# ACID RAIN-INCITED ALTERATIONS ANTHER GERMINATION AND BLOOMING OF SUNFLOWER (HELIANTHUS ANNUUS L.)

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### ABSTRACT

This study inspected the impacts of reenacted corrosive downpour (AAR) of various pH [distilled water-7.0 (control), 5.7, 4.5 and 3.0] on leaf abscission, blossoming and Anther germination in sunflower (*Helianthus annuus* L.). 'Morden' as test framework under field conditions in earth soil with adequate natural matter, light yellow in variety and pH 7.3 and 80 % water holding limit. Corrosive downpour arrangement (30 ml/plant) was splashed on plants at week by week stretches beginning from two leaved stage till inception of blossoming in the early morning under common habitat. Assessment of AAR impacts on maturing, blooming, leaf abscission and Anther improvement uncovered that inception and length of growing and blossoming changed fundamentally in test plant with diminishing pH of corrosive downpour arrangement. Corrosive downpour application showed early leaf abscission and decrease in dust germination rate and dust tube length. Impacts of AAR expanded all the more decisively with the increment of AAR corrosiveness. The review shows the sunflower plant goes to be a corrosive downpour delicate framework and requests for reproducing with corrosive downpour open minded assortments considering extending corrosive downpour geological regions and developing industrialization.

**Keywords:** Artificial acid rain(AAR), Simulated acid rain (SAR) Helianthus annuus, flowering, leaf abscission, Anther germination, sunflower

#### INTRODUCTION

Since the start of civilization, individuals have involved different normal assets for their advantage. To make their life more straightforward, they have delivered offices that utilization a considerable lot of the Earth's energy assets. . On one side this sort of advancement makes our lives more straightforward, however then again it results into contamination by discharge unsafe substance into climate. Corrosive downpour is the most genuine ecological issues arose because of air contamination. Corrosive downpour is especially harming to lakes, streams and woods, and the plants and creatures that live in these environments. Downpour is one of the most fundamental elements for human and creature life. Corrosive downpour affidavit (counting snow and haze), shaped because of response of oxides of sulfur and nitrogen with air dampness, has turned into a worldwide ecological worry in most recent couple of many years. Normal climatic precipitation is marginally acidic because of disintegration of CO2 though acidic precipitations contain grouping of H+ more noteworthy than 2.5 µeq-1 and pH under 5.6 and apply unsafe consequences for living and nonliving parts of earthly and oceanic biological systems. Farming yields show wide scope of aversion to rain water acridity and dust germination and cylinder development are among the delicate natural signs of air contamination. Several studies conducted in field and greenhouse have demonstrated that acid rain exposure of plants results in poor growth, premature leaf abscission, delayed flowering, decreased flower size, reduced pollen fertility, low seed set and yield (Pal and Kumar, 2000; Dursun et al., 2002; Lal and Singh, 2012; Lal, 2016). The acidic precipitation damages various

kinds of vegetation including *Triticum aestivum* (Kausar *et al.*, 2010), Capsicum annuum (Pal and Kumar, 2000), *Solanum lycopersicum* (Dursun *et al.*, 2002) and *Coriandrum sativum* (Kazim, 2007). Herbaceous plants are more sensitive to direct injury by acid rain than woody plants. The effect of acid deposition on higher plants arises in two ways- either through foliage or through roots and affects nutrient storage function of the soil by increasing breakdown of electrostatic bonds responsible for nutrient absorption by soil particles (Naqvi *et al.*, 2010). Verma *et al.*, (2010) observed decreased growth and fruiting in three crops (*Capsicum annuum*, *Lycopersicum esculentum* and *Solanum melongena*) upon exposure to simulated acid rain (SAR). In a recent study on *Capsicum annuum* L., SAR with increased acidity levels has been shown to cause negative effects on the growth and exposure to SAR at pH 4 and below drastically suppressed growth characteristics (Bamidele and Eguagie, 2015). The current review expected to survey the effect of reenacted corrosive downpour of various pH on growing, blossoming, leaf abscission and dust germination in sunflower (*Helianthus annuus* L., Family-Asteraceae), a significant yearly herbaceous oilseed and decorative yield.

### MATERIALS AND METHODS

The present study was carried out with sunflower variety 'CO2' in the experimental plot available at Department of Botany Annamalai university having clay soil with sufficient organic matter, pale yellow in color and pH 7.3 and 70 % water holding capacity. Sowing of sunflower variety 'CO2' was done in second week of April 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 maintained finally. During the period of crop growth the maximum and minimum temperature ranged 38.13 to 24.03°C, atmospheric moisture ranged 64.23 to 34.36% and saturated vapor 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 treatments. Three concentrations of SAR i.e. pH 3.0, 4.5, 5.7 and control (pH 7.0) were applied in respective plots. These plots were irrigated with normal deionized water. Simulated acid rain (SAR) of different concentrations was prepared by mixing conc. of 1N nitric acid (HNO3) and 1N sulphuric acid (H2SO4) 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. The days taken to first flower bud initiation were recorded at peak growth stage in terms of days taken to first bud initiation and duration (period) of bud initiation in days. Days to first flower bud initiation were calculated since sowing of seed to appearance of first sunflower size flower bud and duration of bud initiation were recorded from first to last bud initiation since sowing of seed to appearance of first to last flower bud. The data on flowering were recorded in terms of days taken to fist flower opening, duration (period) of flowering and average flower size (cm). Days taken for the appearance of the first full size flower in each plot was recorded by visual observation since the date of sowing of seed and days taken for flowering were calculated for nine plants selected randomly. The number of days taken from date of first flowering to last flowering constituted duration of flowering and were recorded by taking average of nine plants in each treated plots and control. Flower size was recorded using measuring scale on nine flowers selected randomly in each treatment when the flowers were fully opened and size was expressed as diameter (cm) of flowers.

Observations on the leaves were taken visually and also with the help of the lens. Leaf abscission level was recorded in order to monitor total number of leaves/plant and fallen number of leaves/plant. For total

number of leaves/plant, all fully opened leaves were counted at peak growth stage starting from the base to the apex of the plant in the sampled plants (including fallen leaves). Total fallen numbers of leaves/plants were calculated at peak growth stage and the data in percentage were calculated using the formula given below:

Fallen number of leaves

..... X100

Total number of leaves

The pollen grains from freshly dehisced anthers of treated plants were collected separately. Pollen grain germination was tested on media composed of 20% sucrose and 20 ppm potassium tetra borate (K2B4O7) which was converted into semi-solid medium by adding the 1% agar as a solidifying agent. Pollen grains were placed on a slide with thin layer of medium and then stained with acetocarmine dye. Slides were kept in a 60% humid chamber and incubated at  $25 \pm 3$  °C. The germinated pollen grains were counted under a compound microscope (binocular) 20 minutes after shedding of pollen grains on the medium. The total number of pollen grains and number of germinated pollen grains were counted in microscopic fields. Pollen viability was computed as the ratio of germinated pollen grains to total pollen grains. Any pollen grains with a tube length more than a half of the grain diameter were regarded as germinated. For each treatment, 200-300 pollen grains were counted for germination and lengths of 60 pollen grains were measured in µm using an Ocular Micrometer.

## **RESULTS AND DISCUSSION**

The floral bud formation was found directly affected with AAR concentration. In case of control, the days taken to first flower bud initiation was recorded 52.11 days from sowing of seeds. Bud initiation took minimum days in control and number of days increased at decreasing pH level from 5.7, 4.5 and 3.0 to 53.11, 54.11 and 57.88 days respectively (Figure 1). The maximum difference of first bud initiation was recorded between pH 4.5 and 3.0. There are no reports for the effect of AAR on budding behavior of sunflower. However, Chehregani and Kavianpour (2007) reported that the buds of bean plants showed some developmental abnormalities including smaller embryo sac due to acid rain.



**Figure 1: Effect of AAR on budding behavior in sunflower** (*Helianthus annuus* L.) The plants showed erect type of initiation of budding in control while they turned erect to partial erect at pH 5.7 and pH 4.5 and fully drooping type at pH 3.0. In case of control plants, the duration (period) of

bud initiation (days) was observed 25.88 days. Bud initiation period was decreased with increasing level of acidity i.e. 24.77, 23.44 and 21.66 days at pH 5.7, 4.5 and 3.0, respectively (Figure 2). The pH 4.5 and 3.0 showed significant difference as compared to control. The application of SAR resulted in preponement of floral bud initiation in sunflower. In case of control plants, first flower was opened in 55.88 days. SAR application of pH 5.7 took 56.77 days to have the first flower opening. As the acidity of SAR increased, days taken to first flower opening also increased and it recorded 57.88 in pH 4.5 followed by 3.0 with 62.11 days (maximum).



Figure 2: Effect of AAR on flowering behavior in Sunflower (Helianthus annuus L.)

Duration of flowering was recorded maximum (28.22 days) in case of control and further increase in acidity showed decreasing trend on duration of flowering. In SAR showered plants, period of flowering was 27.22, 25.88 and 24.77 days at pH 5.7, 4.5 and 3.0, respectively. The difference in duration of flowering among treatments was maximum between pH 5.7 (27.22) to 4.5 (25.88). Pal and Kumar (2000) investigated effect of SAR in Capsicum annuum and found that numbers of flowers were decreased in treated plants for all treatments at all pH values. Milton and Abigael (2015) in their phenological observations on okra (Abelmoschus esculentus L.) also reported delayed first flower emergence with increasing SAR concentration. The effect of SAR became more pronounced with increasing acidity and duration of treatments.AAR influenced the flower head diameter significantly. Average head diameter of plant size was recorded 16.04 cm in the case of control plants and decrease over the control in head diameter was recorded by 3.87 cm or 33.87% in pH 3.0, 2.74 cm or 17.08% in pH 4.5 and 1.79 cm or 11.15% in pH 5.7 (Figure 3). Present observation in sunflower on Leaf is the most sensitive plant organ to acid rain. Acid rain treatment at pH 3.0 induced the formation of pale brown necrotic spots on the leaves. Necrotic symptoms first appeared along the vein and spread gradually into the interveinal areas. When young leaves were exposed to acid rain at pH 3.0 during the early development stage, they became severely necrotic, crinkled and wrinkled. However, mature leaves were only slightly injured (Figure 4). The plant leaf abscission was recorded 6.49% in the case of control. At pH 5.7 leaves showed 10.59% abscission and at pH 4.5 leaf abscission was further increased with 12.31%. Maximum leaves abscission were observed at pH 3.0 (15.10%) as compared to control (Figure 5). Verma et al., (2010) studied the impact of simulated acid rain on three crops species viz. Capsicum annuum, Lycopersicon esculentum and

*Solanum melongena* and observed that the leaf number in comparision to control was reduced 60% average flower size are confirmed with the study of Khalid and Qureshi (2004). Delay in opening of the first flower, duration of flowering and reduce flower size seem to be due to toxic effects of acids on sunflower tissues.



Figure 3: Comparative effect of SAR on head diameter of Sunflower (*Helianthus annuus* L.)

in *L. esculentum*, 50% in *C. annuum*, and 70% in *S. melongena*. In another study on *Solanum lycopersicum* L., Eguagie (2015) reported that SAR induced reduction in number of leaves with declining pH value of acid rain. The percentage of fallen leaves was also significantly higher at pH 3.0 than at all other treatments. These studies proved that the old leaves of all these plants are readily exposed and abscission of leaves started on first week of exposure. Sulfur dioxide (SO2) damages cell on the surface of leaves, depending on the concentration and the amount of water present and causes stomata either to close or to open, in either case disrupting the flow of water through plants (Sharma and Kaur, 1994).



Fig. 4: Comparative Study effect of SAR on leaves in sunflower (*Helianthus annuus* L.) (a) Mature leaves, (b) young leaves treated with pH 3.0.





Figure 5: Effect of SAR on % leaf abscission at peak growth stage in Sunflower (*Helianthus annuus* L.)

Figure 6: Effect of AAR on in vitro anther germination in sunflower (Helianthus annuus L.)

The mean of sunflower pollen germination and in vitro pollen tube measurements were significantly influenced by simulated acid rain (Figure 6). In case of control, pollen germination was recorded 86.33% and with increasing acidity a reduction in germination was observed at pH 5.7 (70.88%), 4.5(50.55%) and 3.0 (43.22%). The above observations on in vitro pollen germination are similar to other studies in different crop species. Munzuroglu *et al.*, (2003) treated the pollen of apple (*Malus sylvestris*) with SAR and recorded that pollen germination decreased significantly as the quantity of acid rain increased. Yao *et al.*, (1996) also observed that direct exposure to AAR of pH 4.0 and 3.0 reduced pollen grain germination

in vivo in rice. In vitro pollen tube length was recorded 2.73  $\mu$ m in case of control and pollen tube showed reduction to 2.35  $\mu$ m at pH 5.7. Pollen tube was further reduced at pH 4.5 (2.22  $\mu$ m) and at pH 3.0 (1.36  $\mu$ m) (Figure 7). It is clear from above observation that pollen tube length decreased significantly with increase in level of rain acidity. This reduction in pollen tube length is similar to Monzuroglu *et al.*, (2003) in apple who observed that pollen tube elongation decreased as pH value increased and resulted in complete destruction of pollen tube. Ismail and Zohair (2013) investigated the effect of different pH levels on in vitro pollen germination and pollen tube growth in date palm and identified pH 6.0 to 6.5 suitable for pollen germination with reduction in pollen germination at pH 4.0 and below. The mean of pollen tube length ranged from 42.7 to 275.8  $\mu$ m at pH values 4.0 and 8.0, respectively. These workers observed that pollen germination and pollen tube elongation were more sensitive in comparison to seed set. The AAR at pH 3.0 seems to be sufficiently acidic to cause direct damage to and consequent leakage of solutes from pollen tube. The present results on in vitro pollen tube length in sunflower are supported by Munzuroglu *et al.*, (2003) and Ismail and Zohair (2013).



Figure 7: Effect of AAR on in vitro pollen tube length in Sunflower (Helianthus annuus L.)

## Conclusion

This audit shows that reenacted destructive deluge inhibitorily affects the growing, blooming and residue germination, and progressed leaf abscission in Helianthus annuus which have direct bearing on seed and oil yield and draws thought for exploring preventive and control measures to diminish the effects of destructive deluge on blooming, dust germination and proficiency and raising for destructive storm indulgent varieties in crop plants.

## REFERENCES

**Bamidele JF and Eguagie MO (2015).** Ecophysiological response of *Capsicum annuum* L. exposed to simulated acid rain. Niger. *Journal of Biotechnology*, **30** 48- 52.

Chehregani A and Kavianpour F (2007). Effects of acid rain on the developmental stages of ovules and seed proteins in bean plants. *American Journal of Plant Physiology* **2**, 367-372.

**Dursun A, Kumlay AM, Yildirim E and Guvvenc I (2002).** Effect of simulated acid rain on plant growth and yield of tomato. *Acta Horticulturae* **579**, 245-248.

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**Eguagie MO (2015).** Effects of simulated acid rain on the growth, yield and mineral nutrient relations of Solanum lycopersicum L. *European Journal of Biotechnology and Biosciences*, **3** 15-18.

**Ismail OM and Zohair MM (2013).** Date Palm pollen germination and growth susceptibility to different pH medium. *Journal of Agriculture and Food Technology*, **3** 26-30.

Kausar S, Hussain MA and Khan AA (2010). Response of simulated acid rain on morphological, biochemical and leaf characteristics of wheat. *Trends in Bioscience*, **3** 34-36.

**Kazim I (2007).** Response of coriander to sulphur dioxide and acid rain. MSc Dissertation, Aligarh Muslim University, Aligarh. p. 18.

Khalid N and Qureshi MS (2004). Changes in growth, pigmentation and yield of sunflower in response to environmental pollution of acid rain. *Indus Journal of Biological Science*, **1** 192-195.

Lal N and Singh H (2012). The effects of simulated acid rain of different pH-levels on biomass and leaf area in Sunflower (Helianthus annuus). *Current Botany* **3**(5) 45-50.

Lal N (2016). Effects of acid rain on plant growth and development. *e-Journal Science and Technology*, 11(5) 85-101.

Milton MB and Abigael TO (2015). Effects of simulated acid rain on the morphology, phenology and yield of Okra (*Abelmoschus esculentus* (L). Moench). *Journal of Environmental Science, Computer Science and Engineering Technology*, **4** 501-518.

Munzurolglu O, Obek E and Geckil H (2003). Effects of simulated acid rain on the pollen germination and pollen tube growth of apple. *Acta Biologica Hungarica*, **54** 95-103.

Naqvi SSM, Alam S and Ansari R (2010). Chapter 3. Impact of Soil pH on Nutrient Uptake by Crop Plants. In: Pessarkli, M (ed.) Handbook of Plant and Crop Stress (Third edition, Revised and expanded), CRC Press, Taylor and Francis Publishing Company, Boca Raton, Florida, USA, 51-60.

**Pal S and Kumar N (2000).** Effects of simulated acidic rain on yield and carbohydrate contents of green pepper (Capsicum annuum L.). *Advanced Plant Science*, **13** 85-88.

Sharma BK and Kaur H (1994). Environmental Chemistry (IVth Edition). Goel Publishing House, Meerut, India.

Verma A, Tewari, A and Azami A (2010). An impact of simulated acid rain of different pH levels on some major vegetable plants in India. *Report and Opinion* 2 38-40.

Yao MH, Chen S, Tsai JC and Liang LS (1996). Effect of simulated acid rain on pollination of rice plants. *Journal of Agriculture Research of China*, **45** 105-112.