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Physiological Response of Simulated Acid Rain on Zinnia Elegans L. and Helianthus Annuus L

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Abstract

Acid rain is a major environmental concern and affects plant physiology and overall growth. Zinnia's native habitat is in Central and S. America and Helianthus is also a native of South America and Peru and often exposed to acid rain. Among these, stomatal behavior plays a vital role in determining the acidic stress on plants. A mixture of sulphuric acid and nitric acid in the ratio of 7:3 was artificially prepared and sprayed on plants. This study is focused on derived stomatal parameters- stomatal index (S.I.), arrested cells index (A.C.I), free cells index (F.C.I.), Arrested cells ratio (A.C.R), Free cells ratio (F.C.R), Stomatal ratio (S.R), Arrested cells free cells ratio (A.F.R), Arrested cells stomatal ratio (A.S.R) and Free cells stomatal ratio (F.S.R) in response to different concentrations of pH 5.6(control), 4.5, 3.5 and 2.5 of simulated acid rain (s.a.r.) on widely cultivated annual ornamental plant Zinnia elegans L. and Helianthus annuus L., belonging to family Asteraceae. There were significant variation in stomatal density, aperture, coverage area and a significant reduction in derived stomatal parameters with increased acidity of s.a.r. Zinnia elegans was found to be more sensitive to acidic conditions than Helianthus annuus. The data was analyzed statistically using ANOVA (one-way). The findings exhibit the importance of stomatal adaptations to increase the productivity in acidic stress.

Keywords: Simulated Acid Rain (s.a.r.), Stomatal Index (S.I.), Arrested Cell Index (A.C.I.), Free Cell Index (F.C.I.), Analysis of Variance (ANOVA)

1. Introduction

Acid rain, a global environmental issue caused due to increase in normal level of acidity in rain and snow harms forests, ecosystems, and human beings, has a serious impact on our forests in the northeast and many valued monuments. Zinnia, can be used in the treatment of inflammatory diseases and cancer (Rates et al. 2001). The earliest report about acid rain in the United States came from chemical evidence gathered from Hubbard Brook Valley; public awareness of acid rain in the US increased in the 1970s after The New York Times reported on these findings (Likens, G. E.; Bormann, F. H. 1974),(Keller, C. K.; White, T. M.; O'Brien, R.; Smith, J. L. 2006). The research regarding the antioxidant, hepatoprotective, antifungal and antimalarial activities on Zinnia can be found in literatures (Hafiza et al. 2002, Mohamed et al. 2015, Gomaa et al. 2018). Helianthus is economically important crop, the seeds being the source of sunflower oil used in many foods. This research provides an insight into derived stomatal responses to different concentrations of pH 5.6(control), 4.5, 3.5 and 2.5 of s.a.r. on annual ornamental plants Zinnia elegans L. and Helianthus annuus L. belonging to family Asteraceae.

2. Materials and Methods

2.1 Preparation and spraying of acid water solutions A mixture of sulfuric and nitric acid in the ratio of 7:3 (V/V) of different pH concentrations viz. 5.6 (control), 4.5, 3.5 and 2.5 (Lee et al., 1981) was sprayed with one liter local hand sprayer, thrice a week on 4 sets of plants, with 30 plants in each set on 10 cm high saplings of cultivars obtained from the nursery of Circuit house, Meerut, planted in polythene bags, and data recorded on 20, 40, 60 and 80 days old plants.

2.2 Stomatal Characteristics and Response Epidermal peels of unfolded and mature leaves taken manually stained in Delafield's hematoxyline and mounted in glycerine were microscopically analyzed using 45x objective and 5x ocular and derived parameters were obtained by formulae.

2.3 Statistical Analysis Data was statistically analyzed from ANOVA (one way) in both the treatment and control groups and compared with the tabulated value of F at 5% and 1% levels of significance. The F value obtained from ANOVA, was compared with the tabulated value of F at 5% and 1% levels of significance. Under the above comparison, if the calculated F ≥ F value (tab) at 5% or 1% level, then there was no evidence against Null hypothesis, and the hypothesis was accepted at 5% or 1% level. If the hypothesis was accepted, it means that there was no significant difference among different treatment means, at respective level of significance and we did not require any further analysis. But if the hypothesis was rejected it means that there is significant difference among treatment means and we required further analysis of the data.

From the analysis of variance table, one can only know that there is significant difference among different treatments, but we do not know that which of the treatments is showing effective difference. For this LSD (Least Significant Difference) or CD (Critical Difference) test was used. The LSD or CD was calculated by the following formula:

$CD_{\alpha}\%$ = SE of difference × $t_{\alpha}\%$ (error d.f.)

where, SE = standard error

α% = 5% or 1% level of significance

$t_{\alpha}\%$ = (error d.f.) = tabulated value of 't' at α% level at error degree of freedom.

Standard error of difference = $\sqrt{\frac{2\,MSe}{r}}$

where, MSe = mean sum of square for error ; r = total number of replicants

The difference between control and treatment mean, found equal or more than CD at 5% level and 1% level is shown superscripted with single star (*) and double stars (**) respectively. Critical difference (CD) value was used for any two treatments to know whether there is significant difference between them or not. If the difference between treatment means comes equal or greater to CD, then there is significant difference at respective level of significance. The values calculated on percent basis were treated as non-significant when they were less than 5 percent.

3. Observations

In both crops, leaves are amphistomatic. At 2.5 pH concentration, the percent reduction in stomatal aperture width was 43% in Zinnia and 27% in Helianthus as compared to control indicating Zinnia as more sensitive to acidity. Appreciable reductions were recorded in stomatal complex, stomatal index (S.I.), arrested cell index (A.C.I.), A.C.R (Arrested cells ratio), S.R (stomatal ratio), and A.F.R (Arrested cells free cells ratio) with decrease in pH of s.a.r, while the values for F.C.I. (Free cells Index), F.C.R

(Free cells Ratio) and F.S.R (Free cells Stomatal ratio) increased with increase in pH of s.a.r. for both adaxial and abaxial surfaces. The values for A.S.R. (Arrested cells Stomatal ratio) remained uncertain. Findings of the investigation regarding the effects of the pollutant on stomatal parameters are being presented in tables (I-XIV).

1. Effect of different concentrations of s.a.r. on the number of stomata and their associated cells per unit area (mm^{-2}), change in average distance (mm) between two nearest stomata, size (mm^{-2}) of Guard cell and Stomatal aperture, Density and Coverage area (mm^{-2}) in the leaves (adaxial surface) of Zinnia elegans L.

	Plant age, d							
	20				**40**			
	pH of acid solution							
Derived attribute	**5.6**	**4.5**	**3.5**	**2.5**	**5.6**	**4.5**	**3.5**	**2.5**
Stomata	12.4	8.8	6.4	5	15.6	13.2	10.4	8.6
	±1.14	± 0.836	±1.30	±1.00	±0.894	±0.836	±0.894	± 0.894
Arrested cells	22.8	17.2	12.6	11	28.6	25	20.2	18.6
	±0.894	±0.836	±0.894	±1.00	±0.547	±0.707	±0.836	±0.547
Free cells	16.4	13.2	9.8	9.60^{-4}	22	21	18	17
	±0.894	±0.837	±0.837	±1.34	±1.41	±1.14	±1.00	±1.41
Average distance between two nearest stomata	0.0375	0.0398	0.047	0.0505	0.0327	0.0379	0.0469	0.0445
	±0.0039	±0.0021	±0.0053	±0.0012	±0.0014	±0.0027	±0.0026	±0.0047
Size of guard cell	1.105×10^{-4}	1.051×10^{-4}	1.022×10^{-4}	1.010×10^{-4}	1.702× 10-4	1.036×10^{-4}	1.0321×10^{-4}	1.0310×10^{-4}
Size of aperture	0.831×10^{-4}	0.826×10^{-4}	0.820×10^{-4}	0.811×10^{-4}	0.911×10^{-4}	0.894×10^{-4}	0.860×10^{-4}	0.849×10^{-4}
Density	72.94	51.76	37.65	29.41	91.76	77.65	61.18	50.58
Coverage area	0.051	0.036	0.026	0.021	0.064	0.054	0.043	0.035

± Standard deviation

2. Effect of different concentrations of s.a.r. on the number of stomata and their associated cells per unit area (mm^2), change in average distance (mm) between two nearest stomata, size (mm^{-2}) of Guard cell and Stomatal aperture, Density and Coverage area (mm^{-2}) in the leaves (adaxial surface) of Zinnia elegans L.

	Plant age, d							
	60				**80**			
	pH of acid solution							
Derived attribute	**5.6**	**4.5**	**3.5**	**2.5**	**5.6**	**4.5**	**3.5**	**2.5**
Stomata	18.2	15.6	13.6	11.4	20.6	16.8	15.6	12.6
	±0.447	±0.547	±0.894	±0.548	±0.547	±0.857	±0.894	±0.547
Arrested cells	37.4	33.6	29.6	26	47.4	43	41.4	37.8
	±0.894	±1.940	±0.548	±1.00	±0.547	±0.707	±0.547	±1.640
Free cells	26.4	25.4	28.2	22.2	31.6	31	31	30
	±1.34	±0.894	±1.090	±1.090	±1.34	±1.00	±1.00	±1.00
Average distance between two nearest stomata	0.0265	0.0257	0.0348	0.0309	0.0221	0.0217	0.0328	0.0283
	±0.0012	±0.0048	±0.0032	±0.0012	±0.0012	±0.0021	±0.0014	±0.0013
Size of guard cell	1.909×10^{-4}	1.287×10^{-4}	1.230×10^{-4}	1.220×10^{-4}	2.511×10^{-4}	1.881×10^{-4}	1.830×10^{-4}	1.719×10^{-4}
Size of aperture	0.950×10^{-4}	0.946×10^{-4}	0.944×10^{-4}	0.940×10^{-4}	0.970×10^{-4}	0.966×10^{-4}	0.964×10^{-4}	0.958×10^{-4}
Density	107.06	91.76	80	67.06	121.18	98.82	91.76	74.12
Coverage area	0.075	0.064	0.056	0.047	0.085	0.069	0.064	0.052

± Standard deviation

3. Effect of different concentrations of s.a.r. on the number of stomata and their associated cells per unit area (mm^{-2}), change in average distance (mm) between two nearest stomata, size (mm^{-2}) of Guard cell

and Stomatal aperture, Density and Coverage area (mm^{-2}) in the leaves (adaxial surface) of Helianthus annuus L.

Derived attribute	Plant age, d							
	20				40			
	pH of acid solution							
	5.6	4.5	3.5	2.5	5.6	4.5	3.5	2.5
Stomata	10.4 ±2.60	9.8 ±1.64	8.2 ±1.09	7 ±1.00	11.6 ±2.19	11 ±1.00	10.2 ±1.78	9.6 ±1.52
Arrested cells	24 ±1.00	21.4 ±1.516	17.6 ±1.342	15.2 ±1.30	28.4 ±1.14	25.6 ±0.894	22.6 ±0.894	23 ±1.00
Free cells	34 ±1.20	33 ±1.00	32.4 ±1.52	30 ±1.00	36.4 ±1.67	36 ±1.00	35.8 ±1.30	332.2 ±0.836
Average distance between two nearest stomata	0.1129 ±0.0011	0.1242 ±0.0027	0.1296 ±0.0064	0.1455 ±0.0032	0.941 ±0.0040	0.1057 ±0.0035	0.108 ±0.0018	0.1163 ±0.0028
Size of guard cell	2.252×10^{-4}	2.174×10^{-4}	2.108×10^{-4}	1.923×10^{-4}	2.811×10^{-4}	2.700×10^{-4}	2.625×10^{-4}	2.595×10^{-4}
Size of aperture	1.011×10^{-4}	0.994×10^{-4}	0.922×10^{-4}	0.908×10^{-4}	1.270×10^{-4}	1.264×10^{-4}	1.241×10^{-4}	1.220×10^{-4}
Density	61.18	57.65	48.24	41.17	68.24	64.71	60	56.47
Coverage area	0.0612	0.0576	0.0482	0.041	0.068	0.065	0.06	0.056

± Standard deviation

4. Effect of different concentrations of s.a.r. on the number of stomata and their associated cells per unit area (mm^{-2}), change in average distance (mm) between two nearest stomata, size (mm^{-2}) of Guard cell and Stomatal aperture, Density and Coverage area (mm^{-2}) in the leaves (adaxial surface) of Helianthus annuus L.

Derived attribute	Plant age, d							
	60				80			
	pH of acid solution							
	5.6	4.5	3.5	2.5	5.6	4.5	3.5	2.5
Stomata	12.2 ±2.04	12.8 ±1.09	11 ±1.78	10.4 ±1.342	14 ±1.00	13.6 ±1.14	12.4 ±1.67	10.8 ±1.78
Arrested	31	29.2	27.6	26.4	33.2	31.4	30	28.8

cells	±1.00	±0.0894	±2.07	±1.14	±1.30	±1.64	±1.00	±1.09
Free cells	40 ±1.0	38.2 ±2.49	42.6 ±1.34	36.2 ±1.30	45.2 ±1.64	43.4 ±2.30	42.6 ±1.34	36.2 ±1.30
Average distance between two nearest stomata	0.851 ±0.0025	0.0914 ±0.0033	0.0945 ±0.0066	0.0977 ±0.0084	0.0735 ±0.0094	0.0714 ±0.0050	0.0767 ±0.0019	0.0798 ±0.0019
Size of guard cell	3.954×10^{-4}	3.811×10^{-4}	3.716×10^{-4}	3.622×10^{-4}	4.410×10^{-4}	4.282×10^{-4}	4.154×10^{-4}	4.116×10^{-4}
Size of aperture	1.423×10^{-4}	1.392×10^{-4}	1.349×10^{-4}	1.321×10^{-4}	1.510×10^{-4}	1.472×10^{-4}	1.382×10^{-4}	1.340×10^{-4}
Density	71.76	75.29	64.71	61.18	82.35	80	72.94	63.52
Coverage area	0.072	0.075	0.065	0.061	0.082	0.08	0.073	0.064

± Standard deviation

5. Effect of different concentrations of s.a.r. on the number of stomata and their associated cells per unit area (mm^{-2}), change in average distance (mm) between two nearest stomata, size (mm^{-2}) of Guard cell and Stomatal aperture, Density and Coverage area (mm^{-2}) in the leaves (abaxial surface) of Zinnia elegans L

	Plant age, d							
	20				**40**			
	pH of acid solution							
Derived attribute	**5.6**	**4.5**	**3.5**	**2.5**	**5.6**	**4.5**	**3.5**	**2.5**
Stomata	20 ±1.00	18.2 ±0.836	16.8 ±1.09	14.2 ±1.30	30.25 ±0.957	27.2 ±0.836	26 ±1.00	23.2 ±0.836
Arrested cells	54 ±1.22	49 ±1.22	45.2 ±1.09	41.4 ±1.52	76.17 ±1.83	71.4 ±2.61	68.6 ±1.52	60.8 ±1.00
Free cells	18.6 ±1.14	17.4 ±0.894	16.8 ±1.30	16.6 ±0.894	28.8 ±1.79	28.4 ±1.52	27.2 ±1.64	26.6 ±0.894
Average distance between two nearest stomata	0.0266 ±0.0008	0.0282 ±0.0016	0.031 ±0.0023	0.0367 ±0.0016	0.0259 ±0.0006	0.0254 ±0.0019	0.0298 ±0.0013	0.0317 ±0.0011
Size of guard cell	0.840×10^{-4}	0.816×10^{-4}	0.801×10^{-4}	0.792×10^{-4}	1.275×10^{-4}	1.250×10^{-4}	1.180×10^{-4}	1.110×10^{-4}

Size of aperture	0.511×10^{-4}	0.509×10^{-4}	0.504×10^{-4}	0.498×10^{-4}	0.672×10^{-4}	0.660×10^{-4}	0.655×10^{-4}	0.642×10^{-4}
Density	117.64	107.06	98.82	83.53	177.94	160	152.94	136.47
Coverage area	0.047	0.043	0.039	0.033	0.071	0.064	0.061	0.054

± Standard deviation

6. Effect of different concentrations of s.a.r. on the number of stomata and their associated cells per unit area (mm^{-2}), change in average distance (mm) between two nearest stomata, size (mm^{-2}) of Guard cell and Stomatal aperture, Density and Coverage area (mm^{-2}) in the leaves (abaxial surface) of Zinnia elegans L

Derived attribute	**Plant age, d**							
	60				**80**			
	pH of acid solution							
	5.6	**4.5**	**3.5**	**2.5**	**5.6**	**4.5**	**3.5**	**2.5**
Stomata	34 ±1.003	32.6 ±1.34	30.2 ±0.0447	28.6 ±0.0547	40.8 ±0.0836	40.06 ±1.95	37.4 ±0.894	32.2 ±0.447
Arrested cells	92.6 ±1.67	86.8 ±1.30	85.4 ±1.82	77.2 ±0.837	105.6 ±1.35	99.6 ±1.67	86.6 ±0.894	81.4 ±0.836
Free cells	52 ±1.41	51 ±1.00	47 ±1.41	43.6 ±1.94	57 ±0.707	61.8 ±1.79	62.4 ±1.34	65.8 ±1.10
Average distance between two nearest stomata	0.0201 ±0.0001	0.0212 ±0.0001	0.0229 ±0.0013	0.0231 ±0.0006	0.156 ±0.0005	0.145 ±0.0003	0.0138 ±0.0006	0.0127 ±0.0004
Size of guard cell	1.590×10^{-4}	1.471×10^{-4}	1.452×10^{-4}	1.440×10^{-4}	1.811×10^{-4}	1.720×10^{-4}	1.680×10^{-4}	1.672×10^{-4}
Size of aperture	0.758×10^{-4}	0.755×10^{-4}	0.742×10^{-4}	0.729×10^{-4}	0.782×10^{-4}	0.771×10^{-4}	0.770×10^{-4}	0.765×10^{-4}
Density	205.88	191.76	177.65	168.24	240	238.82	220	189.41
Coverage area	0.082	0.077	0.071	0.067	0.096	0.095	0.088	0.76

± Standard deviation

7. Effect of different concentrations of s.a.r. on the number of stomata and their associated cells per unit area (mm^{-2}), change in average distance (mm) between two nearest stomata, size (mm^{-2}) of Guard cell and Stomatal aperture, Density and Coverage area (mm^{-2}) in the leaves (abaxial surface) of Helianthus annuus L.

Derived attribute	20				40			
	pH of acid solution							
	5.6	4.5	3.5	2.5	5.6	4.5	3.5	2.5
Stomata	44.35 ±1.528	41.67 ±0.577	38.34 ±0.577	33.67 ±0.577	49.8 ±0.500	47.4 ±0.577	44 ±1.15	42.2 ±0.50
Arrested cells	72 ±1.00	68.34 ±0.547	63 ±1.00	56 ±1.00	82.4 ±1.34	78.6 ±1.34	73.2 ±0.836	70.2 ±0.836
Free cells	7.67 ±1.52	7.67 ±1.52	10.01 ±1.00	10.81 ±1.64	9.5 ±0.548	9.2 ±1.64	8.9 ±1.00	8.6 ±1.67
Average distance between two nearest stomata	0.0152 ±0.0025	0.0152 ±0.0025	0.0181 0.0012	0.021 ±0.0036	0.0126 ±0.0009	0.0139 ±0.0020	0.0145 ±0.0021	0.0152 ±0.0024
Size of guard cell	1.640×10^{-4}	1.620×10^{-4}	1.572×10^{-4}	1.362×10^{-4}	1.871×10^{-4}	1.881×10^{-4}	1.776×10^{-4}	1.670×10^{-4}
Size of aperture	0.560×10^{-4}	0.542×10^{-4}	0.528×10^{-4}	0.527×10^{-4}	0.765×10^{-4}	0.760×10^{-4}	0.754×10^{-4}	0.746×10^{-4}
Density	260.88	245.12	225.53	198.06	292.94	278.82	258.82	248.24
Coverage area	0.208	0.196	0.18	0.158	0.234	0.223	0.2007	0.199

± Standard deviation

8. Effect of different concentrations of s.a.r. on the number of stomata and their associated cells per unit area (mm^{-2}), change in average distance (mm) between two nearest stomata, size (mm^{-2}) of Guard cell and Stomatal aperture, Density and Coverage area (mm^{-2}) in the leaves (abaxial surface) of Helianthus annuus L.

	Plant age, d							
	60				80			
	pH of acid solution							
Derived attribute	5.6	4.5	3.5	2.5	5.6	4.5	3.5	2.5
Stomata	58	55.4	51.7	44.2	69.6	60	48.2	33.4
	±0.50	±0.547	±0.50	±0.957	±1.29	±0.816	±0.50	±1.50
Arrested cells	95.6	89.2	83.3	72.4	116.2	98.8	78.2	51.6
	±1.34	±1.30	±0.836	±1.14	±0.836	±0.837	±0.547	±1.14
Free cells	11.4	11.2	10.6	10	13.8	13.4	13.2	12
	±1.520	±1.64	±2.19	±1.00	±2.17	±1.52	±1.92	±1.00

Average distance between two nearest stomata	0.0085	0.0095	0.0135	0.0157	0.0052	0.0073	0.0078	0.0082
	±0.0020	±0.0016	±0.0018	±0.0037	±0.0009	±0.0012	±0.0009	±0.0009
Size of guard cell	1.912×10^{-4}	1.894×10^{-4}	1.862×10^{-4}	1.785×10^{-4}	2.086×10^{-4}	2.080×10^{-4}	2.029×10^{-4}	1.962×10^{-4}
Size of aperture	0.851×10^{-4}	0.850×10^{-4}	0.836×10^{-4}	0.810×10^{-4}	0.976×10^{-4}	0.963×10^{-4}	0.957×10^{-4}	0.942×10^{-4}
Density	341.18	325.88	304.11	260	409.41	352.94	283.53	196.47
Coverage area	0.273	0.26	0.243	0.208	0.328	0.282	0.227	0.157

± Standard deviation

9. Effect of different concentrations of s.a.r. on different indices and ratios like Stomatal Index (S.I.), Arrested cell Index, Free cell Index, Arrested cells ratio (ACR), Free cells ratio (FCR), Stomatal ratio (SR), Arrested cells and Free cells ratio (AFR), Arrested cells and Stomatal ratio (ASR) and Free cells and Stomatal ratio (FSR) in leaves (adaxial surface) of Zinnia elegans L.

	Plant age, d							
	20				**40**			
	pH of acid solution							
Derived attribute	**5.6**	**4.5**	**3.5**	**2.5**	**5.6**	**4.5**	**3.5**	**2.5**
S.I	26.03	24.45	22.23	19.53	24.56	23.3	21.4	19.26
Arrested cell Index.	43.18	42.87	40.75	39.97	42.2	40.23	39.56	31.24
Free cell Index	32.78	33.77	34.03	37.5	32.23	34.47	35.04	36.46
ACR	57.16	56.58	53.85	53.41	56.52	54.35	52.87	52.25
FCR	41.84	43.42	43.75	47.26	43.48	45.65	45.12	47.25
SR	33.63	28.95	28.57	24.27	32.83	28.79	27.43	24.86
AFR	1.69	1.32	1.19	1.15	1.54	1.92	1.32	1.09
ASR	1.84	1.95	1.97	2.2	1.83	1.89	1.94	2.16
FSR	0.132	0.154	0.153	0.192	0.141	0.159	0.173	0.198

10. Effect of different concentrations of s.a.r. on different indices and ratios like Stomatal index, Arrested cell index, Free cell index, Arrested cells ratio(ACR), Free cells ratio (FCR), Stomatal ratio (SR), Arrested cells and Free cells ratio (AFR), Arrested cells and Stomatal ratio (ASR) and Free cells and Stomatal ratio (FSR) in leaves (adaxial surface) of Zinnia elegans L.

Derived attribute	Plant age, d							
	60				80			
	pH of acid solution							
	5.6	4.5	3.5	2.5	5.6	4.5	3.5	2.5
Stomatal index	24.03	22.45	22.23	19.53	23.56	22.3	21.4	19.46
Arrested cell index	44.18	43.87	43.75	42.97	43.2	42.23	41.56	41.24
Free cell index	31.78	33.67	34.03	37.5	33.23	35.47	37.04	38.46
ACR	58.16	56.58	56.25	53.4	56.52	54.35	52.87	52.25
FCR	41.84	43.42	43.75	46.6	43.48	45.65	47.12	47.45
SR	31.63	28.95	28.57	24.27	30.83	28.69	27.23	24.16
AFR	1.39	1.3	1.29	1.15	1.3	1.19	1.12	1.09
ASR	1.84	1.95	1.97	2.2	1.83	1.89	1.94	2.16
FSR	0.132	0.15	0.153	0.192	0.141	0.159	0.173	0.198

11. Effect of different concentrations of s.a.r. on different indices and ratios like Stomatal index, Arrested cell index, Free cell index, Arrested cells ratio (ACR), Free cells ratio (FCR), Stomatal ratio (SR), Arrested cells and Free cells ratio (AFR), Arrested cells and Stomatal ratio (ASR) and Free cells and Stomatal ratio (FSR) in leaves (abaxial surface) of Zinnia elegans L.

	Plant age, d							
	20				40			
	pH of s.a.r.							
Attribute	5.6	4.5	3.5	2.5	5.6	4.5	3.5	2.5
Stomatal index	22.68	21.51	21.32	19.67	22.37	21.42	21.35	21.01
Arrested cell index	58.32	57.92	57.36	57.34	56.33	56.22	56.32	54.97
Free cell index	19.01	20.57	21.32	22.99	21.99	22.36	22.33	24.05
ACR	75.42	73.8	72.9	71.38	72.56	71.54	70.98	69.56
FCR	24.58	26.2	27.09	28.62	27.44	28.46	29.02	30.43
SR	35.06	38.52	4.55	47.02	29.33	27.41	27.09	24.48
AFR	25.09	24.69	24.54	22.33	3.068	2.816	2.69	2.494
ASR	2.571	2.692	2.69	2.915	2.518	2.625	2.615	2.621
FSR	0.838	0.956	1.0	1.169	0.952	1.044	1.069	1.147

12. Effect of different concentrations of s.a.r. on different indices and ratios like Stomatal Index, Arrested cell index, Free cell index, Arrested cells ratio (ACR), Free cells ratio (FCR), Stomatal ratio (SR), Arrested cells and Free cells ratio (AFR), Arrested cells and Stomatal ratio (ASR) and Free cells

and Stomatal ratio (FSR) in leaves (abaxial surface) of Zinnia elegans L.

	Plant age, d							
	60				**80**			
Derived	**pH of s.a.r.**							
Attribute	**5.6**	**4.5**	**3.5**	**2.5**	**5.6**	**4.5**	**3.5**	**2.5**
Stomatal index	19.49	19.13	18.75	18.4	20.76	20.04	19.7	19.14
Arrested cell index	53.23	51.52	51.78	47.75	51.92	49.16	47.43	47.2
Free cell index	28.95	28.64	29.45	29.99	28.02	30.8	32.56	40.31
ACR	66.11	64.59	64.5	61.42	64.94	61.48	59.45	55.06
FCR	33.89	35.41	35.5	38.58	35.06	38.52	40.55	47.02
SR	24.2	23.37	22.81	23.68	25.09	24.69	24.54	22.33
AFR	1.951	1.756	1.758	1.592	1.853	1.596	1.466	1.17
ASR	2.731	2.727	2.795	2.594	2.588	2.453	2.422	2.466
FSR	1.4	1.553	1.589	1.629	1.397	1.537	1.652	2.106

3. Effect of different concentrations of s.a.r. on different indices and ratios like Stomatal Index, Arrested cell index, Free cell index, Arrested cells ratio (ACR), Free cells ratio (FCR), Stomatal ratio (SR), Arrested cells and Free cells ratio (AFR), Arrested cells and Stomatal ratio (ASR) and Free cells and Stomatal ratio (FSR) in leaves (abaxial surface) of Helianthus annuus L.

	Plant age, d							
	20				**40**			
Derived	**pH of s.a.r.**							
Attribute	**5.6**	**4.5**	**3.5**	**2.5**	**5.6**	**4.5**	**3.5**	**2.5**
Stomatal index	35.76	35.41	34.43	33.56	35.14	35.05	34.89	34.88
Arrested cell index	58.06	58.07	56.58	55.81	58.15	58.13	58.05	58.01
Free cell index	6.18	6.52	8.99	10.77	6.7	6.8	7.05	7.11
ACR	0.904	0.865	0.862	0.857	0.896	0.895	0.892	0.891
FCR	0.096	0.097	0.137	0.165	0.103	0.104	0.108	0.109
SR	0.557	0.527	0.525	0.335	0.542	0.54	0.536	0.535
AFR	9.387	8.91	6.294	5.18	8.674	8.543	8.225	8.163
ASR	1.623	1.64	1.643	1.663	1.655	1.658	1.664	1.664
FSR	0.173	0.184	0.261	0.321	0.191	0.194	0.202	0.204

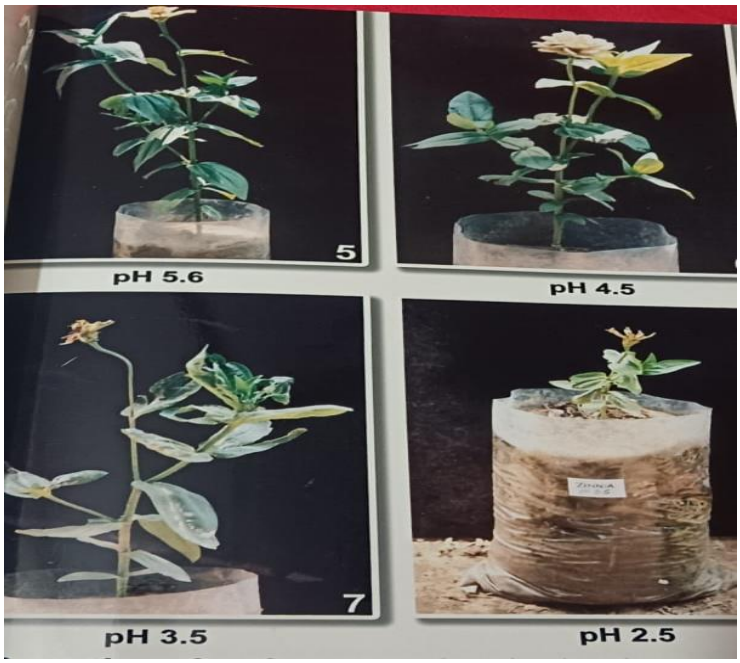

Figure1. Zinnia elegans L. treated with different pH concentrations

Figure2. Foliar injuries seen in Helianthus annuus L. due to simulated acid rain

(XIV). Effect of different concentrations of s.a.r. on different indices and ratios like Stomatal index, Arrested cell index, free cell index, Arrested cells ratio (ACR), Free cells ratio (FCR), Stomatal ratio (SR), Arrested cells and Free cells ratio (AFR), Arrested cells and Stomatal ratio (ASR) and Free cells and Stomatal ratio (FSR) in leaves (abaxial surface) of Helianthus annuus L

	Plant age, d							
	60				**80**			
	pH of s.a.r.							
Attribute	**5.6**	**4.5**	**3.5**	**2.5**	**5.6**	**4.5**	**3.5**	**2.5**
Stomatal index	36.02	35.56	35.51	34.91	34.87	34.84	34.63	34.43

Arrested cell index	59.38	57.25	57.21	57.18	58.22	57.38	56.18	53.2
Free cell index	7.08	7.18	7.28	7.89	6.91	7.78	9.48	12.37
ACR	0.91	0.888	0.887	0.879	0.923	0.881	0.855	0.811
FCR	0.109	0.112	0.113	0.121	0.11	0.119	0.144	0.188
SR	0.552	0.552	0.551	0.536	0.552	0.535	0.527	0.525
AFR	8.386	7.964	7.858	7.24	8.42	7.373	5.924	4.3
ASR	1.648	1.61	1.611	1.638	1.67	1.647	1.622	1.544
FSR	0.197	0.202	0.205	0.226	0.198	0.224	0.274	0.359

4. Discussions

The results indicate that s.a.r. significantly affects stomatal parameters in both Zinnia and Helianthus. Acid rain can alter cellular division patterns and reduce leaf surface integrity (Evans et al., 1994). Zinnia exhibited a sharper decline in derived stomatal parameters compared to Helianthus, indicating its higher sensitivity to acidic stress and Helianthus to be more resistant and having more adaptive traits to acidity. Reduction in the size of stomatal complex, stomatal index, arrested cell index, on both adaxial and abaxial surfaces of the leaves is also reported by Satoh (1996), Kumar (1997) and Paoletti (1998). This is explained due to the phytotoxic components of s.a.r. which penetrate the cuticle and affect the submicroscopic structure of the epicuticular wax layer(s) of the leaves. Reduction in size and efficiency of assimilatory surface due to foliar injury as well as disturbed stomatal regulation are responsible for a decline in growth potential of test crops.

5. Conclusion

Increasing acidity caused reductions in derived stomatal parameters, with Zinnia exhibiting greater sensitivity compared to Helianthus. These findings emphasize the vulnerability of certain plant species to acid rain and underscore the importance of stomatal adaptations in stress tolerance. Future research should explore long-term physiological responses to acid rain to develop mitigation strategies for sensitive plant species. This study highlights the susceptibility of certain plant species to acid rain and underscores the significance of stomatal adaptations as physiological response in stress tolerance. To increase the productivity in acidic conditions, the study of physiological responses in crops is crucial as in near future the acidic soil will limit the use of irrigation water and protective measures can be developed for sensitive plant species.

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