Deionized water was in all the examinations.

Statistical analysis: The results for each pH solution and control group were given as average of values obtained from 5 crops. In addition it was subjected to statistical analysis by counting standard error of average and making variance analysis. SPSS 10.0 version was used in variance analysis.

Results

The recovery rates were determined to be 97.3% for vitamin A, 99.2% for vitamin E, 95.0% for vitamin C. C vitamin levels were given by dividing into 100 on column graphic due to very high levels. The mean ± SD values of antioxidant vitamin (A, E and C) levels are given in the Fig. 1-4.

After 24 and 48 hours implementation of simulated acid rains on the strawberry crops, the changes in A, E and C vitamin levels were determined successively between 0.41±0.12 - 1.30±0.43; 0.75±0.18 - 1.53±0.39; 339±51.8 - 449±64.3 µg/g; 0.37±0.10 - 1.21±0.37; 0.62±0.15 - 1.45±0.32; 293±43.8 - 423±64.6 µg/g (Figures 1 and 2). When these results compared with the control group, changing reductions at the end of 24 and 48 hours were seen successively between 28.87%, 44.38%, 70.63%; 26.43%, 35.57%, 61.43% in A, E, and C vitamin amounts ($p<0.05$, $p<0.01$).

A, E and C vitamin amounts in harvested strawberry crops after 24 and 48 hours based on the applications made to root regions were given in Figure 3 and 4. A, E and C vitamins varied in the range of 0.29±0.08 - 1.24±0.30; 0.64±0.14 -1.55±0.32 ; 291±40.0 - 458±70.3 µg/g for 24 hours and 0.20±0.07 – 1.10±0.32; 0.50±0.13 – 1.44±0.32; 274±36.5 - 425±64.9 µg/g for 48 hours, respectively. When these results were compared with control group, A, E and C vitamin amounts decreased 20.71, 37.86, 60.62 % for 24 hours and 14.28, 30.30 and 57.44 % for 48 hours respectively ($p<0.01$).

Discussion

There are different reports concerning changes happening in some of vitamin levels depending on various stress factors (Havaux and Kloppstech, 2001; Sarita *et al*., 1996; Mishra and Choudhuri, 1998). But there is almost no study made on the vitamin contents of acid rain implemented crops. Rinallo and Mori (1996) reported that simulated acid rain under pH 4 implemented on pear fruits caused decrease in C vitamin levels. Likewise our acid rain implementing

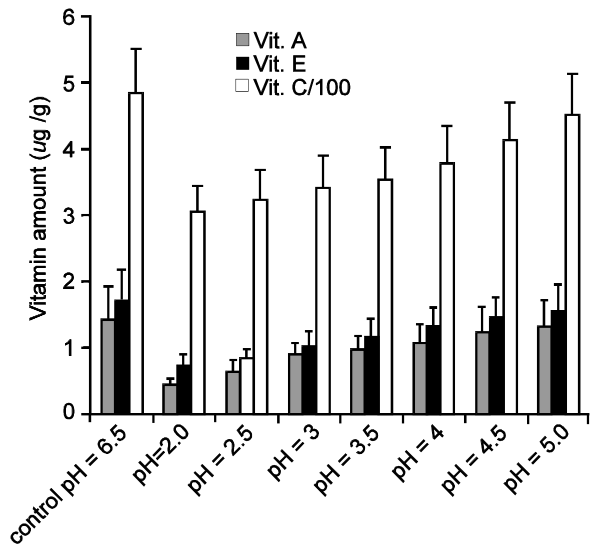

Fig. 1: Column graphic of A, E and C vitamin levels in acid rain implemented strawberry crops in various pH (24 hours)

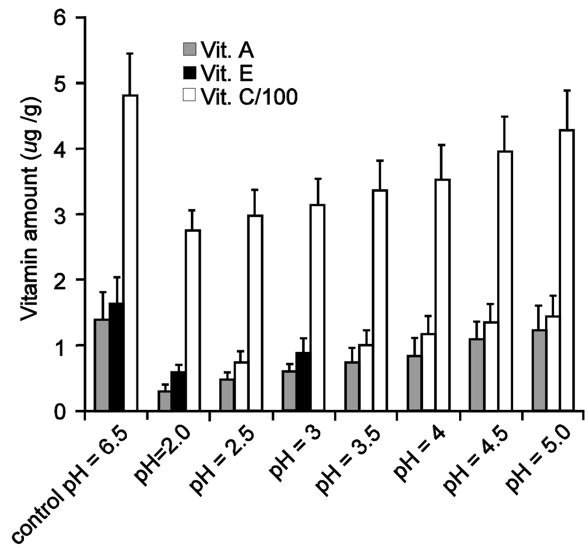

Fig. 2: Column graphic of A, E and C vitamin levels in the fruits that acid rains implemented on strawberry crops in various pH (48 hours)

study on strawberry fruits of ripening phase showed a significant reduction in A, E and C vitamin levels ($p<0.01$).

Free oxygen radical formation is indispensable in living creatures using oxygen gases in their metabolism. It is known that many environmental factors cause stress which eventually increases free radical formation, and in order to remove the negative effects of free radicals, organisms develop various defense mechanisms. Kong *et al.* (2000) determined that acid rains cause an increase in oxygen radicals and a decrease in protein

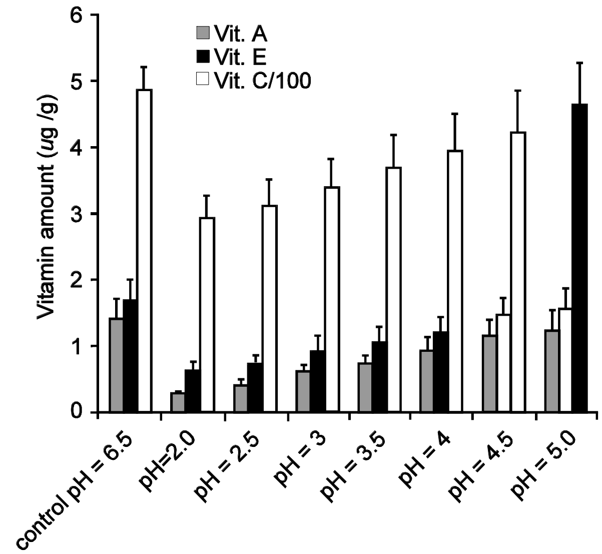

Fig. 3: Column graphic of A, E and C vitamin levels in the fruits that acid rains implemented on strawberry crop's root region in various pH (24 hours).

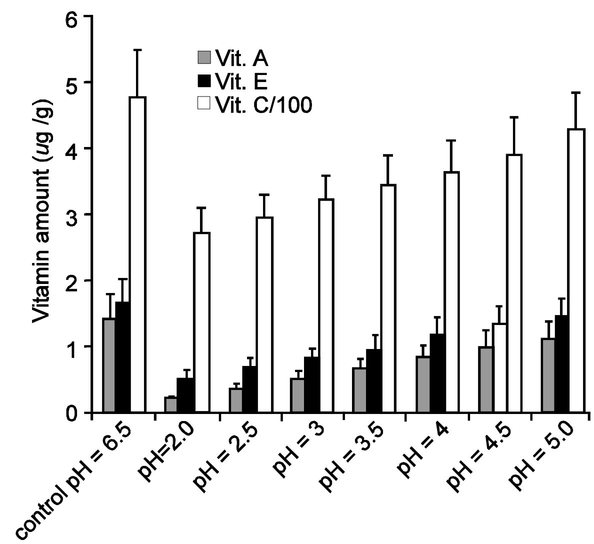

Fig. 4: Column graphic of A, E and C vitamin levels in the fruits that acid rains implemented on strawberry crop's root in various pH (48 hours)

amount in their organs. It is reported that acid rains cause induction in the activity of *Pinus massoniana* superoxide dismutase (SOD), peroxidase (POD) and glutathione (GSH). Velikova *et al*. (2000) determined that acid rains reduce MDA amount which is a signal for lipid peroxidation, and increase peroxides activity while decreasing catalos activity. The data we obtained shows that non-enzymatic defense system (vitamins A, E and C) of crop was affected by acid rains.

In our opinion, there are two basic reasons for the reduction in the antioxidant vitamin (A, E and C) amounts in acid rain implemented strawberry fruits. The first one is the increase in the free oxygen radical formation in the plant and the fruit due to acid rain stress, and plant's use of the antioxidant system in order to resist the stress which results in a loss in vitamin levels. The second is that in autotrophy crops metabolic ways formed by vitamin synthesis are inhibit somewhat by acidic conditions. It is well known that various kinds of crops exposed to stress change their metabolism.

This study showed that, in addition to reduced levels of A, E and C vitamins, acid rain implementation via root is more effective than spraying, that the effect of acid rain continues for a while and acidity degree is of great importance.

As known land crops get water via their roots from soil and distribute to their tissues via xylem. The reason that implementing acid rains via roots is more effective than the spraying can be accounted for the inhibition of the acidity to all tissues easily. In addition, in the spraying method the acid rain evaporates more rapidly while in implementation via root it remains in the soil longer. After acid rain implementation on strawberry crop, the reduction in vitamin levels was less after 48 hours while higher after 24 hours ($p>0.05$) (Fig. 2 and 4). This indicates that acid rain penetrated into plant tissues is effective for a longer time and that crop does not tolerate stress immediately.

In conclusion, simulated acid rains decreased vitamin levels of strawberry fruits in important ratios. We are polluting the environment irresponsibly and causing acid rains wit unrestricted consumptions and wastages. In the near future, unless serious measures are not taken, human beings may face even greater risks. Studies of this kind may help overcome some of the dangers and reduce the risks.

References

Catignani, G.L., 1983. Simultancous determination of retinol and "-tocopherol in serum or plazma by liquid chromatogaphy. Clin. Chem., 2914: 708-712.

Cerhata, D., A. Bauerova and B. Ginter, 1994. Determination of ascorbic acid in serum using high performance liquid-chromatogaphy and its correlation with spectrophometric (colorimetric) determination. Caska. Slov. Farm., 43: 166-168.

Creasy, L.L. and H.J. Swartz, 1981. Agents influencing russet on golden delicious apple fruits. J. Am. Soc. Hort. Sci., 106: 203-206.

Denis, T. DuBay, 1988. The direct effects of simulated acid rain on the sexual reproduction of selected agricultural crops. Environ. Pollut., 53: 422-423.

Douglas, C.J., 1996. Phenylpropanoid metabolism and lignin biosynthesis: from weeds to trees. Trends Plant Sci., 1: 171-178.

Evans, L.C., 1982. Biological effects of acidity in precipitation on vegetation: a review. Environ. Exp. Bot., 22: 155-169.

Ferenbaugh, R.W., 1976. Effects of simulated acid rain on *Phaseolus vulgaris* L. (Fabaceae). Am. J. Bot., 63: 283-288.

Forsline, P.L., R.C. Musselman, W.J. Kender and R.J. Dee, 1983. Effects of acid rain on apple tree productivity and fruit quality. J. Am. Soc. Hort. Sci., 108: 70-74.

Halliwell, B., 1994. Free radical antioxidants in human disease. Curiosity, cause or consequence. Lancet, 344: 721-724.

Havaux, M. and and K. Kloppstech, 2001. The protective functions of carotenoid and flavanoid pigments against excess visible radiation at chilling temperature investigated in Arabidopsis npq and tt mutants. Planta, 213: 953-966.

Kohno, Y. and T. Kobayashi, 1989. Effect of simulated acid rain on the growth of soybean. Water, Air, and Soil Pollut., 43: 11-19.

Kohno, Y., H. Matsumura and T. Kobayashi, 1995. Effect of simulated acid rain on the gowth of japanase conifers grown with or without fertilizier. Water, Air and Soil Pollut., 85: 1305-1310.

Kong, FX., Y. Liu, W. Hu, P.P. Shen, C.L. Zhou and L.S. Wang, 2000. Biochemical responses of the mycorrhizae in *Pinus massoniana* to combined effects of Al, Ca and low pH. Chemosphere, 40: 311-318

Laila, G., A. Yues, H. Bernard, J. Claude, C. Gerard and S. Gerard, 1991. Biological variability of superoxide dismutase, glutathione peroxidase and catalase in blood. Clin. Chem., 37: 1932-1937.

Miller, K.W., N.A. Lorr, and C.S. Yang, 1984. Simultaneous determination of plasma retinol, "-tocopherol, lycopene, "-carotene, and $-carotene by high performance liquid- chromatogaphy. Anal. Biochem., 138: 340-345.

Mishra, A. and M.A. Choudhuri, 1998. Amelioration of lead and mercury effects on germination and rice seedling gowth by antioxidants. Biol. Plant., 41: 469-473.

Munzuroglu, Ö., E. Öbek and H. Geckil, 2003. Effects of simulated acid rain on the pollen germination and pollen tube growth of apple (Malus sylvestris Miller cv. Golden). Acta Biologica, 54: 95-103.

Rinallo, C., 1992a. Effects of simulated acid rain on the foliage and fruit yield of Malus domestica Borkh. J. Hort. Sci., 67: 553-559.

Rinallo, C., 1992b. Effect of acidity of simulated rain on the fruiting of "Summerred" apple trees. J. Environ. Qual., 21: 61-68.

Rinallo, C., G. Modi, A. Ena and R. Calamassi, 1993. Effects of simulated rain acidity on the chemical composition of apple fruit. J. Hort. Sci., 68: 275-280.

Rinallo, C. and G. Modi, 1995. Fruit yield of field-grown pear *Pyrus Communis* (L.) exposed to different levels of rain acidity in Tuscany. Hort. Abst., 65: 9512.

Rinallo, C. and B. Mori, 1996. Damage in apple (*Malus domestica* Borkh) fruit exposed to different levels of rain acidity. J. Hort. Sci., 71: 17-23.

Sarita, S., G. Manisha and C. Prakash, 1996. Bioaccumulation and biochemical effects of mercury in the plant *Bacopa monnieri* L. Environ. Toxi. and Water Quality, 11: 105-112.

Seufert, G., V. Hoyer, H. Wo¨ llmer and U. Arndt, 1990. The Hohenheim long-term experiment. General methods and materials, Environ. Pollut., 68: 205-229.

Shan, Y., Z. Feng, T. Izuta, M. Aoki and T. Totsuka, 1996. The individual and combined effects of ozone and simulated acid rain on gowth, gas exchange rate and water-use efficiency of Pinus armandi Franch. Environ. Pollut., 91: 355-361.

Sverdrap, H. and W. De Vires, 1994. 'Calculating critical loads for acidity with the simple mass balance method.' Water, Air and Soil Pollut., 72: 143-162.

Tavazzi, B., G. Lazzarino, D. Di-Piero and B. Giardina, 1992. Malondialdehyde production and ascorbate decrease are associated to the reperfusion of the isolated postischemic rat heart. Free Radic. Biol. Med., 13: 75-78.

Velikova, V., I. Yordanov and A. Adreva, 2000. 'Oxidative stress and some antioxidant systems in acid rain-treated bean plants Protective role of exogenous polyamines'. Plant Sci., 151: 59-66.

Whetten, R. and R. Sederoff, 1995. Lignin biosynthesis', Plant Cell, 7: 1001-1013.

Willekens, H., D. Inze, M. van Montagu and W. van Camp, 1995. Catalases in plants'. Mol. Breeding, 1: 207-228.