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13

Estimation of some Biochemical Parameters in *Lycopersicon lycopersicum* (L.) cv. Damyanti in Response to Acid Rain

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Abstract

In the present study investigations were done to analyze the effect of acid rain on some biochemical parameters of the tested plants i.e. Lycopersicon lycopersicum (L.) CV. Damayanti. The plants were exposed to different acid water solutions of pH 5.6, 4.5, 3.5 and 2.5. The control set of plants was treated to only distilled water (pH 5.6). The plants were given treatment of acid rain solution after only 5 days of sowing till maturity of crop, after a gap of 20 days interval. It was obvious after the study that exposure to simulated acid rain affected biochemical components of leaves in treated plants and caused them to subside to a great extent. Low pH of simulated acid rain proved more toxic to the green pigments of treated plants as compared to the higher pH. It was also observed that with increasing acidity the values of carbohydrate content, anthocyanin content, was decreased, whereas the values of proline and ascorbic acid content was found to increase as a result of stress developed by increasing acidity in rain.

Keywords- *Acid rain, tomato, biochemical parameters.*

The term "acid-rain" first appeared in a remarkable book authored by Robert Angus Smith "Air and Rain: Beginning of chemical climatology". In the mid 1970's the existence of highly acidic rain became widely known because it appears to be reducing biodiversity through acidification of surface waters. This ecological problem was linked to emissions of oxides of sulphur and nitrogen. Small contributions derive from hydrogen chloride, carbon dioxide and other organic acids found in the air. Analysis of more than 1500 precipitation samples, with a median pH of 4.0, revealed that in 80-100% of the cases, low pH was attributable to sulphuric and nitric acids (Galloway *et al.*, 1976). Likens (1975) observed that precipitation of hydrogen ion contents was 60% due to sulphuric acid, 34% due to nitric acid and 6% due to various organic acids.

Acid deposition became an issue of major concern in Asia in early 1980s, nearly one decade after widespread acid deposition was recognized in Europe and America (Bhatti, 1992). Before the establishment of national monitoring networks for acid deposition in Asia , isolated surveys of acidity level and chemical composition of rain water in some Asian countries (such as China, Japan and India) indicated the occurrence ofacid rain (Bhatti,1992).

Asia is now the Global hotspot of S and N deposition (Vet *et al.*, 2014). Since the early 2000s, the global maximum of both S and N deposition is found in East Asia including regions like eastern China and South Korea. Other areas of high deposition in Asia include sections of Pakistan, India, Bangladesh, Myanmar, Thailand, Laos, North Korea and Japan (Vet *et al.*, 2014). It was reported that the pH of rainfall in China was higher due to high buffering of precipitation acidity by emissions of basic particulate matter, including soil dust (Larssen and Carmichael, 2000), anthropogenic dust (Zhu *et al.*, 2004: Lie *et al.*, 2011), and NH_3 (mainly from agricultural activities : Kang *et al.*, 2016).

Acid rain increases soil acidity, thus affecting flora and fauna both, causing acidification of lakes and streams thus affecting aquatic life, crop productivity and human health. In addition it also corrodes buildings, statues, bridges, fences, monuments, etc. Anthropogenic inputs of S and N into terrestrial ecosystem impact soil and surface water, causing acidification and eutrophication (Bowman *et al.*, 2002). Soil acidification, as indicated by a significant decrease in soil pH and increase in aluminium (Al) mobilization and increased N leaching (Aber*et al.*, 2003), has been commonly reported in East Asia (Larssen*et al.*, 2011: Asano and Uchida, 2005: Fang *et al.*, 2011). The adverse effects of high atmospheric concentrations of SO2 and NO2 on trees and agricultural crops have long been known. Plants sensitive to these gases show necrosis of leaves or needles at exposure to SO2 and NO2 in excess of current levels or anticipated levels as well as, sulphuric acid droplets for short periods. Vegetation serves as natural sink for air pollutants by producing an enormous surface of expanded leaves for absorption and setting of gases and particulate matter .Acid rain causes reduction in agriculture production and their biochemical quality. Relationship between pollutant concentration and response of plants depends upon many factors such as species condition, stage of development, sensitivity of seedling etc.The variation in the plants response to acid rain may be due to interaction of rain treatment with a number of biological, chemicaland climatic factors.

In recent years the acidity of rain and snow has increased sharply all over India. Acid rain with pH 4.8 was reported in Greater Mumbai in 1974-75. (Negi, 1983). Low pH levels have also been reported from Delhi, Maharashtra, Uttar Pradesh, Madhya Pradesh, Tamil Nadu and even the Andaman Island. An analysis of data obtained from ten Indian Background Air Pollution Monitoring Stations (BAPMONS) collected during 1974-85 shows that a few areas are already under stress conditions (Khemani*et al*., 1989). According to researchers at Tata Energy Research Institute (TERI), by the year 2020, the energy demands in India is expected to increase by 300 percent from the present level and SO2 emissions are expected to increase more than four fold between 1990 and 2020. Therefore, in the coming years, large portions of Northern and Western India specifically Delhi, Agra, Chembur, Thane, Trombay, Belapur, Pune, Nagpur, Korba, Singrauli, Kodaikanal etc. are expected to be severely affected with acidic rainfall (Mohan and Kumar, 1998).

Acid rain may lead to leaching of essential cations such as Ca+2 and Mg+2 (Byres and Volk, 1981). An increase in concentration of sulphate (SO_4^-), calcium (Ca+2) and magnesium (Mg+2) in soil leachate was recorded in plants of sugar maple at the acid rain of pH 3.5 (Hutchinson *et al.*, 1999). Strayer and Alexander (1981) reported that CO2 production from glucose was significantly reduced after the soil has been exposed to simulated acid rain (pH 3.2). Glucose mineralization in the test soils (pH values of 4.4–7.1) was inhibited acid rain at pH 3.2 but not at 4.1.

Pea plants showed significant reduction in photosynthesis rates (PN) in response to ambient air pollution (SO2, NO2 and O3) at various sites in peri- urban areas of Varanasi. There was found significant difference in photosynthesis rate among sites (Rajput and Agrawal, 2004). Gaseous pollutants have been shown to inhibit photosynthesis rate depending upon exposure dose and species involved (Darall, 1989).

Sheridan and Rosenstreter (1973) reported that simulated acid rain destroyed chlorophyll and depressed photosynthesis in moss Tortularuralis. The acid treatment also reduced chlorophyll b concentrations in Pine, and to a less significant extent, in giant sequoia also (Westman and Temple, 1989).

Kumar (1997) observed that accumulation of different biochemical components in leaves of Zea mays cultivars got affected in acid rain treated, 60 d and 75 d old plants. Chlorophyll a, b and total chlorophyll were found to be increased at pH 4.5 and 3.5 acid rain and decreased at pH 2.0 acid rain.

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Chlorophyll a was more sensitive to acid rain as compared to chlorophyll b since most pronounced effects were observed in chlorophyll a. Maximum reduction in chlorophyll a as well as chlorophyll b was noticed in cultivar Sweta and minimum reduction of chlorophyll a was noticed in cultivar Ganga-5, and of chlorophyll b in cultivar Ganga-2 at the 2.5 pH acid rain. Carotenoid content in leaves showed significant decrease with an increase in acidity of treatments in both 60 d and 75 d old plants. Maximum reduction was noticed at pH 2.5 in cv. Kanchan after 60, as well as, 75 d and minimum effect was at pH 4.5 in cv. African tall at the age of 60 and 75 d. Chlorophyll content was found to be affected due to low pH levels in Triticumaestivum plant (Raj *et al.,* 2003). Ramakrishnaiah and Somashekhara (2003) also found that chlorophyll content was reduced due to pollution. Rajput and Agrawal (2004) in a study aimed to assess the effect of ambient air pollution on physiological as well as yield characteristics of pea plants grown at different sites in periurban areas of Varanasi, found that total chlorophyll content was less at polluted sites in comparison to reference sites. Carotenoid content was found reduced. Rabe and Kreeb (1979) reported the diminution of protein contents and the increased activities of the enzymes glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, alanine aminotransferase and glutamate dehydrogenase as indicators of low pollution levels, which don't produce visible damage on plants. Sarkar *et al.* (1986) found a close correlation between the distance of plants from the roadside and acceleration in peroxidase and catalase activities.

Table 1: Estimation of some biochemical components in leaves *of Lycopersicon lycopersicum* treated with different pH of acid rain at 20d and 40 d.

	Plant age											
	20 d						40 d					
	pH of acid water solution				CD		pH of acid water solution				CD	
Attribute	5.6	4.5	3.5	2.5	5%	1%	5.6	4.5	3.5	2.5	5%	1%
Leaf extract pH	6.456	6.266	5.706**	5.032**	0.249	0.292	6.363	6.076**	5.433**	5.610**	0.139	0.164
	±0.055	±0.068	±0.180	±0.133			±0.325	±0.159	±0.250	±0.426		
RWC	86.17	84.062	83.333	82.86	-	-	85.65	84.826	83.264	82.164	-	-
Chl*a*	0.05	0.863	0.739	0.779**	0.129	0.151	1.24	0.976**	0.872**	0.847**	0.105	0.124
(mg g^{-1}f.wt.)	±0.035	±0.068	±0.032	±0.041			±0.120	±0.031	±0.060	±0.047		
Chl*b*	0.708	0.627**	0.605	0.58	0.638	0.075	1.03	0.956	0.759**	0.645**	0.109	0.128
(mg g^{-1}f.wt.)	±0.049	±0.026	±0.030	±0.024			±0.061	±0.031	±0.054	±0.043		
Chlorophyll stability index	100	96.136	86.284	86.928	-	-	100	85.632	71.265	65.246	-	-
Carotenoids	0.741	0.81	0.641	0.614*	0.123	0.145	1.603	1.489	1.309**	0.879**	0.154	0.147
(mg g^{-1}f.wt.)	±0.029	±0.037	±0.018	±0.017			±0.019	±0.033	±0.023	±0.048		
Anthocyanin	0.034	0.024	0.018*	0.016*	0.153	0.18	0.04	0.034	0.028**	0.023**	0.009	0.011
(mg g^{-1}f.wt.)	±0.002	±0.001	±0.001	±0.002			±0.004	±0.004	±0.002	±0.003		
Protein	15.749	14.634	13.204**	12.229**	1.829	2.148	19.58	19.333	18.385	15.841*	3.471	4.076
(mg g^{-1}f.wt.)	±0.847	±0.616	±0.758	±1.075			±1.999	±1.894	±1.671	±1.674		
Carbohydrate	22.685	20.598**	19.574**	18.753**	1.661	1.951	29.95	28.04	25.525**	24.398**	2.683	3.151
(mg g^{-1}d.wt.)	±1.024	±1.300	±1.147	±1.243			±2.400	±1.214	±1.107	±0.754		
APTI	8.707	8.503	8.457	8.422	-	-	8.699	8.656	8.553	8.435	-	-

Observations:

The assessment studies were conducted on three economically important vegetable crops of the family Solanaceae to assess their sensitivity to different pH of acid rain viz. 5.6, 4.5, 3.5 and 2.5. The choice of the above concentrations was made to observe the limit of the tolerance of the test plants to simulated acid rain. The plants selected for experiments were:

Lycopersiconlycopersicum (L.) Karsten (= Lycopersicon esculentum Mill.) cv. Damyanti

The seeds were obtained from a local National Seed Corporation (NSC) shop of Meerut. Observations were made on the morphological, reproductive and biochemical makeup of plant species. Seeds of *Lycopersicon lycopersicum* were sown in small polythene bags filled with sandy loam soil. Before filling the bags, soil was well pulverised and homogenised with equal amount of farm manure. During the course of experiment, normal agronomic practices were followed and no pesticide or fertilizer was added.Solutions of different pH values viz. 5.6, 4.5, 3.5, and 2.5 were prepared using a combination of sulphuric acid and nitric acid in the ratio of 7:3 v/v (Lee et al., 1981). The solution of pH 5.6 was taken as control. For the evaluation of response of biochemical machinery of plants to acid rain the following biochemical attributes were studied on the leaves and fruits of two test plant at an interval of 20 days.

Table2: Estimation of some biochemical components in leaves of *Lycopersiconlycopersicum* treated with different pH of acid rain at 60d and 80d.

	Plant age											
	60 d						80 d					
	pH of acid water solution				CD		pH of acid water solution				CD	
Attribute	5.6	4.5	3.5	2.5	5%	1%	5.6	4.5	3.5	2.5	5%	1%
Leaf extract	6.043	5.640**	5.420**	5.153**	0.156	0.183	6	5.8	5.400**	5.246**	0.24	0.278
pH	±0.166	±0.100	±0.100	±0.040			±0.100	±0.100	±0.100	±0.050		
RWC	86.26	82.624	80.256	80.01	-	-	85.555	80.45	78.442	70.24	-	-
Chl*a*	2.631	1.567**	1.186**	1.174**	0.516	0.606	3.303	3.27	1.806**	1.106**	0.28	0.323
($mg\,g^{-1}$f.wt.)	±0.208	±0.167	±0.246	±0.147			±0.185	±0.081	±0.166	±0.150		
Chl*b*	2.022	1.251**	1.094**	1.054	0.633	0.743	1.585	1.324**	1.131	1.001**	0.16	0.19
($mg\,g^{-1}$f.wt.)	±0.693	±0.281	±0.121	±0.162			±0.152	±0.205	±0.220	±0.122		
Chlorophyll stability index	100	60.605	48.69	47.638	-	-	100	97.87	59.938	43.15	-	-
Carotenoids	1.615	1.424**	1.402**	1.365**	0.051	0.107	1.352	1.327	1.064**	0.898**	0.13	0.155
($mg\,g^{-1}$f.wt.)	±0.015	±0.033	±0.002	±0.047			±0.011	±0.020	±0.056	±0.041		
Anthocyanin	0.049	0.047	0.042	0.031**	0.007	0.008	0.058	0.052**	0.045**	0.038**	0	0.002
($mg\,g^{-1}$f.wt.)	±0.003	±0.004	±0.005	±0.003			±0.002	±0.004	±0.004	±0.004		
Protein	25.35	24.13	21.424**	20.040**	3.187	3.743	28.865	26.29	24.361**	20.606**	2.69	3.159
($mg\,g^{-1}$f.wt.)	±2.207	±1.631	±0.625	±1.581			±1.356	±1.040	±1.525	±1.244		
Carbohydrate	41.79	38.582**	36.232**	34.432**	2.086	2.45	48.982	46.66	44.686**	42.503**	3.06	3.588
($mg\,g^{-1}$d.wt.)	0.757	±1.112	±1.380	±2.028			±2.583	±2.433	±2.613	±2.218		
APTI	9.032	8.604	8.345	8.327	-	-	9.259	8.712	8.439	7.618	-	-

Result and Discussion:

Exposure to simulated acid rain affected biochemical components of leaves in treated plants and caused them to subside to a great extent. Low pH of simulated acid rain proved more toxic to the green pigments of treated plants as compared to the higher pH. Chlorophyll *a*, chlorophyll *b* and total chlorophyll was found to be reduced under the stress of acid rain. The simulated acid rain of pH 2.5 was found most effective. Chlorophyll *a* level in the leaves of *Lycopersicon lycopersicum* showed maximum percent reduction value of 66.66 at pH 2.5. Chlorophyll *b* content decreased profoundly and maximum percent decline in *L* was 36.84 at 2.5 pH. Similar effects were observed for total chlorophyll content in the treated plants. The values of total chlorophyll at pH 5.6 and 2.5 of simulated acid rain were 4.788 and 2.004 respectively.

Influence of simulated acid rain on carotenoids, the accessory pigments, meant for photoprotection at different ages of plants was also observed. The values attained (mg g^{-1}f.wt.) in leaves of *Lycopersicon lycopersicum* (80 d old) were 1.327, 1.064 and 0.898 at pH 4.5, 3.5 and 2.5, respectively.

Simulated acid rain also induced considerable alteration in anthocyanin content of experimental crops. The amount of anthocyanin exhibited linear relation with the pH of simulated acid rain and the values observed were 0.092, 0.045 and 0.038 at pH 4.5, 3.5 and 2.5, respectively.

Proline content was also measured in test crops, data analysis shows that simulated acid rain treatment also altered the proline content in the experimental crops. The proline content in 80 d old leaves of the test plants was 0.483, 0.528 and 0.608 at pH 4.5, 3.5 and 2.5, respectively.

The relative water content (RWC) also exhibited reduction in accordance with acidity of simulated acid rain. Percent decline in RWC in the plants was recorded and the values attained were 5.97, 8.31 and 17.89 at pH values 4.5, 3.5 and 2.5, respectively. Carbohydrate and protein contents were also assessed in the plants under observation. The carbohydrate content showed significant reduction with an increase in the acidity of simulated acid rain. Most significant reduction in carbohydrate content was obtained at pH 2.5. The values of percent reduction in carbohydrate in leaves of *Lycopersicon lycopersicum* was 13.19 percent at pH 2.5. Simulated acid rain exerted negative impact on Air Pollution Tolerance Index (APTI) of presently studied crops. The APTI of any plant indicates the tolerance capacity of any plant under stress. The result shows that plants exposed to pH of 5.6 have highest value of APTI, while those treated with APTI at 5.6 and 2.5 pH of simulated acid rain are 9.259 and 7.618 in *Lycopersicon lycopersicum* (80 d old) respectively.

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