Chemical Changes in Leaves of Corn and Bean Plants Exposed to Simulated Acid Rain

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Summary: In this study, the effects of simulated acid rain on vitamin E, vitamin D_2 and fatty acids levels of corn and bean leaves were investigated. It was determined that the level of palmitoleic acid (C16:1) increased in comparison with the control group ($p<0.01$, $p<0.05$), while the vitamin E, D_2 and linoleic acid (C18:2) levels considerably decreased in comparison with the control group ($p<0.001$). From the these results, we can suggest that simulated acid rain can have negative effects on levels of fatty acids, vitamin E and vitamin D_2 of these plant leaves.

Key Words: Simulated acid rain, Fatty Acids, Vitamins E and D_2, corn, bean, leaf.

Introduction

Acid rain is an important and interesting environmental problem. Acid rain is a rain or any other form of precipitation that is unusually acidic, meaning that it possesses elevated levels of hydrogen ions (low pH). It can have harmful effects on plants, aquatic animals, and infrastructure. Acid rain is caused by emissions of carbon dioxide, sulfur dioxide and nitrogen oxides which react with the water molecules in the atmosphere to produce acids. These pollutants originate from human activity such as the combustion of burnable waste and fossil fuels within thermal power plants and automobiles [1].

The acid rain components generate reactive free oxygen radicals that may cause inhibition to photosynthesis, enzymatic breakdown, membrane damage and DNA alterations. All of these effects cause negative plant growth [2]. In addition, simulated acid rain affects the metabolic activity [3], photosynthesis [4] and structure of chloroplasts and mitochondria [5].

Generally, the leaves of bean and corn have been consumed by domestic herbivorous animals; these leaves also have been consumed by human at some rural areas of Turkey. The objective of this study to determine the influences of simulated acid rain on the fatty acids and some vitamins (vitamin E and D_2) of this plant leaves.

Results and Discussion

The levels of vitamin E and vitamin D_2 in leaves are given in Table-1. And fatty acid compositions in the analyzed leaf samples have been listed in Table-2. The concentrations of vitamins and in these samples are quite variable (Vitamin E 3.13–12.00 μg g^{-1} and vitamin D_2 1.54-45.00 μg g^{-1}).

Table-1: The Levels of vitamin in plants leaf (μg g^{-1})

Sample	Vitamin	Control group	Acid rain group
Corn leaf	Vitamin E	12.00±3.25	3.13±1.22[d]
	vitamin D_2	3.87±0.76	1.54±0.42[d]
Bean leaf	Vitamin E	8.12±1.23	4.70±0.98[d]
	vitamin D_2	42.00±5.26	25.87±4.73[d]

a : $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$

Table-2: Chemical composition (relative % peak area) of the fatty acids

Fatty acid	Control group (corn leaves)	Acid rain group (corn leaves)	Control group (bean leaves)	Acid rain group (bean leaves)
16 : 0	11.90±0.22	10.77± 0.18[b]	12.23±1.97[a]	13.98±2.35[a]
16 : 1	3.93±0.36	5.38±0.32[c]	2.22±0.09	2.70±0.12[b]
18 : 0	2.45±0.12[a]	2.35 ±0.17[a]	2.52±0.15	4.34±1.04[c]
18 : 1	1.72±0.11	0.98 ±0.13[d]	1.69±0.03	2.30 ±0.15[d]
18 : 2	12.16±1.13	6.98± 1.03[d]	12.45±1.72	7.64 ±1.63[d]
18 : 3	68.95±0.83	73.46±0.21[b]	66.57±3.17[a]	64.98±2.63[a]
20 : 0	*	*	2.88±0.23	1.76 ±0.13[b]

a : $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$, *:not detectable, Values are means ± SE (n = 6)

It was found that the vitamin E, D_2 and linoleic acid (C18:2) levels of corn and bean leaves exposed with simulated acid rains considerably decreased when compared with the control group ($p<0.001$).

The level of palmitic acid (16:0) of corn plant leaves that simulated acid rain treated decreased when compared with the control group ($p<0.05$), while the level of 16:1 of corn leaves is higher than control group ($p<0.01$). Especially it is found that the

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levels of 18:2 and 18:1 of corn leaves were considerably lower than control group ($p<0.001$) (Fig. 3). In this group, we also found that the levels of 16:1 and 18:0 of the leaves of bean increased when compared with the control group ($p<0.05$ and $p<0.01$ respectively). In addition, it is found that in the acid rain group, the level of 18:2 of the bean leaves is considerably low when compared with the control group ($p<0.001$).

It is known that the acid rain reduce the quality and yields of fruit as one of the most important injury of acid rain on the plants. From the studies related to rain pH, it was obtained that acid rain caused a decreasing of quantity of yield and caused spots on the peel and pulp of the apple fruits [6-8].

In the studies carried out on apples and pears, it is reported that the levels of dry matter, minerals, yield and vitamin C decreased in the application of acid rains at pH<4, while the levels of acidic organic compounds, nitrogen, and heavy metal ions increased [9,10]. A similar result was observed by Munzuroglu [11] who determined a significant decline in the levels of the vitamins A, E and C of strawberry exposed with simulated acid rain. The cause of these decreasing is related two aspects. First: Due to the acid rain stress, plants have used the antioxidant system in order to resist the stress, and so, free oxygen radical formation has increased in plant and fruit. For this reason, vitamin levels have begun decreasing. Second is that in autotrophy crops metabolic ways formed by vitamin synthesis are inhibiting somewhat by acidic conditions. It is well known that various kinds of crops exposed to stress change their metabolism [11]. Similarly, in our study, the effects of simulated acid rain revealed a decreasing in the levels of vitamin E and D_2 of leaves of corn and bean.

Velikova reported that acid rains have negative affected on the level of fatty acids of bean plant [12]. In this study, we didn't found a statistically significant difference between the levels of (16:0) and (18:3) of bean leaves of control and acid rain groups ($p> 0.05$). However, we found that the levels of (16:1), (18:0) and (18:1) fatty acids have increased in comparison with the control groups ($p<0.05$; $p<0.01$; $p<0.001$) and also the levels of (18:2) and arachidic acid (20:0) decreased in comparison with the control groups ($p<0.05$; $p<0.001$). We also observed that the levels of (16:0), (18:2) and (18:1) fatty acids have decreased and the level of (16:1) has increased in corn leaves in acid rain group compared to those of control group ($p<0,01$).

From the obtained results it is understood that acid rains affected the metabolism of fatty acid which is important for development of the plants. We think that acid rains have affected on the activities of enzyme which has important role in the synthesis of the fatty acids. We found that acid rains especially affected on (16:1) and (18:2) fatty acids in both bean and corn plants. We can also say that negative changes in these fatty acids are results from lipid peroxidation on the plant leaf.

In conclusions; from the results of the study, it is concluded that plants exposed to acid rain have suffered and their structure have been affected as molecular level. Since the plants suffer from the severe stress caused by acid rain, the balance of ecosystems might be damaged and thus life process of living things will be negatively affected.

Experimental

Six seeds were planted in each of two pots in dimension of 60x40x15 and grown standard conditions for 30 days. Experiments were conducted on the leaves of bean and corn using control and acid groups. Control group was in normal conditions. In acid group, plants were placed in closed system and SO_2 gas formed as a result of reaction of $NaSO_3+H_2SO_4$ was given to this system (the concentration of SO_2 gas was 10 mg L^{-1}) for 24 hours. At the same time, acid rain was simulated by given water vapor to the system. At the end of time, the samples were taken out from leaves cut up pieces by homogenizer.

Extraction of vitamins in leaves was realized according to Sahin et al [13]. Leaves extracts were re-dissolved in 1 mL methanol. Samples were transferred to auto sampler vials of the HPLC (Shimadzu HPLC-VP Series and Supelcosil LC 18 DB Column) instrument. Vitamins, the mixture of acetonitrile/methanol (3/1, v/v) was used as the mobile phase and the elution was performed at a flow-rate of 1 mL/min. The temperature of column was kept at 40 ˚C. Quantification was according to Miller et al.[14] using absorption spectra of 202 and 246 nm for Vitamin E and Vitamin D_2 respectively. Identification of the individual vitamins was performed by frequent comparison with authentic external Standard mixtures analyzed under the same conditions.

Extraction of Plant Materials

Fatty Acid and Vitamin Analysis

1 g plant leaves materials for fatty acid and vitamin analyses were finely ground in a mill and were then extracted with hexane/isopropanol (3:2 v/v) [15]. The lipid extracts were centrifuged at 10.000 *g* for 5 minutes and filtered. The solvent was then removed on a rotary evaporator at 40°C. The extracted lipids were stored at -25°C until further analysis. The experiment was repeated three times.

Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol [16]. The fatty acid methyl esters were extracted with n-hexane. The methyl esters were then separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver.3) coupled to a Glass GC 10 computer software. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter) (Permabound 25, Macherey-Nagel, Germany) using nitrogen as a carrier gas (flow rate 0.8 ml/min.). The temperatures of the column, detector and injection valve were 130-220, 240, and 280°C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions.

Samples were analyzed in duplicate and results averaged. Area % of the fatty acid methyl esters were determined by summing the peak areas corresponding to the seven fatty acids identified. The experimental results were reported as mean ± S.E.M. Statistical analysis was performed using SPSS Software. Analysis of variance (ANOVA) and an LSD test were used to compare the experimental groups with the controls.

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