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Effects of Simulated Acid Rain on Germination and Seedling Growth of some Wild and Cultivated Species

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Abstract
The effects of simulated acid rain (SAR) on germination and early seedling growth of five species were studied. Two treatments of SAR were given - pH 3.0 and 4.0 while deionized distilled water served as control. SAR treatment at both the pH decreased the rate as well as the final germination percentage of *Clitorea ternatea* L. Germination of *Senna holosericea* (Fresen.) Greuter and *Adenanthera pavonina* L. remained unaffected by SAR while that of *Leucaena leucocephala* (Lam.) de Wit. and *Senra incana* Cav. increased over the controls. Except for *Senra incana* where root growth was stimulated, SAR markedly inhibited root as well as shoot growth of the test species. The possible causes of differential effects of SAR on plants are discussed.

Key words: Acid rain, germination

Introduction
Acidic precipitation is a major polluting agent possibly harmful to terrestrial and aquatic ecosystems. Despite more than two decades of research little is known regarding how acid rain affects the plants and the ecosystems.

Rain falling through unpolluted atmosphere in equilibrium with CO_2 has a pH of about 5.6 but the pH of acid rain in heavily polluted areas-ranges between 3 to 4 or even lower. There have been seveals efforts to assess the effects of acidic rain on crop plants. However, the results in general are controversial.

In field studies on the radish cultivar "Champion", hypocotyl dry weights iw acid treatment were increased over the pH 5.6 (used as control) (Lee and Neely, 1980).

Lee *et al*. (1981) working with field grown "Cherry Belle" radish reported a 25% greater yield (i.e. an increase in hypocotyl weight) at pH 3.5 but not at pH 3.0 when compared with the growth at pH 5.6 (serving as control). Increased in yield occurred when sulphate was varied to account for the acidity, keeping nitrate constant.

In greenhouse experiments, Harcourt and Farrar (1980) demonstrated decreased hypocotyl growth of radish subjected to simulated acid rain (SAR) of pH between 3.5 to 2.5.

Kohno *et al*. (1995) studied the effects of SAR on the growth of "Japanese conifers" grown with or without fertilizer. SAR at pH 2.0 induced visible injuries but no visible symptoms at pH 3.0 or higher.

Faret *et al*. (1990) studied the effects of SAR on reproductive attributes of red-spruce (*Picea rubens* Sarg.) and reported that growth and reproduction is declining in many areas of Eastern United States. The percentage of,. filled seeds per cone was significantly reduced as the pH of SAR solution declined.

Johnston and Shriner (1985) reported that some cultivars of wheat exhibited reduction or stimulation in productivity of immature plants depending on cultivars selected and the level of acidity applied.

Rochefort and Vitt (1988) working on mosses treated with SAR at pH 3.3 found significant increase in growth and chlorophyll "b" content in *Tomenthypnum nitens* but growth and chlorophyll "b" content of *Scorpidium scorpioides* remined unaffected.

Funk and Bonde (1986) showed that acidification from sulphate and nitrate compounds may stimulate yield by providing additional nutrients.

The objective of this study was to test the relative susceptibility of some wild and cultivated species to simulated acid rain (SAR) at the level of germination and early seedling growth.

Materials and Methods
Seed collection: Seeds of the test species were collected during June 1996 to December 1997 from different localities of Karachi city i.e., *Clitoria ternatea* L. Seeds (November, 1996) were collected from a hedgerow at Urdu Science College (Gulshan-e-lobal), *Senra incana* Cav. (October, 1996) was collected from a halophytic community at Karachi University. Seeds of *Senna holosericea* (Fresen.) Greuter were collected (September, 1997) from a disturbed community at SITE, Karachi *Adenanthera pavonina* L. and *Leucaena leucocephala* (Lam.) De Wit. Seeds were collected (December, 1997) from the trees growing at road sides in Karachi University Campus. Collected seeds were stored for 2-4 weeks in refrigerator in air-tight glass bottles.

Since the seeds of all test species possess hard seed coat, they were chemical or mechanically scarified. Chemical scarification was done by placing the seeds in concentrated sulphuric acid (48%) for 2-3 minutes and then the seeds were washed in running tap water. *Leucaena leucocephala* and *Adenanthera pavonina* seeds were scarified chemically.

Mechanical scarification was done by rubbing seeds on sand paper. *Senna holosericea*, *Senra incana* and *Clitoria ternatea* seeds were mechanically sacrificed. Simulated acid rain (SAR) solution was prepared in accordance with Caporn and Hutchinson (1986). SAR solution contained 20 μmol dm^{-3} KOH, 27 μmol dm^{-3} $CaSO_4$, 10 μmol dm^{-3} NaOH, 7.8 μmol dm^{-3} KOH, 27 μmol dm^{-3} $FeC1_3$, 0.1 μmol dm^{-3} $PbCl_2$, 1.5 μmol dm^{-3}, $3ZnC1_2$, 0.18 μmol dm^{-3} $MnCl_2$, and 0.15 μmol dm^{-3} $CuCl_2$.

The desired pH was adjusted by using a mixture of 50 μmol dm^{-3} H_2SO_4 and HNO_3. The experiments were conducted between September to December, 1997. Germination was performed in 14 cm diameter sterilized petri plates having a Whatman No. 1 filter paper. Twenty seeds were placed in each petri plate and were treated with 10 ml of simulated acid rain solution of pH 3.0 or 4.0, while control plates received deionized distilled water. Treatments and controls were replicated thrice. The petri plates were kept in a growth chamber maintained at $25 \pm 2°C$ day temperature and $18 \pm 2°C$ night temperature. Light intensity at the top of dishes was 2000 Lux (14th day length). Small amounts of solutions or deionized distilled water were added when it was obvious that petri plates were begining to dry out.

Germination was recorded at two day interval until germination remained constant on 3 or more recording occasions. A seed was considered germinated when the radicle had attained a length of not less than 1.5 mm (Taylor, 1942).

After the completion of seed germination, root and shoot lengths of all the seedlings were measured.

Data On germination and root and shoot lengths were subjected to factorial analysis of variance (FANOVA). Percentage germination data was arc-sine transformed before performing FANOVA. Duncan's multiple range test was employed to compare the means at 0.05, 0.01 and 0.001 probability levels (Sokal and Rohlf, 1995). Computer programs used were developed by the senior author in Microsoft FORTRAN 77 and are available on request.

Results

Germination: The five test species differed significantly in the rate and the final percentage germination in response to simulated acid rain (SAR) treatment ($F = 422.78$, $p < 0.001$). SAR treatment also had differential effect upon germination ($p < 0.001$) (Fig. 1). The rate as well as the final germination percentage of *Clitona ternatea* was suppressed by SAR solutions of both pH 3 and 4 ($p < 0.05$) (Fig. 1a). The germination of *Sena holosericia* and *Adenanthera pavonina* did not change significantly over the controls due to SAR treatment (Fig. 1b, c). On the other hand, the final percentage germination of *Leucaena leucocephala* and *Senra incana* increased significantly over the controls in response to SAR ($p < 0.05$ and $p < 0.001$ respectively) (Fig. 1d, e).

Root and shoot growth: With the exception of *Senra incana* shoot growth of all the test species was significnatly

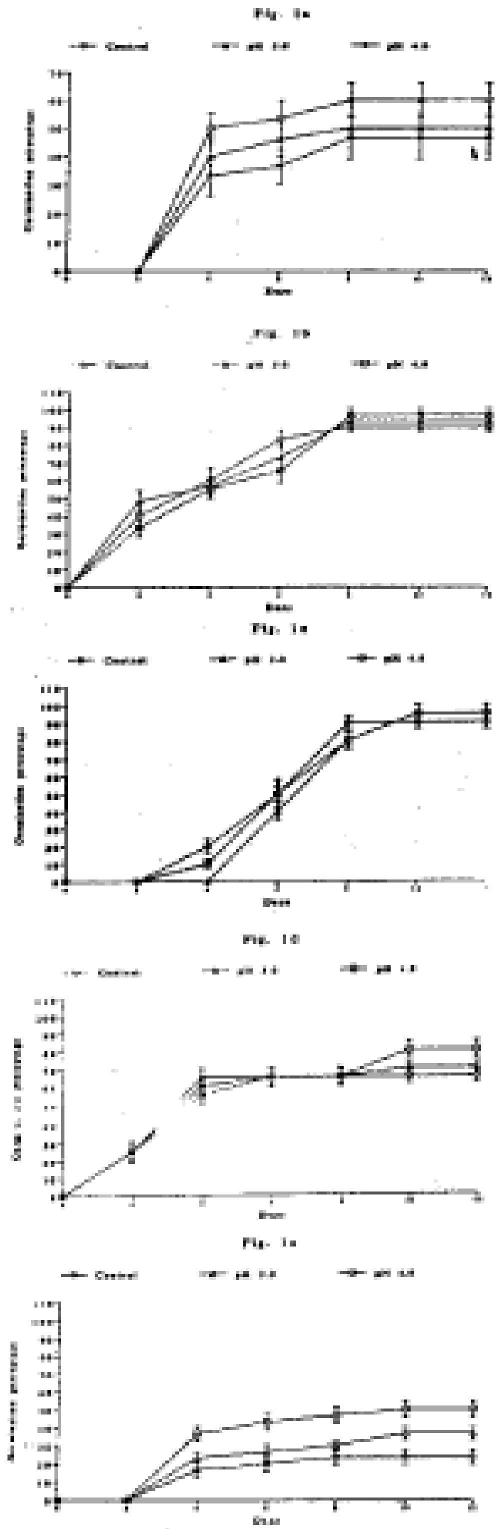

Fig. 1(a-e): Effect of simulated acid rain of pH 3.0 and pH 4.0 on germination with time (days) of five speices (a) *Clitoria ternatea*, (b) *Sena holosericea*, (c) *Adenanthera pavonia*, (d) *Leucaena leucocephala* and (e) *Senra incana*

Table 1: Effect of simulated acid rain on root and shoot growth of five test species. Means are followed by ±SE

	Control	pH 3	PH 4
Clitoria ternatea			
Root length (cm)	3.27±0.23	2.44±0.18	1.19±0.12
Shoot length (cm)	5.78±0.28	2.80±0.21	3.20±0.26
Sena holosericea			
Root length (cm)	4.17±0.31	1.20±0.15	0.2±0.18
Shoot length (cm)	4.34±0.32	2.85±0.26	2.47±0.2
Adenanthera pavonia			
Root length (cm)	1.35±0.15	1.22±0.18	1.30±0.12
Shoot length (cm)	1.25±0.11	1.03±0.09	1.20±0.17
Leucaena leucocephala			
Root length (cm)	4.02±0.55	3.63±0.32	2.59±0.25
Shoot length (cm)	1.80±0.21	1.77±0.23	1.33±0.16
Senra incana			
Root length (cm)	1.77±0.25	2.45±0.28	2.52±0.30
Shoot length (cm)	2.81±0.18	2.96±0.35	2.40±0.14

reduced by simulated acid rain (SAR) solution compared to controls (p at the most 0.05). Root growth was also significantly inhibited by SAR (p at the most 0.05) except in *Senra incana* where it was significantly promoted ($p<0.05$) (Table 1).

Discussion

Germination response to SAR treatment of various test species was differential. The final germination percentage of *Clitoria ternatea* was significantly reduced but that of *Leucaena leucocephala* and *Senra incana* was stimulated by SAR treatment.

These results corroborate the findings of Kim (1992), who examined the effects of artificial acid rain (pH 2.90, 3.0, 4.0 and 5.0) on seed germination of far species and found that germination of *Pinus densiflora* was highest in pH 5.0 whereas germination in *Alianthus altissima* was highest in control and that of *Magnolia sieboldi* in acid rain of pH 3.0. Schernatskoy *et al*. (1987) found germination rate under laboratory conditions is not generally influenced by a single SAR treatment. Similar results were found for tree seeds exposed to continued aciddifcation.

Like the germination inhibition in *Clitoria ternatea* found in this study, other workers including Lee and Weber (1979), McColl and Johnson (1983), Percy (1986) and Schernatskoy *et al*. (1987), have also demonstrated some reduction in germination after continued exposure to SAR of pH 2.0-3.0.

Exposure to acid is a well accepted technique for breaking seed dormancy in the laboratory (Schopmeyer, 1974). Therefore, acid rain may help in breaking dormancy under field conditions.

A number of workers (Raynal *et al*., 1982; McColl and Johnson, 1983; Percy, 1986) have demonstrated that germination response is less sensitive than the subsequent seedling growth to acidification.

At seedling growth level the plants showed highest seedling growth in controls. The SAR treatment caused reduction in seedling growth by inhibiting both root and shoot length in all the species except *Senra incana* where root length was enhanced by SAR.

The reduction in seedling growth obtained in this study has been previously reported by various workers (Lee and Weber, 1979; Harcourt and Farrar, 1980; Lee *et al*., 1981; Olson *et al*., 1987). On the other hand increase in root growth has also been demonstrated by Funk and Bonde (1986) and Rochefort and Vitt (1988).

According to Caporn and Hutchinson (1986) the epicuticular waxy substances present on cotyledons and leaves may be a major factor determining the deleterious effects of SAR. Plants showing poor development of waxy coating on their cotyledons and young leaves are particularly vulnerable. The susceptible species in this study namely *Clitoria ternatea* had the least waxy coating on the seeds. By contrast *Senra incana* showed increased seedling growth presumably due to more waxy substances on seed surface.

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