Asian Resonance
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. 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 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

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 nature 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 60-100% 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
Asian Resonance

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. Yangmai 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 decreased greatly. 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 at pH 5.7, 4.5 and 3.7, and Singh (2015) observed that acid rain caused a marked decline in Chi a, chi 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. Kauser 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 stomatal behaviour 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 Iberis amara, Impatiens balsamina, Cosmos bipinnatus as well as in Antirrhinum majus. Mandal (2006) studied roadside plants like Nerium indicum Mill., Boerhaavia diffusa L., Amaranthus spinosus L., Cephalandra indica and Tabernaemontana divaricata 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
Asian Resonance

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.

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 (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 (cm²)**

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.

**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 spread over it. It was allowed dry for a few seconds and then to the dried surface was fixed a celloptape for one minute. Then celloptape 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 replications were used. Stomatal analysis was made for both the dorsal and ventral surfaces of leaves.**

**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)**

Stomatal index was calculated by the following formula-


clogging in *Nerium* and *Tabernaemontana* help these plants in preventing the entry of pollutants. Stomatal area is seen to decrease significantly in *Nerium*, *Bohraavan* and *Cephalandra*. Number of stomata remaining open per mm² 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 ectotopic 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 Agrawal (2003) and Waker *et al.* (2004). In SO₂ 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 SO₂ 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 crop 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:

- *Lycopersicon lycopersicum* (L) Karsten (= *Lycopersicon sculentum* Mill.) cv. Darnyanti
- 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.
Asian Resonance

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

Observations

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

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Plant age</th>
<th>pH of acid water solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 d</td>
<td>40 d</td>
</tr>
<tr>
<td>No. of leaves plant(^{-1})</td>
<td>9.036 ± 0.055</td>
<td>8.746 ± 1.035</td>
</tr>
<tr>
<td>Leaf fresh wt. (g)</td>
<td>0.180 ± 0.003</td>
<td>0.158 ± 0.006</td>
</tr>
<tr>
<td>Leaf dry wt. (g)</td>
<td>0.019 ± 0.004</td>
<td>0.017 ± 0.006</td>
</tr>
<tr>
<td>Single leaf area (cm(^2))</td>
<td>5.620 ± 0.144</td>
<td>5.703 ± 0.204</td>
</tr>
</tbody>
</table>

± Standard deviation

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

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Plant age</th>
<th>pH of acid water solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 d</td>
<td>80 d</td>
</tr>
<tr>
<td>No. of leaves plant(^{-1})</td>
<td>31.060 ± 0.026</td>
<td>31.923 ± 0.026</td>
</tr>
<tr>
<td>Leaf fresh wt. (g)</td>
<td>2.460 ± 0.032</td>
<td>2.320 ± 0.098</td>
</tr>
<tr>
<td>Leaf dry wt. (g)</td>
<td>1.750 ± 0.030</td>
<td>1.650 ± 0.036</td>
</tr>
<tr>
<td>Single leaf area (cm(^2))</td>
<td>64.666 ± 1.258</td>
<td>64.630 ± 2.608</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>pH of acid water solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6</td>
</tr>
<tr>
<td>No. of Stomata</td>
</tr>
<tr>
<td>LE</td>
</tr>
<tr>
<td>No. of epidermal cells</td>
</tr>
<tr>
<td>LE</td>
</tr>
<tr>
<td>Stomatal Index</td>
</tr>
<tr>
<td>LE</td>
</tr>
</tbody>
</table>

Result and Discussion

In general, *Lycopersicon esculentum* 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 are 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 lower rate 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 Bytnowicz (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 rain, the cells 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 Lycopersiconlycopersicumshow 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 1). It was also reported by Irina Gostin (2009) that there occurs a stomatal index increase in Trifolium repens and T. montana lower epidermis in response to air pollution from cement factory.

References

Asian Resonance


Asian Resonance


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