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The effects of simulated acid rain of different pH-levels on biomass and leaf area in Sunflower (*Helianthus annuus*)

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Abstract

The effects of simulated acid rain (SAR) of different pH [distilled water-7.0 (control), 5.7, 4.5 and 3.0] were studied using sunflower (*Helianthus annuus*) cv. 'Morden' as test system. Sunflower plants were sprayed with 30 ml acid rain solution at weekly intervals starting from two leaved stage till initiation of flowering in the early morning under natural environment. Evaluation of SAR effects on plant roots, shoots and leaves at peak growth and maturity stages revealed that biomass and lengths of the studies plant parts decreased with decreasing pH of acid rain solution. Comparison of biomass and length at peak growth and maturity stages recorded maximum difference in control the difference narrowed with increasing acidity. The differences at acidic treatments were well-marked with leaves followed by roots and shoots, respectively. In case of length, roots and shoots were more adversely affected as compared to leaves. Acid rain application caused reduction in leaf area which has direct bearing on growth of roots and shoots, and overall plant growth. Effects of SAR on sunflower increased more dramatically with the increase of SAR acidity and were correlated with exposure times and doses of SAR. The study indicates the sunflower plant to be an acid rain sensitive system and demands for breeding acid rain tolerant varieties in view of growing industrialization and expanding acid rain geographical areas.

Keywords: Simulated acid rain, Sunflower, Roots, Shoots, Leaves, Biomass, Leaf area

INTRODUCTION

The impact of industrial civilization on the environment may be unparalleled in history of the biosphere. Indiscriminate and ever-growing use of energy may not only cause wide spread degradation of natural resources but may also influence our life support system. Acid rain, a serious problems resulting from rapid industrialization, is a major polluting agent possibly harmful to terrestrial and aquatic Ecosystems. The sources of atmospheric deposition can be categorized as either natural or anthropogenic. Unlike the case of fluoride that is emitted by a fewer industries such as the aluminum ones, there are many anthropogenic sources that acidify rain water (Horner and Bell, 1995). Nitrogen and sulphur oxides are the major sources of atmospheric acidity; both are products of combustion, and both are converted in the atmosphere to strong acids, mainly nitric and sulphuric acids that acidify the rain water (Cowling and Linthurst, 1981). Rain that presents a concentration of H^+ ions greater than 2.5 μeq^{-1} and pH values lower than 5.6 is considered acid rain (Evans, 1984). The effects of acid rain on growth and development of plants not well understood, but nevertheless visible injuries and loss of yield has been observed in crops after treatment with simulated acid rain both, in laboratory and field situations. Evans et al. (1981) and Evans and Lewin (1981) reported reduced growth and yield of several crop species due to simulated acid rain under glashouse conditions. Likewise, Field grown soybeans given with simulated acid rain of pH 207 to 4.1 were found to show reduced growth rate and 3 to 23% less seed yield in comparision to plants treated with rain of pH 5.6 (Evans et al. 1983, 1984). Monocots are reported to be less affected by simulated acid rain than dicots (Kuitel and Pell, 1991). In a recent study on vegetable plants species viz. *Capsicum annuum, Lycopersicon esculentum* and *Solanum melongea,* Verma *et al.* (2010) reported that growth parameters and fruiting was severely curtailed in all the three species by simulated acid rain. In the current study, we have tried to assess the impact of simulated acid rain of different concentration (pH) on the growth and biomass production in sunflower (*Helianthus annuus*).

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MATERIALS AND METHODS

The present study was carried out with sunflower variety 'Morden' in the experimental plot available at Oilseed Farm, C.S.A.U.A.T., Kanpur. The soil of experimental plots was sandy loam with sufficient organic matter, pale yellow in colour, sandy loam texture, pH 7.3 and 65 % water holding capacity. Sowing of sunflower variety 'Morden' was done on 12 April 2006 at an inter-row spacing of 60 cm. The seeds were sown at a depth of about 5 cm in the soil. After 15 days of sowing, thinning operation was done and 60 cm inter-row and 30 cm inter-plant spacing (within the row) was finally maintained. During the period of crop growth the maximum and minimum temperature ranged 38.13 ºC to 24.03 ºC, atmospheric moisture ranged 64.23 % to 34.36 % and Saturated Vapour Pressure ranged 20.2 to 18.96. The Average rainfall, Evaporation and Sunshine were 1.11 mm, 8.15 mm/d and 7.43 hour, respectively. After pre-sowing irrigation in the first week of April, two additional irrigations were provided in mid-May and first week of June, respectively. Field experiment was laid out in a randomized block design and comprised of four treatments including control with three replications. There were five rows of ten plants in each treatment to

create different SAR concentrations. Three concentrations of SAR i.e. pH 3.0, 4.5, 5.7 and control (pH 7.0) were applied in their respective plots. These plots were then irrigated regularly with normal deionized water.

Formulation of SAR

Simulated acid rain (SAR) of different concentrations was prepared by mixing conc. 1N nitric acid (HNO_3) and 1N sulphuric acid (H_2SO_4) in 1: 2 molar ratios. The mixture was diluted with deionized water for preparation of solutions of pH 5.7, 4.5, and 3.0, respectively. The plants sprayed with distilled water (pH 7.0) were used as control. Acid rain sprayings were given in the early morning to avoid its application under high temperatures and high irradiance during the day. All treatments of SAR and control plants were treated with 30 ml solution/plant of different pH (SAR treatments), starting from two leaves stage till initiation of first flower buds at weekly intervals.

Plant Sampling Techniques

At the time of sampling, the nine randomly selected plants from each plot were uprooted gently and washed in clean running tap water to remove any foreign material sticking to it. The plants were then wiped off by pressing between the folds of filter paper, were weighed for the fresh weight of root, shoot and leaf and kept in paper bags. The cut plant parts (roots, shoots and leaves) were subjected to oven drying by placing them in aluminium foils and maintained in an electric oven at 80 °C till they gained constant weight.

The cut plant parts (roots, shoots and leaves) were taken for measurement of length. Lengths of roots were measured from ground level to tip of the root at peak growth stage and maturity stage with the help of the meter scale on randomly chosen plants/treatment. Length of the shoots was measured from ground level to the base of the petiole of the upper most leaf during the peak growth stage and to the base of the flower head during the maturity stage. Length of the leaf was measured from the base of the petiole of the leaf to terminal leaflet at peak growth and maturity stage.

RESULTS

Analyses of Variance (ANOVA) for Variables of the Experiments

The analyses of variance (ANOVA) for various variables (not shown) revealed highly significant differences of SAR treatments for the characters like root biomass (fresh weight and dry weight) and length at peak growth and maturity stage, shoot (biomass and length) at peak growth and maturity stage, leaf (biomass and length) at peak growth and maturity stage and leaf area at peak growth stage, These values showed that means have wide variations for all the characters under study.

Root biomass at peak growth and maturity stage

Root fresh weight at peak growth stage in control (pH 7.0) was 19.35 g (Figure 1). SAR application caused reduction in fresh weight of root at pH 5.7 (18.41 g), 4.5, (16.82 g) and 3.0 (12.46 g). Root fresh weight at maturity stage increased in comparison to root fresh weight recorded at peak growth stage. Root fresh weight at maturity stage in control (pH 7.0) was 27.10 g (Figure 3). At pH 5.7 root weight reduced to 18.90 g and showed significant difference from control. The increasing level of acidity to pH 4.5 and 3.0 further showed reductions in root fresh weight to 17.08 g and 13.81 g, respectively. Comparison between root fresh weight at peak growth and maturity stages showed marginal (pH 5.7, 4.5 and 3.0) to significant (pH 7.0) increase in root fresh weight (Figure 3). The difference of pH 5.7 treated root fresh weight with control at maturity stage showed more reduction as compared to root fresh weight at peak growth stage. Comparison of different SAR treatments at both the stages showed that the fresh weights of root decreased gradually with increasing acidity. Tyagi *et al.* (2004) in pea and Sirohi and Khan (2006) in *Trifolium alexandrium* plants observed that root fresh weight decreased with increasing acidity. Pragati and Dhaka (2006) reported that root fresh matter decreased as the pH of the acid rain decreased and duration of exposure increased in *Zinnia elegans* plants. These authors found that root fresh weight was inhibited at all acid rain treatments and same is evident in the present study.

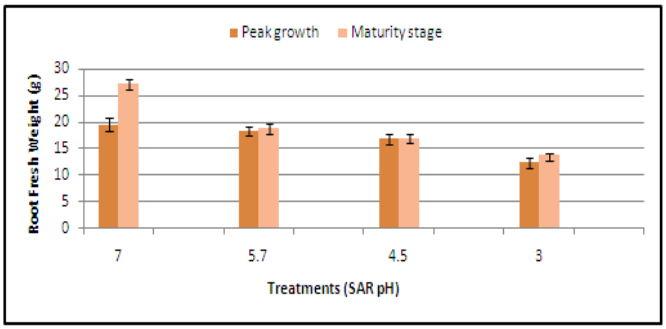

Fig 1. Effect of SAR on root fresh weight at peak growth and maturity stages in sunflower (CD value at Peak growth 1.01 and at maturity stage 0.709). Vertical bars represent SD.

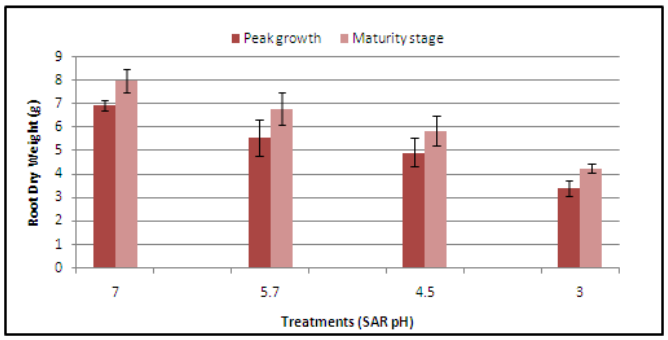

Fig 2. Effect of SAR on root dry weight at peak growth and maturity stages in sunflower (CD value at Peak growth 0.556 and maturity stage 0.553). Vertical bars represent SD.

In case of control, root dry weight at peak growth stage recorded 6.95 g (Figure 2). SAR treatment caused reduction in dry weight of root at pH 5.7 (5.56 g). Root dry weight was further reduced at pH 4.5 to 4.93 g and at pH 3.0 to 3.40 g. In case of pH 3.0 sprayed plants, the root dry weight showed maximum reduction. Root dry weight at maturity stage slightly increased in comparison to root dry weight recorded at peak growth stage. In case of control root dry weight at maturity stage was recorded 7.99 g. SAR applications caused reduction in dry weight of root at pH 5.7, 4.5 and 3.0 and it was 6.81 g, 5.88 g and 4.25 g, respectively. Root dry weight showed significant difference in pH 3.0 from control (Figure 2) and showed more reduction in root dry weight in comparison to other

SAR treatments at maturity stage. The speed of reduction in root dry weight at peak growth and maturity stages was quite slower at both the stages till pH 4.5 whereas subsequently it reduced rapidly at pH 3.0. Jacobson *et al.* (1985) studied effect of episodic exposure of SAR on radish seedling and found reduction in dry mass of shoot and hypocotyls. Hosono and Nouchi (1992) recorded observations on hypocotyls dry weight of radish plants exposed to simulated acid rain and found that it was significantly smaller than control. Chung *et al.* (1994) studied *Perilla frutescens* plants sprayed by SAR and recorded reduced root dry weight. Tyagi *et al.* (2004) assessed effect of SAR on seedling growth and found that dry weight of the root was inhibited by acidity in *Pisum sativum* plants. Sirohi and Khan (2006) studied on *Trifolium alexandrium* plants and found that dry weight of the root decreased due to SAR. Pragati and Dhaka (2006) reported that root dry weight decreased as the pH of the acid rain decreased and the duration of exposure increased in *Zinnia elegans* plants. Shaukat and Khan (2008) demonstrated that simulated acid rain at pH 3.0 and 4.0 significantly suppressed root dry weight of tomato plants. The reduction in root dry weight due to an increase in absorption of nitrate-N through the leaf surface and soil during acid rain treatment as has been proposed by Kohno and Kobayashi (1989) in case of soybean. The reduction in biomass accumulation due to SAR may also be a consequence of reduced photosynthesis (Singh and Agrawal, 1996).

Root length at peak growth and maturity stage

In case of control, root length at peak growth stage was recorded 10.9 cm (Figure 3). SAR application caused reduction in root length at pH 5.7 (9.10 cm), 4.5 (7.81 cm) and 3.0 (6.67 cm). Root length at maturity stage increased in comparison to root length at peak growth stage. Root length at maturity stage in control was recorded 14.70 cm. Root length in the entire treatments showed decreasing trend with increasing level of acidity. At pH 5.7, 4.5 and 3.0, root lengths were 11.05, 9.70 and 7.83 cm, respectively. These values showed significant reductions in root length as compared to control. The comparative study of two stages for root length showed that root length increased significantly at control at maturity stage from peak growth stage but at other pH it was increased marginally. It might be due to accumulation of acid in cell sap of root tissues which restrict the free movement of nutrients from source to sink. The toxic effects of acidity on roots metabolic activities has already been studied by several scientists in different crop species such as in beans (Singh *et al.* 1992), wheat (Singh and Agrawal, 1996, 2004), rice (Suneela and Thakre, 2001), pea (Tyagi *et al.* 2004), mash (Imran and Hussain, 2004), fodder crops (Sirohi and Khan, 2006), *Zinnia elegans* (Pragati and Dhaka, 2006), barley (Morikawa and Saigusa, 2006), *Vigna unguiculata* (Han, 2009) and wheat (Kausar *et al*. 2010). In all the studies discussed above, acid rain application recorded reduction in root lengths. The observations on root length in sunflower plants in present study are supported by the above studies.

Shoot fresh weight at peak growth and maturity stage

Shoot fresh weight at peak growth stage in control (pH 7.0) was 178.00 g (Figure 4). SAR application caused reduction in fresh weight of shoot at pH 5.7 (151.66 g), while at pH 4.5, shoot weight was further reduced 110.66 g and at pH 3.0 to 98.33 g. The pH 3.0 SAR sprayed plants showed minimum shoot fresh weight. Shoot fresh weight at maturity stage increased in comparison to shoot fresh weight at peak growth stage in all the treatments. Shoot fresh weight at maturity stage in control (pH 7.0) was 182.00 g (Figure 4). SAR application caused reduction in fresh weight of shoot at pH 5.7 to 159.00 g and differed insignificantly from control. The increasing level of acidity to pH 4.5 and pH 3.0 showed further reductions in shoot fresh weight to 113.33 g and 100.66 g, respectively.

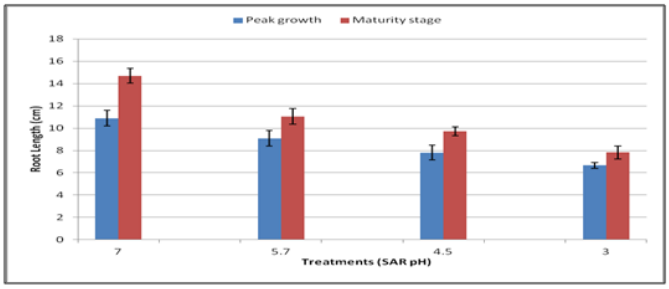

Fig 3. Effect of SAR on root length at peak growth and maturity stages in sunflower (CD value at Peak growth 0.524 and at maturity stage 0.641). Vertical bars represent SD.

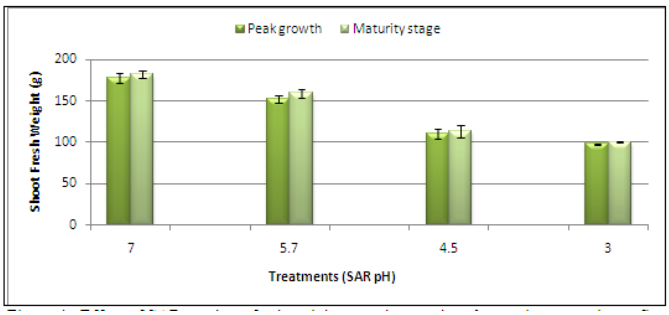

Fig 4. Effect of SAR on shoot fresh weight at peak growth and maturity stages in sunflower (CD value at Peak growth 4.526 and at maturity stage 5.006). Vertical bars represent SD.

The comparative evaluation of shoot fresh weight between peak growth and maturity stages showed maximum difference at pH 5.7. The minimum difference of shoot fresh weight was recorded at pH 3.0. Comparison between shoot fresh weight at peak growth stage and maturity stage showed reduction from control to pH 3.0 at both the stage. The results obtained regarding shoot fresh weight in present study are supported by observation of other studies in different crops. Tyagi *et al.* (2004) and Pragati and Dhaka (2006) reported that shoot fresh weight was inhibited by acidity in pea and *Zinnia elegans*, respectively. Sirohi and Khan (2006) observed that the effect of simulated acid rain water showed decreasing trend (pH 4.0>3.0>2.0) in fresh weight of shoot in *Trifolium alexandrium.* The observations on shoot fresh weight in sunflower plants in present study are supported by observation of Tyagi *et al.* (2004), Sirohi and Khan (2006) and Pragati and Dhaka (2006). The reductions in shoot fresh weight observed at high acidity may be due to acid-auxin interaction as reported by Wood and Borman (1974).

Shoot dry weight at peak growth and maturity stage

Shoot dry weight at peak growth stage in control was 73.55 g (Figure 5). SAR treatments caused reduction in dry weight of shoot at pH 5.7 (47.33 g). Shoot dry weight was further reduced to 40.66 g at pH 4.5 and 21.66 g at pH 3.0. Shoot dry weight at maturity stage slightly increased in comparison to shoot dry weight at peak growth stage. In case of control, shoot dry weight at maturity stage was

recorded 79.33 g (Figure 5). SAR applications caused reduction in shoot dry weight at pH 5.7, pH 4.5 and pH 3.0 to 51.06 g, 44.66 g and 24.66 g, respectively. Tyagi *et al.* (2004) assessed seedling growth of *Pisum sativum* subjected to simulated acid rain and found that dry weight of the shoot was inhibited by acidity. Sirohi and Khan (2006) reported that dry weight of the shoot revealed the decreasing trend (pH 4.0>3.0>2.0) with increasing acidity in *Trifolium alexandrium* plants. Pragati and Dhaka (2006) observed that shoot dry weight decreased as the pH of the acid rain decreased and the duration of exposure increased in *Zinnia elegans* plants. Shaukat and Khan (2008) demonstrated that simulated acid rain at pH 3.0 and 4.0 significantly suppressed shoot dry weight of tomato plants. In alfalfa, acid fog (pH 2.5 or 3.2) was found to inhibit both, transpiration and photosynthesis (Temple *et al.* 1987).

Shoot length at peak growth and maturity stage

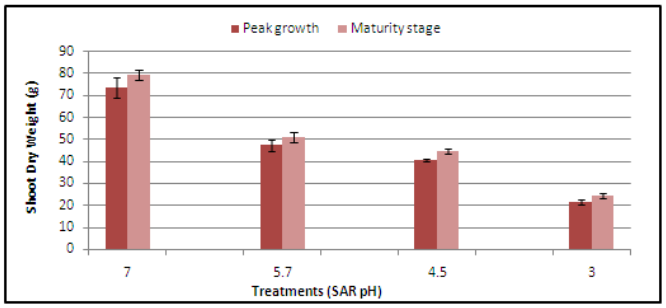

Fig 5. Effect of SAR on shoot dry weight at peak growth and maturity stages in sunflower (CD value at Peak growth 2.735 and maturity stage 1.732). Vertical bars represent SD.

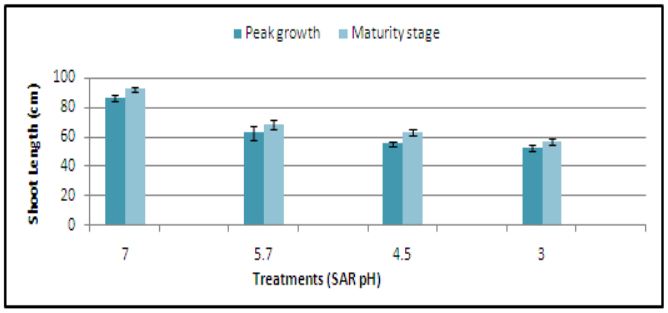

Fig 6. Effect of SAR on shoot length at peak growth and maturity stages in sunflower (CD value at Peak growth 2.86 and at maturity stage 2.347). Vertical bars represent SD.

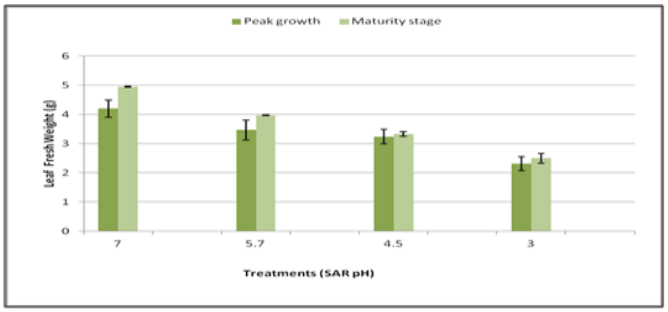

Fig 7. Effect of SAR on leaf fresh weight at peak growth and maturity stages in sunflower (CD value at Peak growth 0.281 and at maturity stage 0.093). Vertical bars represent SD.

Shoot length at peak growth stage in control was recorded 86.2 cm. (Figure 6). SAR application caused reduction in shoot length at pH 5.7 (62.66 cm). Shoot length was further reduced to 53.33 cm at pH 4.5 and 52.66 cm at pH 3.0. Shoot length at maturity stage was increased in comparison to shoot length at peak growth stage. Shoot length at maturity stage in control was recorded 92.33 cm. Shoot length in SAR applied plants showed decreasing trend at increasing level of acidity and were 68.66, 63.66 and 57.33 cm at pH 5.7, 4.5 and 3.0, respectively.

Leaf fresh weight at peak growth and maturity stage

Leaf fresh weight at peak growth stage in control (pH 7.0) was 4.03 g (Figure 7). SAR application caused reduction in fresh weight of leaf at pH 5.7 (3.47 g), Leaf weight was further reduced at pH 4.5 (3.15 g) and at pH 3.0 (2.31 g). In case of pH 3.0 sprayed plants, the leaf showed minimum fresh weight. Leaf fresh weight at maturity stage slightly increased in comparison to leaf fresh weight recorded at peak growth stage (Figure 7). It was recorded 4.95 g in control (pH 7.0) and was reduced to 3.53 g at pH 5.7 and showed insignificant difference from control. The increasing level of acidity to pH 4.5 and 3.0 further showed reductions in leaf fresh weights to 3.33 and 2.50 g, respectively. Comparison between leaf fresh weight at peak growth and maturity stages showed highest increase in control (pH 7.0) and marginal increase at pH 5.7, 4.5 and 3.0 (Figure 7). Reduction in leaf fresh weight accumulation due to SAR could be due to thin and small mesophyll cells, and reduced photosynthesis. Kumaravelu and Ramanujam (1998) studied impact of SAR on leaves biomass of green gram and observed that SAR caused reduction in leaf fresh weight and affected leaves were thinner with smaller mesophyll cells. Liang *et al.* (2008) reported that weight of fresh leaf per unit area was greatly declined with visible injury when a pH 3.1 operation was conducted in rape. Reduction in leaf fresh weight in present study conforms to observations of Kumaravelu and Ramanujam (1998) and Liang *et al*. (2008).

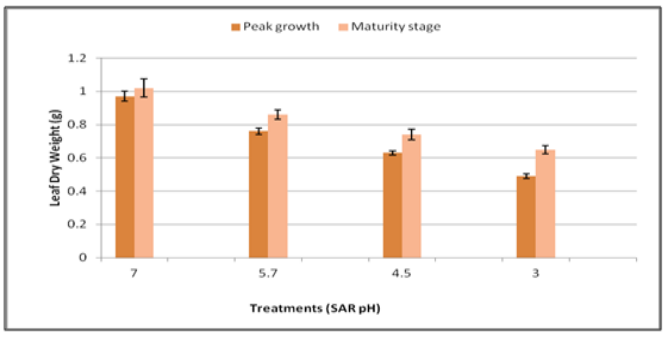

Fig 8. Effect of SAR on leaf dry weight at peak growth and maturity stages in sunflower (CD value at Peak growth 0.024 and at maturity stage 0.040). Vertical bars represent SD.

Leaf dry weight at peak growth and maturity stage

In case of control, leaf dry weight at peak growth stage recorded 0.97 g (Figure 8). SAR treatments caused reduction in dry weight of leaf at pH 5.7 (0.76 g), Leaf dry weight was further reduced at pH 4.5 to 0.63 g and at pH 3.0 to 0.49 g. Comparison of different SAR treatments showed that the dry weight of leaf reduced gradually with increasing level of acidity from control (0.97 g) to pH 3.0 (0.49 g). Leaf dry weight at maturity stage slightly increased in comparison to

peak growth stage. The pH 3.0 showed more reduction of leaf weight in comparison to other SAR treatment in case of leaf dry weight at maturity stage. Chung *et al.* (1994) observed the effect of SAR in *Perilla frutescent* on leaf dry weight which was significantly reduced. Kloseiko *et al.* (2001) reported that the lowest leaflet dry weight was 86 % of the control at a dry matter content of 90 % of the control at flowering and at ripening stages. Kohno and Kobayashi (1989) observed that acid rain treatment at pH 2.0 caused a decrease in leaf dry weight of soybean plants within a relatively short period of time after beginning exposure. It may provide useful information in making preliminary assessments of the severity of acid rain in plants. Leaf dry weight reduction may be due to severe degradation of the epidermal layer and depletion of cytoplasm in the palisade cells of SAR treated sunflower leaves or due to thinner leaves with smaller mesophyll cells (Kumaravelu and Ramanujam, 1998).

Leaf length at peak growth and maturity stage

Leaf length at peak growth stage in control was recorded 9.96 cm. SAR applications caused reduction in leaf length at pH 5.7 (9.36 cm). Leaf length was further reduced at pH 4.5 to 8.50 cm and at pH 3.0 to 6.65 cm. In case of pH 3.0 sprayed plants, the leaf showed shortest length as compared to control. The minimum difference was recorded between control and pH 5.7 at peak growth (Figure 9). Leaf length at maturity stage was increased in comparison to peak growth stage. Leaf length at maturity stage in control was recorded 10.36 cm. SAR applied leaf lengths showed decreasing trend with increasing level of acidity and at pH 5.7, 4.5 and 3.0, leaf lengths were 9.80 cm, 8.85 cm and 6.94 cm, respectively. The maximum difference was recorded between (pH 4.5 and pH 3.0) in leaf length at maturity stage. Similar observations are reported in different crops species including soybean (Kohno and Kobayashi, 1989), *Perilla frutescent* (Chung *et al.* 1994), wheat (Singh and Agrawal, 1996, 2004) and *Trifolium alexandrium* (Sirohi and Khan, 2006).

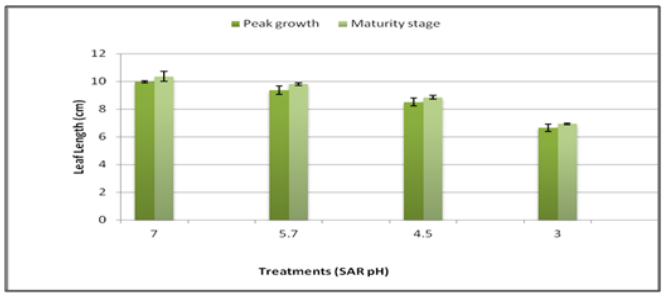

Fig 9. Effect of SAR on leaf length at peak growth and maturity stages in sunflower (CD Value at Peak growth 0.524 and maturity stage 0.641). Vertical bars represent SD.

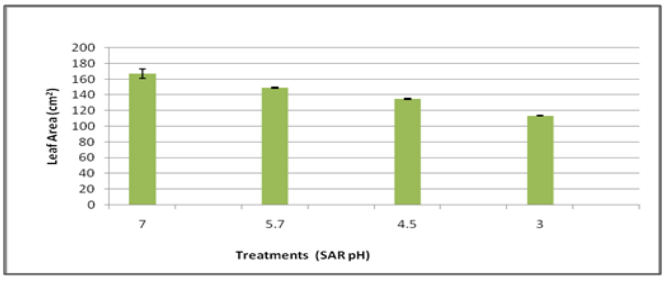

Fig 10. Effect of SAR on leaf area at peak growth stage in sunflower (CD value at Peak growth stage 3.007). Vertical bars represent SD.

Leaf Area at Peak Growth

In case of control, leaf area at peak growth was recorded 167.18 cm². The simulated acid rain caused reduction in leaf area at pH 5.7 (149.35 cm²), 4.5 (135.08 cm²) and 3.0 (113.61 cm²). It showed significant difference as compared to control (Figure 10). Leaf area decreased from control (167.18 cm²) to pH 3.0 (113.61 cm²). Kohno and Kobayashi (1989) observed that acid rain treatment at pH 2.0 caused a decrease in leaf area of soybean plants within a relatively short period of time after beginning exposure. Chung *et al.* (1994) reported that leaf area was reduced in *Perilla frutescent* with increasing level of acidity. Singh and Agrawal (1996) studied that leaf area was reduced in wheat (*Triticum aestivum* L.) cv. Malviya 206 and 234. Singh and Agrawal (2004) observed that leaf area declined at pH 4.0 and 3.0 in M 213 at 45 and 75 days (both) and at 75 days in Sonalika. Sirohi and Khan (2006) reported in *Trifolium alexandrium* plants that total leaf area of the plants exposed to simulated acid rain reduced significantly. Liang *et al.* (2008) observed that leaf area is greatly declined with visible injury when a pH 3.1 operation was conducted in rape. Kausar *et al.* (2010) reported that simulated acid rain exposure caused adverse effect on leaf area of wheat and highest suppressions were reduced at pH 3.0 level. Leaf area reduction may be due to improper distribution of food and mineral to all parts of the lamina or thin leaves and small mesophyll cells, reduced transpiration and photosynthesis (Temple *et al.* 1987).

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