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The Effect of Simulated Acid Rain on *Glycine max*

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Abstract**—**Acid rain occurs when sulphur dioxide (SO_2) and nitrogen oxides (No_x) gases react in the atmosphere with water, oxygen, and other chemicals to form various acidic compounds. The result is a mild solution of sulfuric acid and nitric acid. Soil has a greater buffering capacity than aquatic systems. However excessive amount of acids introduced by acid rains may disturb the entire soil chemistry. Acidity and harmful action of toxic elements damage vegetation while susceptible microbial species are eliminated. In present study, the effects of simulated sulphuric acid and nitric acid rains were investigated on crop *Glycine max.* The effect of acid rain on change in soil fertility was detected in which pH of control sample was 6.5 and pH of 1%H_2SO_4 and 1%HNO_3 were 3.5. Nitrogen nitrate in soil was high in 1% HNO_3 treated soil & Control sample. Ammonium nitrogen in soil was low in 1% HNO_3 & H_2SO_4 treated soil. Ammonium nitrogen was medium in control and other samples. The effect of acid rain on seed germination on 3rd day of germination control sample growth was 7 cm, 0.1% HNO_3 was 8cm, and 0.001% HNO3 & 0.001% H2SO4 was 6cm each. On 10th day fungal growth was observed in 1% and 0.1%H_2SO_4 concentrations, when all plants were dead. The effect of acid rain on crop productivity was investigated on 3rd day roots were developed in plants. On12th day *Glycine max* showed more growth in 0.1% HNO_3, 0.001% HNO_3 and 0.001% H_2SO_4 treated plants growth were same as compare to control plants. On 20th day development of discoloration of plant pigments were observed on acid treated plants leaves. On 38th day, 0.1, 0.001% HNO_3 and 0.1, 0.001% H_2SO_4 treated plants and control plants were showing flower growth. On 42th day, acid treated *Glycine max* variety and control plants were showed seeds on plants. In *Glycine max* variety 0.1, 0.001% H_2SO_4, 0.1, 0.001% HNO3 treated plants were dead on 46th day and fungal growth was observed. The toxicological study was carried out on *Glycine max* plants exposed to 1% HNO_3 cells were damaged more than 1% H_2SO_4. Leaf sections exposed to 0.001% HNO_3 & H_2SO_4 showed less damaged of cells and pigmentation observed in entire slide when compare with control plant. The soil analysis was done to find microorganisms in HNO_3 & H_2SO_4 treated *Glycine max* and control plants. No microorganism growth was observed in 1% HNO_3 & H_2SO_4 but control plant showed microbial growth.

Keywords**—**Acid rain, *Glycine max*, HNO_3 & H_2SO_4, Pigmentation.

I. Introduction

ACID deposition penetrates deeply into the fabric of an ecosystem, changing the chemistry of the soil and streams and narrowing the space where certain plants and animals can survive [1]. Seventy-six rain samples from forty-seven rain events and TSP aerosol samples throughout the entire year of 2005 were collected in Shanghai, China. The annual mean pH in rain was 4.49 with the lowest pH of 2.95, and the frequency of acid rain was 71% in 2005. The acidity of rain increased more than 15 times in the past 8 years compared to 1997 [11]. The high fuel consumption from urbanization and the rapid increase of vehicles resulted in the high emission of SO_2 and NO_x, which were the precursor of the high concentration of acidic ions NO_3^- and SO_4^{2-}. It was the main reason of the severe acid rain in Chengdu [7]. Acidity and harmful action of toxic elements damage vegetation while susceptible microbial species are eliminated. The soil loses its normal functions such as decay and decomposition of organic debric and the capacity of a balanced regulation of nutrients [4]. Simulated acid rain has been reported to cause physiological changes in various plant species. Studies were conducted in 1983, 1984, and 1985 to determine the effect, of acid rain on physiological parameters in two soybean [*Glycine max* (L.) Merr.] cultivars [10]. The effects of simulated acid rain on two soybean [*Glycine max* (L.) Merrill] cultivars were studied in field experiments in central Illinois. The cultivars, 'Amsoy 71' in one of three years, seeds/plant, seeds/pod, and chaff dry weight were reduced by increasing acidity of the simulated rain [13]. Simulated acid rain treatment at pH 3.0 or higher did not significantly affect yield compared to pH 5.6; however, plants exposed to simulated acid rain at pH 4.0 tended to yield more than those treated with pH 5.6 rain [12]. Soybean was grown in 1984, 1985, and 1986 in field plots in east central Illinois. Early in each growing season visible leaf injury was noted for all 20 cultivars, and level of injury was significantly higher for plants receiving the more acidic treatment (pH 3.0) [3]. Acid rain stress had a considerable inhibitory effect on germination energy and germination rate of Glycine max, and the inhibitory effects generally increased with pH reduced, but it had no inhibitory effects on germination of *Arachis hypogaea*. Acid rain stress had the same inhibitory effects on the growth of plumule and radicle of two leguminous crops [14]. The pH 3.5 treatment also caused foliar damage and decline in pH of the leaf sap, leading to growth inhibition of the seedlings [6]. Chemical composition of precipitation collected from a rural forest station near Bhubaneswar in the east coast of India during 2005–2007 was studied. NH_4^+ and NO_3^- were observed to be the dominating cation and anion respectively [9]. In present this study, I the effects of simulated acid rain on *Glycine max* variety exposure and its effects on the germination and growth of the plant are examined.

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II. Materials and Method

A. To Detect the Effect of Simulated Acid Rain on Soil Fertility

I. Acid rain simulation:- Different concentrations of acids were made. Distilled water of pH 6.5 was used as the control and as diluents for preparation of acid concentrations [12].

II. pH of the soil:-1gm soil sample was weighed and 2ml distilled water added to it in the test tube. The soil sample was mixed thoroughly. Then the soil sample was filtered through Whatmann paper 1 and pH of the sample was detected. The plastic pots containing approximately 750gms of soil to which separate concentration of acid samples were added and pH of the each soil sample were noted.

III. Ammonium nitrogen in soil and Nitrogen nitrate in soil. Soil analysis was done by kit method. Kits were procured from HiMedia, India.

B. To Detect the Effect of Acid Rain on Seed Germination

Soaked seeds of Glycine max were kept in a humidity chamber. The humidity chamber was made by keeping filter paper in petriplates. Different concentrations of acids were made and poured 5ml each day in respective petriplates. Observations were made every alternate day.

C. To Investigate the Effect of Acid Rain on Crop Productivity

Soaked seeds of *Glycine max* was ploughed in plastic 7-5 cm diameter pots containing750gms of soil and allowed to become established. Supplementary watering of the plants was provided at the rate of 40ml/day' using simulated acid rain solution of pH2.5- 4.5 (Different concentrations of acids were made from sulphuric and nitric acids 1% to 0.001% concentrations) and poured every day in respective pots. Observations were made every alternate day. The pH of the rain solution was comparable with that for rainfall in upland areas of India [2], [12].

D. To Detect the Effect of Acid Rain on Health of the Plants

Toxicological studies, on plant variety were carried out; Plant varieties were exposed to different concentration of HNO_3 and H_2SO_4 for 46^{th} days. Leaves were removed by cutting the petiole near the base cut petioles were coated in petroleum jelly to prevent water loss from the open wounds and then the leaves were placed in open Petri dishes on a laboratory bench [2]. After 15^{th} days of exposure of plants to different concentrations of acids showed decolouration of plant pigment on leaves so leaf sections were taken and observed under LOBO High magnification microscope.

III. Result and Discussion

A. The Effect of Acid Rain on Soil Fertility

TABLE I
PH OF SOIL SAMPLE

	Sample	Observed pH of soil sample	Colour of Soil sample
1	1% H_2SO_4	3.5	Pink
2	0.1% H_2SO_4	4.0	Yellow
3	0.01% H_2SO_4	4.0	Pinkish Brown
4	0.001% H_2SO_4	4.5	Pink
5	Control	6.5	Yellow

TABLE II
PH OF SOIL SAMPLE

	Sample	Observed pH of soil sample	Colour of Soil sample
1	1% HNO_3	3.5	Red
2	0.1% HNO_3	4.5	Yellow
3	0.01% HNO_3	4.5	Pinkish Brown
4	0.001% HNO_3	4.5	Pink
5	Control	6.5	Yellow

TABLE III
AMMONIUM NITROGEN IN SOIL

HNO_3	Ammonium nitrogen in soil	H_2SO_4	Ammonium nitrogen in soil
1%	Low about 15	1%	Low about 15
0.1%	Medium about 75	0.1%	Medium about 75
0.01%	Medium about 75	0.01%	Medium about 75
0.001%	Medium about 75	0.001%	Medium about 75
Control	Medium about 75	Control	Medium about 75

Ammonium nitrogen in soil was low in 1% HNO_3 & H_2SO_4 treated soil. Ammonium nitrogen was medium in control and other samples.

TABLE IV
NITROGEN NITRATE IN SOIL

HNO_3	Nitrogen nitrate in soil	H_2SO_4	Nitrogen nitrate in soil
1%	High about 50	1%	Low about 10
0.1%	Medium about 30	0.1%	Very low about 4
0.01%	Low about 10	0.01%	Very low about 4
0.001%	Low about 10	0.001%	Medium about 20
Control	High about 50	Control	High about 50

Nitrogen nitrate in soil was high in 1% HNO_3 treated soil & Control sample. Nitrogen nitrate was low in lower concentration of HNO_3, medium in 0.001% H_2SO_4 treated soil and very low in other samples.

TABLE V
AVAILABLE PHOSPHATE IN SOIL KG PER HECTARE AS (P_2O_5)

HNO_3	Available phosphate in soil	H_2SO_4	Available phosphate in soil
1%	Medium about 22to 56.	1%	Medium about 22to 56.
0.1%	Medium high about 56 to 73	0.1%	Medium high about 56 to 73
0.01%	Low less than22	0.01%	Medium high about 56 to 73
0.001%	Medium about 22to 56.	0.001%	Medium about 22 to 56
Control	Low less than22	Control	Low less than 22

Available phosphate in soil was low in 0.01%HNO_3 treated sample and control sample. 0.1% HNO_3, 0.1% & 0.01% H_2SO_4 Medium high phosphate content was found but rest of the samples showed medium

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B. The Effect of Acid Rain on Seed Germination

TABLE VI
3RD DAY READINGS FOR GLYCINE MAX

HNO_3	Germination of seeds	H_2SO_4	Germination of seeds
1%	0.8cm	1%	0.2cm
0.1%	8.0cm	0.1%	4.0cm
0.01%	3.5cm	0.01%	1.8cm
0.001%	6 cm	0.001%	6cm
Control	7cm	Control	7cm



Fig. 1 Germination of *Glycine max* seeds treated with HNO_3 concentrations



Fig 2 Germination of *Glycine max* seeds treated with H_2SO_4 concentrations

TABLE VII
5TH DAY READINGS FOR GLYCINE MAX

HNO_3	Germination of seeds	H_2SO_4	Germination of seeds
1%	1.4cm	1%	1.0cm
0.1%	11cm	0.1%	3.2cm
0.01%	3.9cm	0.01%	2.8cm
0.001%	8.5 cm	0.001%	9.5cm
Control	10cm	Control	10cm

TABLE VIII
7TH DAY READINGS FOR GLYCINE MAX

HNO_3	Germination of seeds	H_2SO_4	Germination of seeds
1%	1.5cm	1%	1.0cm
0.1%	15cm	0.1%	3.0cm
0.01%	4.5cm	0.01%	2.5cm
0.001%	10 cm	0.001%	10cm
Control	13cm	Control	13cm



Fig. 3 Germination of *Glycine max* seeds treated with HNO_3 &H_2SO_4 concentrations on 7th day

On 10th day fungal growth was observed in 1% and 0.1%H_2SO_4 concentrations when all plants were dead.

C .The Effect of Acid Rain on Crop Productivity

On 3rd day roots were developed in *Glycine max* varieties of plants.



Fig. 4 HNO_3 treated *Glycine max* plants on 3rd day

TABLE IX
7TH DAY READINGS FOR GLYCINE MAX

HNO_3	Germination of seeds	H_2SO_4	Germination of seeds
1%	4.5cm	1%	12cm
0.1%	16cm	0.1%	14cm
0.01%	14.5cm	0.01%	11.5cm
0.001%	14. 5cm	0.001%	13.5cm
Control	14.58cm	Control	14.5cm



Fig. 5 Length of *Glycine max* variety was more in0.1% HNO_3treated plants as compare to control plants

Length of *Glycine max* variety was 50cm in 0.1% H_2SO_4 treated plants more than control plants (44cm). 0.1%$HNO_3$and 0.01% H_2SO_4 showed 36and40cm length of the plant.

1% H_2SO_4 treated plant had died on 20th day after development of discoloration of plant pigments was observed on leaves. HNO_3 treated plants were died on26th day.



Fig. 6 Decolouration of plant pigment was observed in *Glycine max* plant

On 38th day 0.1, 0.001% H_2SO_4, 0.1, 0.001% HNO_3 treated plants and control plants were showing flower growth. On 42th day 0.1, 0.001% H_2SO_4 , 0.1, 0.001% HNO3 treated plants of *Glycine max* variety and control plants were showed seeds on plants.

On 46th day 0.1, 0.001% H_2SO_4, 0.1, 0.001% HNO_3 treated *Glycine max* plants were dead and showed fungal growth.



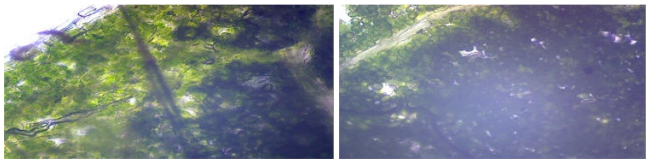
Fig. 7 Dead plants of *Glycine max*

D The Effect of Acid Rain on Health of the Plants

Glycine max plants exposed to 1% HNO_3 were died on 15th day. Cells were damaged in 1% H_2SO_4. Leaf sections exposed to 0.001% HNO_3 & H_2SO_4 showed less damaged of cells and pigmentation observed in entire slide when compare with control plant. Damage was found more with HNO_3 than H_2SO_4.



Fig. 8 Leaf section of *Glycine max* plant exposed to 1%H_2SO_4 concentration

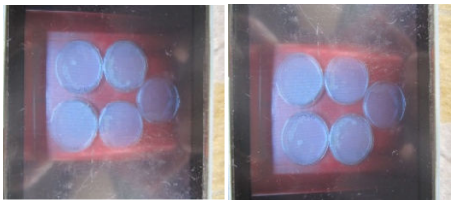


(a) (b)

Fig. 9 (a) Leaf section of *Glycine max* plant exposed to 0.001%H_2SO_4 concentration. (b) Leaf section of *Glycine max* plant exposed to 0.001%HNO_3 concentration



Fig. 10 Leaf section of *Glycine max* Control plant



(a) (b)

Fig. 11 No microorganism growth was observed in 1% HNO_3 & H_2SO_4

The variation of pH to 0.1 to 0.001% HNO_3 and H_2SO_4 acid treated soil samples was shown in Tables I and II. The pH values below 5.60 and the lowest pH of the rain event reached 2.95, reflecting the high loading of the anthropogenic

Pollutants was reported by other scientists [15] and [10] observed the acidity of the precipitation in Shanghai was considerably high with the annual mean pH value of 4.49 and the frequency of acid rain was 71%.

In present investigations, soil fertility was detected Tables III-V listed Ammonium nitrogen was low at 1% HNO_3 and H_2SO_4 but medium in 0.1 to 0.001% HNO_3 and H_2SO_4 as well as control samples. Nitrogen nitrate was high in 1% HNO_3 and control sample but low in 1% to 0.001% H_2SO_4. Available nitrogen was less in control samples but increased in acid treated samples. The soil fertility is found increasing in higher concentrations of acid from 0.001 to 0.1% H_2SO_4 & HNO_3.The findings were different from others [9], [4], [7].

Germination of the seed and seedling growth was more in acid treated samples. As shown in Table VI at pH 2.5 the germination of seeds was fast and plumule developed early than control plants. When pH was increased from 4.5 to 2.5 leaf and root growth was normal. This was in agreement to observations made by other workers [1].The results were different from the findings made by others *Glycine max* (Amsoy71 cultivar) seeds/plant, seed/pod and chaff dry weight were reduced by increasing acidity of the stimulated rain [12], [14].

Figs. 1 and 2 showed that the seedling growth was stimulated at pH levels between 2.5 to 4.5. Similar reports were observed by other workers [7]. In Fig. 3 showed more growth plants exposed to simulated acid rain at pH 4.0 tended to yield more than those treated with pH 5.6 rain [14]. The results were not in agreement with others where they mentioned that the simulated acid rain of pH 2.5 could significantly reduce the germination ratio and survival ratio of alligator weed and cause eyeable damages to leaves [5], [6], [14].

Figs. 4, 5 and Tables VII and VIII showed that *Glycine max* showed more growth in 0.1% HNO_3 treated plants as compare to control plants.

The leaf and root growth of crop variety (*Glycine max*) was normal for 15 days. Though the growth was equally good in 44 days of the study, The results were same as observed by [11] Applications of stimulated acid rain (pH 3.2,4.2 and 5.2), alone or in combination with gaseous pollutants did not significantly affect photosynthesis, transpiration, stomatal

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conductance of water vapor or chlorophyll content at periodic intervals during the 1984 season.

Fig. 6 showed that the leaves were found dipigmented at a larger scale on 20th day of study. The results were different than others stimulated acid rain did not affect chlorophyll content or seed yield etc. [11].

Fig. 7 observed seeds appeared on 42th day of experiments. The result showed that in *Glycine max* variety 0.1%, 0.01% HNO_3, 0.01% H_2SO_4 treated plants were dead on 46th day and fungal growth was observed and showed fungal growth. Results were appeared much faster than gaseous pollutant and acid mist [2].

Fig. 8 showed the leaf sections with rupture of cells were observed in the sections of *Glycine max* variety at 1% H_2SO_4 concentrations but Fig. 9 (a) and (b) showed less damage in 0.001% HNO_3 and H_2SO_4 concentration as compare to control plants as showed in Fig 10. Plant cells were ruptured, uneven distribution of chlorophyll pigments were observed in others study [8], [6]. Fig. 11 No microorganism growth was observed in 1% HNO_3 & H_2SO_4 but control plant showed microbial growth.

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