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Stomatal Study And Foliar Response To Simulated Acid Rain Treatment In *Lycopersicon Lycopersicum(L.)* Plants

Abstract

Experiments were conducted to analyze the effect of acid rain on stomatal behavior of the tested plants i.e. *Lycopersiconlycopersicum* (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 observed after the study that treatment of plant with different pH of simulated acid rain reduced the number of stomata and epidermal cell per unit area on both adaxial and abaxial surfaces of leaves but the impact was more pronounced on upper surface. Decrease in both the number of stomata and epidermal cells got substituted by the increase in the size of epidermal cells. The leaves showed abnormal features in response to prolonged exposure to simulated acid rain. Simulated acid rain caused chlorotic patches in the leaves, which later on get converted into dark necrotic lesions. In the plants, the number of leaves per plant exhibited decline in response to simulated acid rain of different pH values. The reduction was not only confined to number of leaves per plant but also fresh- and dry- weight and leaf surface area.

Keywords: Simulated Acid Rain, Foliar Study, Stomatal Response.

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Introduction

After the onset of industrial revolution, air pollution has emerged as one of the major byproducts. As the world becomes more and more industrialized, it developed various ways for ease of life consequentially more and more harmful ingredients are released into the atmosphere. These various inputs in the form of chemicals and gases disturbed the natural balance of Earth's atmosphere. This change in natural composition of Earth's atmosphere leads to various kinds of pollution. The various instruments of industrialization and urbanization lead to increased energy demands. Burning of fossil fuels in industries and transport sector, industrialization and urbanization had resulted in increased concentrations of gaseous and particulate pollutants in atmosphere leading to air pollution. Air pollutants produced in any air shed are not completely confined, but at times trespassing all geographical boundaries, hence do not remain a problem of urban centers, but spread and affect large rural areas supporting large productive agricultural land (RichaRai, 2011).

Review of Literature

Acid rain is one of the most serious environmental problems emerged as an outcome of air pollution. Any rain, snow or hail with a pH value less than 5.6 is considered as acidic. Analysis of more than 1500 precipitation samples, with median pH of 4, revealed that in 80-100% of the cases low pH was attributable to sulphuric and nitric acids (Galloway *et al*.,1976). High levels of sulphuric and nitric acid can affect the growth of vegetation, leaf and species composition, soil buffering capacity among other components of terrestrial ecosystems (Larssen*et al*., 2006). Asia over the time has emerged as 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 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).

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.Most of the studies conducted on influence of environmental pollution on plants emphasized on physiological and ultrastructural aspects (Heumann, 2002). Studies were also done by different authors on anatomy of vegetative organs under stress conditions of pollution (Alves*et al*., 2008; Ahmad *et al*., 2005 and Verma *et al.*, 2006).

Leaves of a plant are the organs to be primarily affected by environmental changes such as air pollution. The gaseous pollutants compete with CO_2 for uptake through stomata. Greater leaf size/area results in greater interception of the simulated rain, increasing the possibility of injury. In a study conducted by Hosono and Nouchi (1992) on plants of radish (*Raphanussativus*), spinach (*Spinaceaoleracea*) and bush beans (*Phaseolus vulgaris*) exposed to simulated acid rain of different pH it was found that visible injury occurred on leaves of all test plants exposed to acid rain of pH 3.0.In another study on the yield and yield components of tomato (*Lycopersiconesculentum*) Dursun*et al*., (2002) tested the effects of simulated acid rainunder greenhouse conditions. Simulated acid rain with 2.5 and 3.5 pH induced wilting of leaves followed by the appearance of necrotic lesions on leaf surface. Singh and Agrawal (2004) reported a field based experiment conducted to assess the effect of simulated acid rain of different pH i.e. 5.6, 5.0, 4.5, 4.0 and 3.0 on two wheat (*Triticumaestivum*) cultivars Malviya 213 (M 213) and Sonalika). Leaf area declined at pH 4.0 and 3.0 in M 213 at both the ages and at 75 days in Sonalika. Sirohi and Khan (2006) conducted a field experiment to investigate the effect of acidification on the environment and on fodder crop *Trifoliumalexandrium*cv. Mascot grown in nearby agricultural fields. Necrotic lesions were first observed on the leaves of the plants treated with pH 2.5 after 40 days. Total leaf area of the plants exposed to simulated acid rain was also reduced significantly.In a similar study carried out by Irina NetaGostin (2009) on air pollution effects on leaf structure of some Fabaceae species it was found that stomata decrease in size and increase in density in leaves from high polluted sites and dark phenolic deposits were also reported in mesophyll cells of leaf.

In an experiment on effect of simulated acid rain treatments on yield and carbohydrate contents of *Capsicum* cv. NP-46 A. Pal and Kumar (2000) observed that flowering was promoted in simulated acid rain treated plants. Numbers of flowers and fruits per plant were decreased in treated plants for all treatments at all pH values, carbohydrate contents of stem and leaf fractions were affected adversely. The effect of simulated acid rain became more pronounced with increased acidity and duration of treatment. Mai *et al*., (2008) conducted a field experiment to estimate simulated acid rain stress effects on growth and development in winter wheat cv. Yamgmai 12 and observed that simulated acid rain had considerable effect on wheat growth and yield. The growth of leaf area as well as the mass of fresh leaf per unit area declined greatly at pH 3.5, and the yield was significantly lower than control. The plant height was obviously lowered, and the visible injury on leaf surface was observed at pH 2.5. Under acid rain stress, leaf chlorophyll a, chlorophyll b and carotenoid contents, especially chlorophyll a decreased obviously. Acid rain also suppressed the synthesis of soluble sugar and reduced sugar, and the suppression was stronger at pH 3.5, and became much stronger with increasing acidity. The total free amino acid and soluble protein contents in leaves decreased with increasing acidity, and were significantly lower than control when the pH was 3.5 and 4.5, respectively. In a similar study using simulated acid rain of pH 5.7, 4.5 and 3.0, Lal and Singh (2015) observed that acid rain caused a marked decline in Chl a, chl b and carotenoids in sunflower leaves at peak growth stage.Shaukat and Khan (2008) investigated the effect of simulated acid rain (SAR) on growth, yield and physiological parameters in tomato. SAR exposure (pH 3.0 and 4.0) caused white-to-tan spots on the abaxial and adaxial surface of tomato leaves. Kausar et al. (2010) reported that simulated acid rain (SAR) exposure caused adverse effect on morphological, biochemical and leaf epidermal parameters of wheat cv. HD-2329.

Stomata being the primary pathway for exchange of gases between internal leaf surfaces and the atmosphere play an important regulatory role in physiological processes of leaf.Acid precipitation was considered to affect the rate of stomatal gas exchange (Flagler *et al*., 1994). Since guard cells may be injured preferentially upon initial exposure to acid rain along with other epidermal cells, it was postulated that acidic precipitation might affect gas exchange rates as well. Satoh (1996) treated 10 species including weeds and crops to simulated acid rain of pH 2.5 and 3.5. Different species respond differently but as a whole the rate of stomatal gas exchange decreased at and below pH 3.0.In *Zea mays*, a number of changes in stomatalbehaviour have been reported by Kumar (1997) in response to simulated acid rain. A number of characters such as number of stomata, number of epidermal cells, distance between nearest stomata, size of stomata complex, stomatal index and density was found to be affected. Mittal (2002) also reported a decline in number of stomata and epidermal cells in *Iberisamara, Impatiens balsamina, Cosmos bipinnatus* as well as in *Antirrhinum majus.*Mandal (2006) studied roadside plants like *Neriumindicum* Mill., *Boerhaaviadiffusa* L., *Amaranthusspinosus* L., *Cephalandraindica*Naud. and*Tabernaemontanadivaricata* and found that these plants can easily avoid the effects of air pollution by altering their physiological pathways pertaining to photosynthesis and respiration. Stomatal closure in *Boerhaavia, Amaranthus, Cephalandra* and stomatal clogging in *Nerium* and *Tabernaemontana* help these plants in preventing the entry of pollutants. Stomatal area is seen to decrease significantly in *Nerium, Boerhaavria* and *Cephalandra*. Number of stomata remaining open per mm of leaf surface decrease in all the plants under study, except Nerium which, however, possesses sunken stomata.

Several leaf cuticular features such as stomatal frequency, stomatal size, trichome length, type and frequency, and subsidiary complex respond to environmental pollution in different ways and hence can be used as indicators of environmental pollution in an area. Several modifications in cuticular features under polluted environment seem to indicate ectotypic or survival significance for the plant species under investigation (Sharma, 1977).It was also reported by Mittal (2002) that with a decrease in number of stomata, the distance between two nearest stomata got increased. Similar findings were reported by Verma (1999) in three species of Vigna, in green gram by Kumaraveluand Ramanujam (1998) and Rajput and Agrawal in pea plants (2004).High Sulphur di oxide concentrations are phytotoxic and disturb stomatal behavior, photosynthesis, transpiration and formation of secondary metabolites as reported by Agarwal (2003) and Wali*et al*., (2004). In SO_2 exposed plants sulphur accumulation mainly occurs in aerial parts through open stomata on leaves (Mandal, 2006). In another experiment on response of trichome and stomatal frequency of leaves to exposure of aqueous SO_2 by SEM in pigeonpea and amaranth, it was observed that stomatal frequency of leaves increased in both the plants whereas trichome frequency was increased in pigeonpea and decreased in amaranth (Sujatha et al., 2016).

Aim of the Study

The aim of study is to assess the impact of air pollutants such as an acid rain on the most vulnerable part of the plants i.e. leaves which acts as an organ of plants that are foremost to be affected by environmental changes including air pollution. The present investigation aims at;

1. Determining the impact of acid rain on stomata in leaves
2. The present study also helps to investigate the effect of simulated rain on leaves of plant.

Material and Methods

The assessment studies were conducted on economically important vegetable cropof 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 (= *Lycopersiconesculentum* 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. Effects of different pH of acid rain were also studied on leaf and stomatal behavior in the above mentioned vegetable crops.

Seeds of *Lycopersiconlycopersicum* 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.

Experiments on the cultivars were carried out for long term studies. The plants were divided into different sets. Four sets were given the treatment of pH 5.6, 4.5, 3.5 and 2.5 of acid rain solution .The set which is given treatment of pH 5.6 was used as control and it was run in identical conditions of temperature, light, etc. but without exposure to acid solution. The plants were given the treatment of acid rain from the fifth day of sowing to maturity of the crops once in a week with the help of a sprayer.

Preparation of Acid Water Solution

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.

Growth Response

The plants taken for growth studies in response to simulated acid rain were carefully dug out of the soil with intact root and shoot system and were thoroughly washed under tap water in order to remove all the soil from it. Then the intact plants were dried on blotting paper.

Number of Leaves

Leaves were counted per plant for all concentrations at different ages of the plant in both the plant species.

Leaf Area (cm^2)

For calculating the single leaf area, an outline of the leaf was drawn on the graph paper and the area was determined by means of a manual planimeter.

For analysis of stomatal characters the replica technique (conservative facsimile technique) was adopted (Prakash and Kumar, 1995). First the leaf surface (dorsal/ventral) was made clean with the help of cotton and then a thin layer of adhesive quick fix spreaded over it. It was allowed dry for a few seconds and then to the dried surface was fixed a cellotape for one minute. Then cellotape along with epidermal imprint was pulled off from the leaf surface and placed over a glass slide. The slide was observed under microscope using different powered objective and oculars. The various stomatal attributes were studied and for each attribute three replicates were used. Stomatal analysis was made for both the dorsal and ventral surfaces of leaves.

Following stomatal characters were studied-

No. of Stomata per Microscopic Field Area

Number of stomata were counted in three randomly selected field area under high power lens of microscope and mean value was derived.

No. of Epidermal Cell per Microscopic Field Area

Number of epidermal cells except those of guard cells was counted in three randomly selected microscopic field areas.

Stomatal Index (SI)[1]

Stomatal Index was calculated by the following formula-

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$$\text{Stomatal Index}=\frac{\text{No. of stomata per unit area}}{\left(\text{No. of stomata per unit area}\right)+\left(\text{No. of epidermal cells per unit area}\right)}\times 100$$

Observations

Table 1a: Number of leaves per plant, leaf fresh wt., leaf dry wt., single leaf area in *Lycopersiconlycopersicum* in response to acid water solution at 20 d and 40 d plant age.

Attribute	Plant age							
	20 d				40 d			
	pH of acid water solution							
	5.6	4.5	3.5	2.5	5.6	4.5	3.5	2.5
No. of leaves plant^{-1}	9.036 ±0.055	8.746 ±1.035	8.386 ±0.200	7.590 ±0.448	17.570 ±0.147	17.037 ±0.138	16.920 ±0.166	15.386 ±0.250
Leaf fresh wt. (g)	0.180 ±0.003	0.158 ±0.006	0.105 ±0.003	0.097 ±0.001	1.140 ±0.010	1.276 ±0.075	0.933 ±0.056	0.765 ±0.016
Leaf dry wt. (g)	0.019 ±0.004	0.017 ±1.527	0.014 ±0.001	0.012 ±0.002	0.553 ±0.040	0.546 ±0.035	0.460 ±0.036	0.380 ±0.026
Single leaf area (cm^2)	5.620 ±0.144	5.703 ±0.204	5.403 ±0.090	5.090 ±0.100	33.810 ±1.917	31.483 ±1.914	32.800 ±2.029	29.066 ±1.823

± Standard deviation

Table 1b: Number of leaves per plant, leaf fresh wt., leaf dry wt. and single leaf area in *Lycopersiconlycopersicum* in response to acid water solution at 60 d and 80 d plant age.

Attribute	Plant age							
	60 d				80 d			
	pH of acid water solution							
	5.6	4.5	3.5	2.5	5.6	4.5	3.5	2.5
No. of leaves plant^{-1}	31.060 ±0.026	31.923 ±0.502	30.386 ±0.309	28.233 ±0.145	45.166 ±1.722	42.000 ±1.000	41.000 ±1.000	36.666 ±1.527
Leaf fresh wt. (g)	2.460 ±0.052	2.320 ±0.098	2.083 ±0.030	1.920 ±0.062	4.283 ±0.070	4.206 ±0.095	3.763 ±0.229	3.426 ±0.170
Leaf dry wt. (g)	1.750 ±0.030	1.660 ±0.036	1.327 ±0.072	1.083 ±0.064	2.476 ±0.025	2.226 ±0.055	1.993 ±0.015	1.533 ±0.160
Single leaf area (cm^2)	64.666 ±1.258	64.630 ±2.608	63.200 ±1.868	63.000 ±2.645	96.666 ±1.527	94.500 ±0.916	94.333 ±0.550	92.400 ±0.458

Table 2: Effect of simulated acid rain on stomatal behaviour of *Lycopersiconlycopersicum.*

		pH of acid water solution			
		5.6	4.5	3.5	2.5
No. of Stomata	UE	19.333	19	18.333	16.333
		±1.527	±1.000	±1.54	±1.527
	LE	26	24.66	24	19.666
		±3.464	±3.055	±2.000	±1.154
No. of epidermal cells	UE	64	65.666	68	70.666
		±1.000	±3.785	±1.000	±1.527
	LE	82	86	87.333	89
		±2.000	±2.000	±3.055	±2.645
Stomatal Index	UE	30.0207	29.687	26.96	23.332
	LE	31.707	38.54	27.906	22.096

Result and Discussion

In general, *Lycopersiconlycopersicum* exposed to simulated acid rain showed poor growth. The leaves showed abnormal features in response to prolonged exposure to simulated acid rain. Simulated acid rain caused chlorotic patches in the leaves, which later on get converted into dark necrotic lesions. In the plants, the number of leaves per plant exhibited decline in response to simulated acid rain of different pH values (Tables 1a-1b). Maximum percent reduction in number of leaves per plant was shown at pH 2.5 and it was 18.81 percent in *Lycopersiconlycopersicum.* The reduction was not only confined to number of leaves per plant but also fresh- and dry- weight and leaf surface area. The percent reduction in leaf fresh weight and dry weight was 20.0 and 37.13 percent, respectively in *Lycopersiconlycopersicum.* The percent reduction in surface area of leaf at pH 2.5 was 4.41 percent in *Lycopersiconlycopersicum*at final harvest day.

Test plants exposed to simulated acid rain exhibited considerable decline in root, shoot and total plant length fresh- and dry- weight of root, shoot, leaf and whole plant, single leaf area and number of leaves per plant, in comparison to their respective controls. The effect was dependent on both the pH of simulated acid rain and age of plants. Acid rain, in general, showed its effects at crop maturity. It's most harmful effect was observed at pH 2.5 as against the other pH values.

The effects of acid rain were found to be leaf- tissue age dependent. Mature leaves were found to be more susceptible than younger leaves. Similar results have been obtained by Evans and Curry, 1979; Paparozzi and Turkey (1983). This can be attributed to the fact that mature leaves being in degradation phase, their degradation is accelerated by pollutant leading to their greater susceptibility to simulated acid rain. On the other hand, young leaves, being in synthetic phase, activity synthesise metabolites against the pollutant and hence and resistant.

An Important feature of plant growth and productivity is leaf growth. Area of single leaf and number of leaves per plant is found to be reduced in both *Lycopersiconlycopersicum*. The reduction in number of leaves in treated plants is significant for plants adaptation to pollutant stress by limiting the absorption area. Decreased number of leaves per plant hampers the productivity of the plant. The reduction in number of leaves due to acid rain treatment may be because of dormancy and ultimately the death of leaf buds, perturbations in biochemical processes, resulting in lowering of cell division capacity of the leaf may cause reductions in leaf area. Reduction in leaf area due to acid rain has also been reported by Takemoto*et al.* (1988) in *Capsicum annuum* and by Takemoto and Bytnerowicz (1993) in *Pinus ponderosa*.

Simulated acid rain was also found to cause foliar injury in *Lycopersiconlycopersicum*. The area of leaf in contact with rainfall acidity may determine the amount of foliar injury. Leaf wettability is also an important determinant of foliar damage by acid rain. The physical and chemical characteristics of leaf surface determine the wettability of most leaves (Evans, 1982). Acidic rain may change the surface characteristics of foliage by erosion of cuticular waxes. This enhances the wettability of leaf surface thereby enhancing the penetration of simulated acid rain. This was also reported by Shriner (1977) and Natalie Buch (2014).

Acute injury in leaves may be accounted for the accumulation of nitrite and sulphite ions in the mesophyll tissue. These ions bring about metabolic changes, as in enzyme activity and destruction of chloroplast with the continuous exposure to acid water, the cells first get inactivated with or without chlorosis and then killed .The tissue collapses and then dries up leaving a characteristic pattern of interveinal and marginal acute injury. If only a few cells in an area get injured the area becomes chlorotic accruing to chronic injury. This was also supported by Thomas (1961).

Stomata represent the main avenue for the flow of gases into and out of the leaves. In the present study *Lycopersiconlycopersicum*show alteration in stomatal characteristics, standard number and stomatal index was found to decrease as a result of exposure to simulated acid rain. These finding are in consonance with the observations of Kumar (1997), Verma (1999) and Irina Gostin (2009). The decrease in number of stomata is also regarded as a protective measure against the polluted environment. This would reduce the amount of pollutants entering the leaves, thus preventing the plants from their injurious effects (Sharma and Tyree, 1973).

The alteration in stomatal parameters, decrease in number of leaves and leaf area may be considered responsible for a decline in growth potential of test crops. The main avenue, for entry of pollutant into the plant system, is through stomata, which are minute openings in the epidermal layers and primarily meant for gaseous exchange and transpiration. Treatment of plants with different pH of simulated acid rain reduced the number of stomata and epidermal cell per unit area on both adaxial and abaxial surfaces of leaves but the impact was more pronounced on upper surface. Decrease in both the number of stomata and epidermal cells got substituted by the increase in the size of epidermal cells. Decline in number of stomata were recorded on both the surfaces of leaves, but the decline was more pronounced on abaxial surface than on adaxial surface of leaves. The number of stomata on abaxial surface of leaves of *Lycopersiconlycopersicum* were 19.00, 18.33 and 16.33 at pH 4.5, 3.5 and 2.5, respectively (Table 2). It was also reported by IrinaGostin (2009) that there occurs a stomatal index increase in *Trifoliumrepens and T. montana* lower epidermis in response to air pollution from cement factory.

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Footnotes

1. *These values were calculated according to the formula given by Salisbury and Ross (1972).*