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Impacts of Simulated Acid Rain on the Growth and the Yield of Soybean (*Glycine max* (L.) Merr.) in the Mountains of Northern Vietnam

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Abstract: In the mountains of Northern Vietnam, frequent and intense acid rain affects the crops. This paper assesses the impacts of simulated acid rain (SAR) on the growth and the yield of soybeans (*Glycine max* (L.) Merr.) in Hoa Binh province. A field study in the summer–autumn seasons in 2017 (from May to August) in an area of 189 square meters was arranged according to a Randomized Complete Block Design (RCBD) with three repetitions including six treatments and a control. The experimental area was protected from ambient rain. Soybean plants were exposed three times a week to SAR at pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 (control). The results show that the growth parameters such as germination rate, stem length, and the number of main branches of the plants dramatically decreased in a dose–effect experiment. Gradual declines in the chlorophyll content (indirectly determined by SPAD) and the leaf area index (LAI) were observed as the acidity increased. The actual yield and yield components also tended to decrease when the pH of the rainwater fell, especially in the experimental plots treated at pH 3.0. The growth and yield of soybean were adversely affected when the plants were exposed to simulated acid rain, especially from a pH value of 3.5 and lower. This is the first study to evaluate the effects of acid rain on the growth and the yield of soybean grown in the mountains of Northern Vietnam.

Keywords: simulated acid rain; soybean; growth; yield; treatments; control; Northern Vietnam

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1. Introduction

Industrialization leads to a number of environmental effects including air pollution and acid rain [1–3]. Acid rain has been a problem in highly industrialized areas such as Europe and North America since the 1970s [4]. Over the past two decades, it has become an increasing problem in Asia [5]. An acidified environment affects plants and crops. Acid rain damages the leaves, stems, and roots of plants [6–10]; reduces crop yields [11–14]; decreases the photosynthesis of plants [15]; and reduces their germination and chlorophyll content [16–18]. Simulated acid rain damages plants if the pH is below 3.4 [19–23]. The adverse effects of simulated acid rain on the growth parameters of yellow birch, corn, rice, tomato, pea, sunflower, and pollen of apple have been documented [24–30]. The results show that the pollen tube is destroyed from a pH of 3.1 and lower. When the pH was near 3.0, pollen germination stopped. The impact of simulated acid rain was studied with a range of pH values on the seeds and seedlings of legumes including *Phaseolus radiatus* L. and *Phaseolus vulgaris* L. in Kerala, India. The results show that, at a pH of 2.0, the germination rate of both species was reduced and the leaves of the plants showed signs of yellowing and necrotized areas. Additionally, the chlorophyll content decreased with decreasing pH value.

However, the phenol content of both species tends to increase when the pH decreases from 4.0 to 2.0. *Phaseolus vulgaris* L. is more sensitive to acid rain than *Phaseolus radiatus* L. [17]. The number of roots tends to decrease as pH decreases [31]. Young trifoliate leaves are strongly affected by acid rain below pH 3.0. However, an opposite finding concerning the stimulation of seedling emergence and growth by simulated acid rain at pH values from 2.3 to 4.0 was noticed by Lee and Weber (1979) [32]. Direct damage to leaves occurs if the accumulation of sulfate on the leaf reaches toxic levels. Early symptoms of acid rain on the leaves of broadleaf tobacco are small spots when exposed to simulated acid rain at pH 2.0 [33]. An experiment on *Genipa americana* L. indicates that necrotized areas on the leaves appear with exposure to simulated acid rain at pH 3.0 for 10 consecutive days [34]. Brown necrotic lesions appeared on rice leaves when exposed to SO_2 dry deposition [35]. The positive or negative impacts of acid rain on crops depend on the concentration of SO_4^{2-} in rainwater. The top growth of crops exposed to simulated acid rain occurs if the sulfate absorbed by the leaves has a fertilizing effect; inhibition occurs if the accumulated sulfate reaches toxic levels or if the acid causes direct injury to the leaves [32]. The effects of simulated acid rain (pH 3.0–4.5 with 70:30, H_2SO_4:HNO_3) on germination, seedling growth, and oxidative metabolism in *Trichilia dregeana* were investigated. At pH 3.0, the seedlings showed signs of stress typically associated with acid rain such as leaf tip necrosis, abnormal bilobed leaf tips, areas of leaf necrosis and chlorosis, reduced leaf chlorophyll concentration, increased stomatal density, and indications of oxidative stress [20]. A study about the effects of acid rain with different SO_4^{2-}/NO_3^{-} (S/N) ratios and a range of pH values on the growth rate of Chinese fir [16] showed evidence that the chlorophyll a (Chla) and chlorophyll b (Chlb) contents with S/N 1:5 were significantly below those with S/N 1:0 at pH 2.5. The root activities first increased and then decreased as the pH decreased, with S/N ratios of 1:1, 1:5, and 0:1. A lab-scale cultivation experiment and a glasshouse cultivation experiment on rice (*Oryza sativa* L.) with rice blast (*Pyricularia oryzae*) both before and after simulated acid rain at pH 2.0, 3.0, 4.0, and 5.0 were set up. The results showed that the severity of rice blast disease increases significantly with increasing acidity [28].

In Vietnam, acid rain is still a novel topic locality and has not receive much attention from local authorities and the people despite the increasing pressure of the problem. Hoa Binh is a mountainous province, is the gateway to the Northwest region, and is located 76 km from Hanoi capital. Hoa Binh is a province with a low economic growth rate. The economic structure is built on agriculture, industries, and services. Agriculture and forestry are of key importance to the economy, contributing to the stability of the province [36]. Similar to most cities in Vietnam, Hoa Binh faces challenges from the development process: environmental degradation, especially air quality, in which acid rain play a part. In Hoa Binh, the intensity of acid rain (the average pH value by month is less than 5.6) is quite high and there was strong variation between months and seasons during the period from 2000 to 2015. The highest acidity was 81.8% in 2000, and the lowest one was 16.7% in 2008 [37]. The monitoring data from the last five years (2015–2018) in Hoa Binh obtained from the Acid Deposition Monitoring Network in East Asia (EANET) shows high intensities of acidity (50%, 66.7%, 33.3%, 50%, and 72.7%, respectively). Therefore, acid rain likely has effects on the environment and ecosystems in Hoa Binh. Agricultural crops are more sensitive than other natural plants when their foliage are directly destroyed by acid rain. The intensity of acid rain on plants varies with the chemical composition of the rainwater, soil properties, cultivars, climatic conditions, and the variability of crops. It has been shown that vegetables including soybean are sensitive to acid rain [7,13,38–41]. Soybean (*Glycine max* (L.) Merr.) is one of the most popular crops in Hoa Binh. Its growth is optimal when pH conditions range between 6.0 and 7.0. Soybean is very sensitive to acid and alkaline soil conditions [42]. Therefore, soybeans are sensitive to acid rain at a range of pH levels. The production of soybean in select districts of Hoa Binh declined during recent years [43]. To which extent does acid rain affect the growth and yield of soybeans in Hoa Binh? How can soybean plants adapt to acid rain to increase the yield and to enhance the local economy? In response to these questions, we study the acid rain effects on soybean and identify the theoretical basis of the relationship between acid rain and agricultural crops. This study exposes soybean crops grown in the field to simulated acid rain to determine its impact on the growth and yield of soybean. The results warn of the adverse effects and the cost of acid rain on agricultural crops. Adaptation incentives for soybeans under acid rain stress in Hoa Binh province are proposed. The few Vietnamese studies about the impacts of acid rain include its effects on brown mustard (*Brassica juncea*) [44] and common beans (*Phaseolus vulgaris* L.) [45] in pot-scale experiments. Most studies covered the inherent limitations in evaluating the effects of acid rain on plants. The results provided a theoretical basis of the relationship between acid rain and plants but were not applicable to the various conditions in our study area. Important growth parameters and physiology have not been studied yet. This knowledge gap on the effects of acidity on plants needs to be investigated. This is the first study in Vietnam to assess the impact of acid rain on soybeans. The research has both scientific and practical implications and contributes to environmental protection and sustainable development in mountainous areas such as Hoa Binh province, in particular, and Vietnam as a country, in general.

2. Materials and Methods

2.1. Study Area

The districts Yen Thuy and Lac Thuy in Hoa Binh province were previously planned as specialized areas for peanut and soybean cultivation. During recent decades, however, the soybean area in both districts declined. This study was carried out in the summer–autumn seasons in 2017 (from May to August) in fields located at 20°28′49″ North latitude and 105°47′41″ East longitude in Chi Ne Town of the Lac Thuy District, Hoa Binh, Vietnam (Figure 1). Lac Thuy district is located in the southeastern part of Hoa Binh province. This area experiences a tropical monsoon climate with an average annual temperature of 23 °C, a maximum temperature of 28 °C, and a minimum temperature of 17.2 °C. During the rainy season, rainfall is relatively high (1681 mm), mainly concentrated in June and July, and the humidity ranges between 75 and 86% [36]. The land structure of Lac Thuy includes 5455 hectares of agricultural land (accounting for 18.6% of the district area) and 12,766 hectares of forests (accounting for 43.51%). In general, the arable soil layer here is thin, originating from limestone, granite, sandstone, and sediments. The temperature, humidity, and soil layer are beneficial for many crops such as soybeans, sugarcane, oranges, lemons, peanuts, various types of fruit trees, and industrial plants [43].

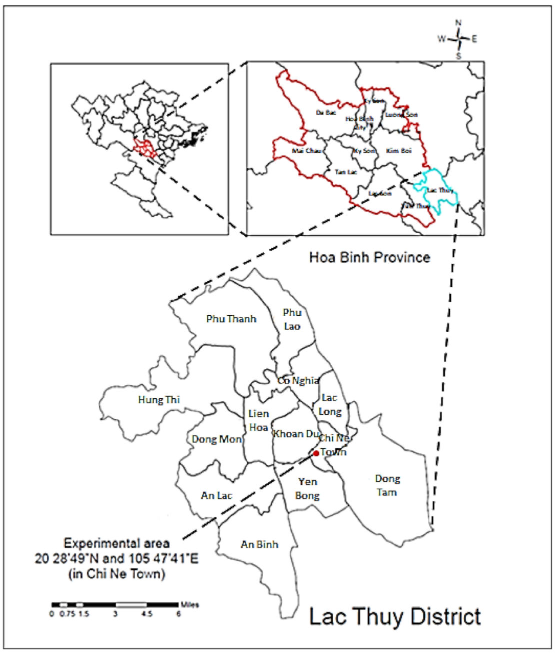


Figure 1. Location of the study area in the Lac Thuy district, Hoa Binh province (Vietnam).

2.2. Experimental Plants

The variety DT84 was developed from the hybrid combination DT-80/DH4(DT96) using a sexual hybridization method combined with experimental mutation by a gamma Co^{60} Krad agent on F3-D333 hybrid lines at the Institute of Agricultural Genetics [46]. DT84 has an average growth duration of 95–115 days. With regard to its morphological characteristics, it has a well-developed main stem (approximately 50–60 cm) with brown hairs, two main types of leaves (primary and trifoliate leaves), a typical papilionaceous purple flower, yellow pods borne in clusters on short stalks, and large and yellow seeds. The average yield is 1.5–2.5 ton/ha, and high intensive farming can achieve up to 3.0 ton/ha [42,46].

DT84 became a national standard variety in 1995. The variety adapts to different ecological regions and shows good resistance to drought, cold, and rust disease. It is one of the most popular soybeans grown in Hoa Binh. The variety was never screened for its acid rain sensitivity. It provides big, firm, and uniform soybeans. Soil preparation, bed raising, and sowing are practiced in its cultivation.

2.3. Experimental Design

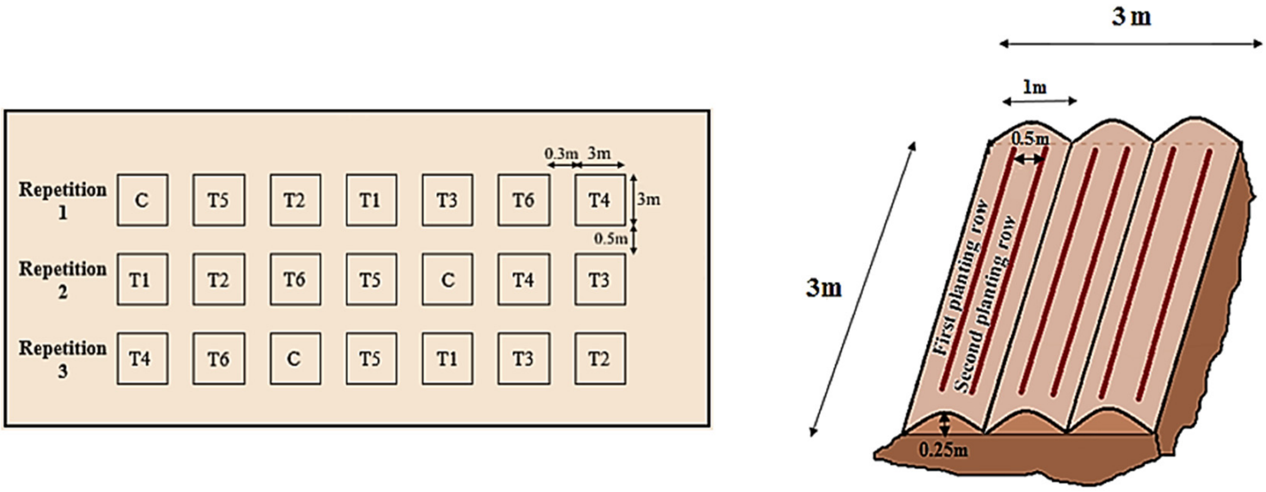
2.3.1. Soil Preparation

The experiment was set up on 189 m^2 of land. Particle size (mechanical analysis), pH, CEC, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, SO_4^{2-}, K, N, P, Mn^{2+}, and OM (organic matter) of the 20 cm topsoil were determined. The soil samples were analyzed at the Laboratory of the Department of Soil Resources and Environment, Faculty of Environmental Sciences (VNU University of Science, Hanoi). The methods used to determine the soil properties included mechanical analysis by the Robinson tube method, pH_{KCl} measurement, hydrogen-selective electrode and classification according to the rating scale of the Southeast Asian Network of Soil Management, OM% calculation, Walkley–Black method, Ca^{2+} and Mg^{2+} extraction by ammonium acetate and quantification by the complexometric titration method, bio-availability of nitrogen assessment using the Chiurin–Cononova method, bio-availability of phosphorus assessment using the Oniani method; CEC measurement using the Schachtschabel method, potassium–ammonium acetate method, SO_4^{2-}–barium chromate method, Al and Fe extraction using an oxalate mixture at pH = 3 (ratio 1:40), Mn^{2+} extraction by H_2SO_4 0.1 N (ratio 1:10), and analysis on the ICP-OES Optima 7300 V (USA). The mechanical analysis showed a sandy clay loam (clay: 23.4%, loam: 8.6%, and sand: 67.6%). The soil reacted neutrally with pH_{KCl} = 6.56 and pH_{H2O} = 7.15, which is favorable for plants to absorb nutrient minerals. The soil OM was 3.18% and the CEC was 11.3 meq/100 g. Ca^{2+} and Mg^{2+} in the soil were 5.75 meq/100 g for Ca^{2+} and 2.95 meq/100 g for Mg^{2+}. The N and P contents were 8.12 mg/100 g and 69 mg/100 g, respectively. The K content was 12.1 mg/100 g of soil. The content of SO_4^{2-} was 59 ppm (0.0059%), Mn^{2+} was 3.16 mg/100 g, and Al^{3+} and Fe^{3+} were 52.6 mg/100 g and 98.5 mg/100 g, respectively [47].

2.3.2. Experimental Set-Up

The experimental land was subdivided into 21 plots; each plot was 3 m $\times$ 3 m. The total plot area was approximately 189 m^2. The distances between two plots in the same repetition and between iterations were 30 cm and 50 cm, respectively. The experimental design was arranged on a Randomized Complete Block Design (RCBD) with three repetitions, including six treatments (T1–T6) and a control (C). The IRRISTAT 5.0 program (developed by the International Rice Research Institute (IRRI) determined the experimental layout (Figure 2a). The field was prepared prior to soybean sowing following standard agronomic practices in Hoa Binh [42]. Each plot was divided into three beds; each of them was 3 m $\times$ 1 m $\times$ 0.25 m. Two rows were 0.5 m apart in each bed (Figure 2b). Poke slits in a cultivated row were used to plant soybean seeds about 0.06 m apart and 0.02 m deep. Each slit was supplemented with a thin layer of fertilizer and filled with soil and sowing seeds on the top, and soil covered the seeds. A density of 45 plants/m^2 was ensured. The amount of fertilizer used throughout the experiment were as follows: decomposed organic fertilizer (136.8 kg), urea nitrogen (1.1 kg), superphosphate (5.7 kg), potassium chloride

(2.3 kg), and lime powder (4.8 kg). During the treatment, the fertilizer was supplemented for two main periods: basal fertilizer and top dressing. The basal fertilizer was applied before sowing and consisted of 100% organic fertilizer + 100% phosphate + 10% nitrogen + 30% potassium + 100% lime powder. The top dressing consisted of three stages: the first one (when the plant had 2–3 true leaves) added 30% of nitrogen, which was stirred up and covered by fertilizer to limit volatile nitrogen loss; the second stage (pre-flowering) applied the remaining 60% of nitrogenous fertilizer + 50% potassium fertilizer combined with stirring up and weeding; and the third stage (fruiting) used the remaining potassium accompanied with hilling of the root to cover the fertilizer and to avoid collapse of the plant. Each plot was irrigated in a similar way to provide similar water quantities and to maintain the soil moisture at about 65–70%.



(**a**) (**b**)

Figure 2. (**a**) Experimental layout diagram, (**b**) Bed design in each plot.

The experimental area was protected from ambient rainfall. Based on rainwater pH monitoring data in the study area for the period 2000–2017 [48], simulated rain was used at different pH values. The soybean plants were exposed three times a week to simulated acid rain (SAR) at pH 3.0 (T1), 3.5 (T2), 4.0 (T3), 4.5 (T4), 5.0 (T5), 5.5 (T6), and pH 6.0 (control (C)). The frequencies and rainfall were similar for each treatment. The frequency and amount of rain used in the study were obtained from the experimental month's average values over 17 monitoring years (2000–2016). Based on the monitoring of acid deposition from the Acid Deposition Monitoring Network in East Asia (EANET), this experiment used a 33% frequency of acid rain (pH < 5.6) during the summer–autumn season, and the total amount of acid rain was 190 mm. The water used in this experiment was collected in rainwater reservoirs in the study area, on which ion analysis was performed (once every 2 weeks) as shown in Table 1. The water samples were analyzed at the Laboratory of Environment Analysis of Faculty of Environmental Sciences of VNU University of Sciences, Vietnam National University, Hanoi. The water for the simulated rain entailed sufficient 1 M H_2SO_4 and 1 M HNO_3 (according to the ratio 2:1; V:V) to decrease the pH to experimental levels. Supplemental irrigation with water from a well was provided to maintain the necessary moisture in each plot. The simulated rain was applied using a stainless steel nozzle mounted at 1.0 m above ground in the center of the plot at an average rate of 6.7 mm per hour, 1.5 h per day, 3 days per week, for a total of 30 mm/week.

Table 1. Rainwater composition during the experimental months at Hoa Binh [47].

Component	Concentration (mg/L)
NO_3^-	4.56–4.61
Cl^-	0.25–0.36
SO_4^{2-}	3.56–3.66
NH_4^+	0.46–0.62
Na^+	0.61–0.66
K^+	0.50–0.56
Ca^{2+}	2.80–2.93
Mg^{2+}	0.42–0.58

2.3.3. Growth Measurement

Four periods of growth were observed: growth–blossoming, blossoming–finish blossoming, finish blossoming–firm fruit, and firm fruit–ripen fruit. The growth–blossoming period is calculated from the moment 50% of the plants sprout until more than 50% of the plants blossom in the plot. This period lasts for the first 46–47 days. The blossoming–finish blossoming stage is calculated from the blossom time until over 50% of the last flowering plants wither in the experimental plots (about 7–9 days later). The finish blossoming–firm fruit stage takes place during the next 17–18 days and starts from the moment more than 50% of the plants with one fruit reach their maximum size; only the fruits on the main stem of the soybean plants were counted. The firm fruit–ripen fruit period takes place 30 days before the soybeans are harvested. This stage finishes when about 95% of the fruits in each plot have a dry shell.

This study observed and assessed the effects of acid rain on soybean growth using growth (including germination rate, stem length, the number of basic branches, leaf area index, and yield components) and the physiological parameters (including chlorophyll content) [49,50]. These parameters are measured as follows:

(1) The germination rate (%) is determined by the ratio between the number of germinated seeds and the total number of seeds sowed, with 350 seeds/plot used for our study.
(2) Stem length (cm) is measured using a tape rule from the ground to the top of the tallest leaf.
(3) The number of basic branches (branches/tree): the number of branches that grow from the main trunk. This parameter is measured during the branching stage and the blossoming–finish blossoming stage.
(4) Chlorophyll content is determined indirectly using the SPAD index (an index positively correlated with chlorophyll content in leaves). For each treatment, three plants were selected, and on each plant, fully opened leaves were chosen to measure chlorophyll. This study uses CCM-200 plus (developed by Opti-Sciences, Inc., Hudson, NH, USA) to measure this index in the four mentioned growth stages.
(5) Leaf area index (m^2 leaf/m^2 land) is the leaf area of a plant (m^2/plant) × density (plant/m^2). The leaf area is measured directly by the Cl-202 Portable Laser Leaf Area Meter (developed by CID Bio-Science, Inc., Camas, WA, USA)
(6) For yield, six yield components were determined in this experiment: the number of fruits per plant, firm fruits percentage (%), rate of fruits with 1 seed/plant (%), rate of fruits with three seeds/plant (%), dry weight of 1000 seeds (gram), and seed dry weight/plot (gram).

The actual yield (gram/m^2) is determined by the following formula:

$$\text{Actual yield} = \frac{\text{Seed dry weight/plot}}{\text{plot area}}\ (\text{gram/m}^2)$$

Tukey's HSD test (developed by John Wilder Tukey, USA) was used to determine the difference between at least one group from the other groups. Based on the difference between means, Tukey's HSD test is frequently used for specific comparisons and frequently used for pairwise comparisons [51]. In this study, the statistical meaning of the results was determined by using variance analysis ANOVA and Tukey's HSD test to determine if the relationship between all pairwise sets of data are statistically significant at $p < 0.05$ using IBM SPSS Statistics for Windows, version 20.0 of IBM Corp., New York, NY, USA.

3. Results

3.1. The Effect of Simulated Acid Rain on the Growth of Soybeans

3.1.1. The Effect on the Germination Rate

Table 2 shows the effects of simulated acid rain on the germination rate. A significant fall in the germination rate is observed with increasing levels of acidity. The germination rate of plots treated with SAR is below that of the control. The germination rates of T1 (78%) and T2 (83%) are significantly lower than that of the other treatments.

Table 2. Germination rate of soybean after exposure to a range of concentrations of simulated acid rain.

Treatment	T1	T2	T3	T4	T5	T6	Control
Average germination rate (%)	$78^{a} \pm 1.63$	$83^{b} \pm 0.82$	$86^{c} \pm 1.63$	$88^{c} \pm 0.82$	$91^{d} \pm 0.82$	$94^{d} \pm 1.41$	$97^{e} \pm 0.82$

Note: Each value is a mean ± standard error of three replicates. Means followed by the same letter are not significantly different at the 95% confidence level (i.e., $p > 0.05$) from each other using Tukey's HSD test.

3.1.2. The Effect on the Stem Length

Stem lengths for different pH treatments were monitored at four stages: growth–blossoming, blossoming–finish blossoming, finish blossoming–firm fruit, and firm fruit–ripen fruit. Table 3 shows a significant decline in stem length with decreasing pH. The stem lengths of the T1, T2, and T3 treatments are significantly lower than that of other treatments.

Table 3. Stem length of soybean after exposure to different concentrations of simulated acid rain.

Treatment	Average Stem Length (cm)			
	Growth–Blossoming	Blossoming–Finish Blossoming	Finish Blossoming–Firm Fruit	Firm Fruit–Ripen Fruit
T1	$27.48^{a} \pm 0.30$	$35.35^{a} \pm 0.23$	$45.53^{a} \pm 0.24$	$48.02^{a} \pm 0.18$
T2	$29.75^{b} \pm 0.52$	$35.95^{a} \pm 0.11$	$47.68^{b} \pm 0.16$	$49.59^{b} \pm 0.51$
T3	$31.68^{c} \pm 0.27$	$36.61^{b} \pm 0.08$	$47.81^{b} \pm 0.25$	$50.25^{b} \pm 0.07$
T4	$32.97^{d} \pm 0.48$	$38.64^{c} \pm 0.39$	$49.19^{c} \pm 0.19$	$51.53^{c} \pm 0.08$
T5	$33.39^{d,e} \pm 0.30$	$40.89^{d} \pm 0.31$	$51.25^{d} \pm 0.17$	$53.71^{d} \pm 0.09$
T6	$33.82^{e,f} \pm 0.33$	$43.42^{e} \pm 0.23$	$53.06^{e} \pm 0.40$	$55.53^{e} \pm 0.18$
Control	$34.80^{f} \pm 0.32$	$46.75^{f} \pm 0.12$	$54.49^{f} \pm 0.17$	$56.70^{f} \pm 0.16$

Note: Each value is a mean ± standard error of three replicates. Means followed by the same letter are not significantly different at the 95% confidence level (i.e., $p > 0.05$) from each other using Tukey's HSD test.

3.1.3. The Effect on the Number of Basic Branches

The basic branches of representative soybeans in seven treatments were analyzed from the branching and flowering–finish flowering stages to evaluate the effects of simulated acid rain on the growth of the soybean. Although the number of basic branches experienced a slight increase between the T1 and T6 treatments, there was no significant difference in the number of basic branches between the treatments (Table 4).

Table 4. Number of basic branches of soybean after exposure to different concentrations of simulated acid rain.

Treatment	No. of Basic Branches/Plant	
	Branching Stage	Blossoming–Finish Blossoming
T1	0.57 ^{a} ± 0.10	1.39 ^{a} ± 0.13
T2	0.65 ^{a} ± 0.06	1.63 ^{a} ± 0.09
T3	0.88 ^{a,b} ± 0.15	1.68 ^{a,b} ± 0.27
T4	1.10 ^{b,c} ± 0.09	2.01 ^{b} ± 0.03
T5	1.25 ^{b,c} ± 0.27	2.09 ^{b} ± 0.10
T6	1.43 ^{c,d} ± 0.29	2.33 ^{b,c} ± 0.19
Control	1.76 ^{d} ± 0.18	2.52 ^{c} ± 0.15

Note: Each value is a mean ± standard error of three replicates. Means followed by the same letter are not significantly different at the 95% confidence level (i.e., $p > 0.05$) from each other using Tukey's HSD test.

3.1.4. The Leaf Area Index (LAI)

This study monitors and evaluates the correlation between pH and the leaf area index at four stages: growth–blossoming, blossoming–finish blossoming, finish blossoming–firm fruit, and firm fruit–ripen fruit. Table 5 shows the effect of SAR on the leaf area index of soybean. In all four stages of plant growth when the pH decreased, the leaf area index decreased.

Table 5. Leaf area index of soybean after exposure to different concentrations of simulated acid rain.

Treatments	Leaf Area Index (m^2 Leaf/m^2 Land)			
	Growth–Blossoming	Blossoming–Finish Blossoming	Finish Blossoming–Firm Fruit	Firm Fruit–Ripen Fruit
T1	0.19 ^{a} ± 0.03	0.67 ^{a} ± 0.05	0.97 ^{a} ± 0.09	1.12 ^{a} ± 0.09
T2	0.29 ^{a,b} ± 0.03	0.84 ^{b} ± 0.07	1.17 ^{b} ± 0.10	1.30 ^{b} ± 0.03
T3	0.36 ^{b,c} ± 0.06	0.94 ^{b,c} ± 0.09	1.31 ^{b} ± 0.04	1.54 ^{c} ± 0.03
T4	0.43 ^{c} ± 0.10	1.04 ^{c} ± 0.07	1.59 ^{c} ± 0.05	1.72 ^{d} ± 0.03
T5	0.58 ^{d} ± 0.05	1.27 ^{d} ± 0.04	1.60 ^{c} ± 0.05	1.91 ^{e} ± 0.09
T6	0.66 ^{d,e} ± 0.06	1.30 ^{d} ± 0.05	1.71 ^{d} ± 0.05	2.01 ^{e,f} ± 0.10
Control	0.79 ^{e} ± 0.06	1.49 ^{e} ± 0.08	1.94 ^{d} ± 0.13	2.15 ^{f} ± 0.13

Note: Each value is a mean ± standard error of three replicates. Means followed by the same letter are not significantly different at the 95% confidence level (i.e., $p > 0.05$) from each other using Tukey's HSD test.

3.1.5. Leaf Chlorophyll Content

The chlorophyll contents shown by SPAD values are presented in Table 6. At each stage from treatment T1 to the control, the chlorophyll index increased. Significant differences in SPAD values between the pH values were observed during the soybean growth stages except for the growth–blossoming period.

Table 6. SPAD parameters for soybean after exposure to different concentrations of simulated acid rain.

Treatment	SPAD Value			
	Growth–Blossoming	Blossoming–Finish Blossoming	Finish Blossoming–Firm Fruit	Firm Fruit–Ripen Fruit
T1	13.35 ^{a} ± 0.61	23.08 ^{a} ± 0.34	21.01 ^{a} ± 0.26	20.01 ^{a} ± 0.25
T2	15.37 ^{b} ± 0.53	26.12 ^{b} ± 0.24	22.95 ^{b} ± 0.32	21.32 ^{b} ± 0.25
T3	16.61 ^{b} ± 0.98	28.03 ^{c} ± 0.30	25.20 ^{c} ± 0.16	22.15 ^{c} ± 0.35
T4	17.98 ^{c} ± 0.31	31.14 ^{d} ± 0.46	28.56 ^{d} ± 0.22	25.26 ^{d} ± 0.25
T5	19.37 ^{d} ± 0.56	34.60 ^{e} ± 0.39	30.15 ^{e} ± 0.29	26.85 ^{e} ± 0.54
T6	20.45 ^{d} ± 0.55	37.06 ^{f} ± 0.23	33.29 ^{f} ± 0.30	30.12 ^{f} ± 0.20
Control	22.46 ^{e} ± 0.24	39.28 ^{g} ± 0.61	35.82 ^{g} ± 0.17	32.06 ^{g} ± 0.21

Note: Each value is a mean ± standard error of three replicates. Means followed by the same letter are not significantly different at the 95% confidence level (i.e., $p > 0.05$) from each other using Tukey's HSD test.

3.2. The Effect on Yield and Yield Components of Soybeans

The results of the yield components of soybeans in this experiment are presented in Table 7. The values of the six yield components tend to increase when the pH of the rainwater increases. There is no big difference between iterations. The number of fruits varies from 8 to 13 fruits per plant. The number of firm fruits on each tree is quite high, particularly for the pH 3.0 and 3.5 treatments with rates below 50% (38.93% and 41.15%, respectively). The number of fruits containing one seed accounts for the majority of the total number of firm fruits collected per tree, while the rate of fruits with three seeds only accounts for a small part (less than 25%). The mass of 1000 random seeds in the control treatment is the highest (162.15 g), while treatment T1 provides the opposite result (71.79 g). The volume of dry seed/experimental plot produced more yield than 1000 g/plot, in which the seed dry weight of the control plot was the highest (1472.67 g).

Table 7. Yield components of soybean after exposure to different concentrations of simulated acid rain.

Treatment	No. of Fruits Per Plant	Firm Fruits Percentage (%)	Rate of Fruits with 1 Seed/Plant (%)	Rate of Fruits with 3 Seeds/Plant (%)	Dry wt of 1000 Seeds (Gram)	Seed Dry wt/plot (Gram)
T1	$8^{a} \pm 0.82$	$38.93^{a} \pm 1.17$	$51.48^{d,e} \pm 0.53$	$3.38^{a} \pm 0.29$	$71.79^{a} \pm 0.24$	$1111.67^{a} \pm 20.17$
T2	$10^{a,b} \pm 1.08$	$41.15^{b} \pm 0.68$	$50.06^{d} \pm 0.61$	$4.93^{b} \pm 0.46$	$77.77^{b} \pm 0.39$	$1205.67^{b} \pm 19.07$
T3	$10^{a,b} \pm 0.24$	$55.49^{c} \pm 0.74$	$43.11^{c} \pm 0.47$	$10.69^{c} \pm 0.55$	$91.75^{c} \pm 0.32$	$1256.57^{c} \pm 15.28$
T4	$11^{b,c} \pm 0.65$	$56.97^{c} \pm 0.38$	$38.09^{a} \pm 0.38$	$10.96^{c} \pm 0.41$	$100.12^{d} \pm 0.10$	$1322.00^{d} \pm 10.71$
T5	$11^{b,c} \pm 0.71$	$66.76^{d} \pm 0.32$	$52.99^{f} \pm 0.54$	$10.33^{c} \pm 0.45$	$130.57^{e} \pm 0.27$	$1371.33^{e} \pm 11.61$
T6	$12^{b,c} \pm 0.22$	$70.47^{e} \pm 0.46$	$52.06^{e,f} \pm 0.67$	$12.37^{d} \pm 0.32$	$145.68^{f} \pm 0.15$	$1424.33^{f} \pm 15.97$
Control	$13^{c} \pm 0.92$	$72.01^{e} \pm 0.74$	$41.28^{b} \pm 0.46$	$24.83^{e} \pm 0.44$	$162.15^{g} \pm 0.06$	$1472.67^{g} \pm 18.93$

Note: Each value is a mean ± standard error of three replicates. Means followed by the same letter are not significantly different at the 95% confidence level (i.e., $p > 0.05$) from each other using Tukey's HSD test.

Table 8 presents the results of the soybean yield of the seven treatments in this study. The trend in the changes in soybean yield from pH 3.0 to the control is similar to the results of the growth index: the soybean yield tends to decrease when the pH value decreases.

Table 8. Yield of soybean after exposure to different concentrations of simulated acid rain.

Treatment	Actual Yield (Gram/m^2)
T1	$123.67^{a} \pm 4.11$
T2	$134.33^{b} \pm 5.44$
T3	$140.67^{b,c} \pm 2.87$
T4	$147.67^{c,d} \pm 4.50$
T5	$152.67^{d,e} \pm 4.92$
T6	$158.33^{e,f} \pm 4.64$
Control	$164.33^{f} \pm 3.86$

Note: Each value is a mean ± standard error of three replicates. Means followed by the same letter are not significantly different at the 95% confidence level (i.e., $p > 0.05$) from each other using Tukey's HSD test.

4. Conclusions and Discussion

Soybean is one of the most commonly consumed crops in Vietnam, in general, and in the mountains of Northern Vietnam, in particular, and contribute significantly to the national and regional agriculture and economy. The wide use of soybean ranges from food for humans and animals to industrial applications [52]. Soybean is traditionally known for its health benefit, which is related to its rich protein content and a wide range of phytochemicals such as isoflavones and phenolic compounds [53]. However, the growth and yield of soybeans in the study area are affected by environmental pollution such as acid rain.

The acidity of simulated acid rain affects crops positively or negatively. The negative effects of acid rain largely depend on the pH of the water. Rain with a pH below 3.0 may cause significant damage to plants [54]. This study shows the detrimental effects of acid rain on the growth of soybean (*Glycine max* (L.) Merr.). Soybeans were exposed to a range of pH values: 6.0 (control), 5.5, 5.0, 4.5, 4.0, 3.5, and 3.0. The germination process depends not only on the internal factors within the seed but also on the external conditions, including water, temperature, oxygen, and light or shadow [55]. Germination starts with the uptake of water, which is pH-dependent. The germination rate is dramatically reduced in SAR seeds compared with the control treatment. This result is in agreement with the studies conducted by Mohamad et al. (2008), Huang et al. (2005), and Wertheim and Cracker (1987) [29,56,57]. The stems of soybean plants play a major role in transferring water and nutrients from the roots to the leaves. In woody and herbaceous plants, the stem also contributes to sustaining the plant. Therefore, healthy stems provide a sound basis for the development of the plant and facilitate the photosynthesis process. Consequently, the stem length can be used to evaluate the growth and development of the crop. With increasing pH, the plants grow higher, and vice versa. However, pH affects not only the height of the plants but also the leaves and the stem. The plants treated at pH = 3.0, pH = 3.5, and pH = 4.0 are shorter and show more damaged leaves than the plants treated at higher pH levels. Odiyi et al. (2014) made a similar finding when studying the effects of simulated acid rain on *cowpea* growth. They found that stem height was significantly reduced when the pH of the simulated acid rain decreased [58]. The number of basic branches also decreased slowly when the pH values fell between pH 5.5 and pH 3.0. This reduction confirms the observation of Rani (2017) [59]. The effects of acid rain on the leaf area are usually evaluated via the leaf area index (LAI). In this study, the highest leaf area index in all four monitored stages was the control treatment and the lower the pH, the lower the index. Previous studies made similar conclusions on the effect of pH of simulated rainwater on sunflowers and rapeseed leaves [60,61].

The chlorophyll content provides information on the physiological state of plants [62,63]. In this study, the chlorophyll content was assessed using the SPAD index (a correlated index of chlorophyll content in leaves). A significant decrease in the chlorophyll index was found with decreasing pH of the acid rainwater. Our study results are similar to previous studies on the effects of acid rain on chlorophyll in other crops [64–66]. The reduction in chlorophyll content is explained by foliar leaching of nutrient elements, especially the removal of Mg^{2+} in the chlorophyll molecules by H^{+} [66,67]. The decrease in photosynthesis is caused by the reduction in leaf size or chlorophyll content [56]. A similar observation is the significant decrease in the chlorophyll content in plants under environmental stress such as salinity, antibiotics, or water stress. In detail, the chlorophyll content in *A. thaliana* declined when the plants were exposed to sulfonamides, which affected photosynthesis and inhibited chlorophyll synthesis [68]. A study on the effect of tetracycline in *Iberis sempervirens* L. grown in soil and in agar showed that the antibiotic induced inhibition of the photosynthetic activity [69]. Chlorophyll a and b were also reduced with increasing NaCl levels from 0 to 6 ds/m in a previous study by Mostafa Heidari in 2012 [70]. Drought stress is the main limitation to the net photosynthetic rate and photosynthetic pigment content in the lily (*Lilium*) [71].

The leaves were in direct contact with simulated acid rainwater. Therefore, signs of acid rain influence on soybean plants were clearly shown on the leaf surface [72]. Leaf changes were observed during simulated acid rain treatment, and pathological phenomena appeared on the leaves. At the more intense T1 (pH 3.0) and T2 (pH 3.5) treatments, black spots as well as discolored leaves and curled leaf edges appeared on the soybean leaf surface and some of the leaves were punctured with small holes. In particular, at T1 (pH 3.0), the leaves were necrotic. Visual observation of the color of the leaves showed that, the lower the pH, the more the green color of the leaves were faded. Under simulated acid rain, the growth of the leaves was affected by evapotranspiration and essential nutrient absorption [73], leaf characteristics, moisture in leaves, and environmental factors [74]. The humidity of large soybean leaves may be a factor in increased crop susceptibility for acid rain [75,76]. Signs of leaf lesions during the spraying of simulated acid rain on soybean plants were also observed. Simulated acid rain at pH 2.8 or pH 2.4 causes some white or tanned wounds on leaves [77]. Necrotic patches of different sizes were noted on primarily young leaves of soybean plants after three weeks of acid rain treatment at pH below 3.0 [13]. Leaf lesions were also recorded in 20 soybean cultivars when they were exposed to simulated acid rain at pH 5.6 and 3.0. This study indicates that leaf damage is greater at pH 3.0 [78]. Plant pigment changes were observed in soybean leaves after 20 days of exposure to simulated acid rain at pH 3.5 (1% H_2SO_4 and 1% HNO_3) [79].

The effect of acid rain was evidenced on the yield and yield characteristics of soybean plants. The yield results indicate that acid rain adversely affects soybean yields: actual yield and yield components decreased as the pH of the rainwater decreased. This result is similar to the literature results on the response of soybean yield with simulated acid rain [80,81]. However, the acidity of acid rain affecting soybeans differs among studies. This difference can be explained because soybean cultivars have different levels of sensitivity, plant growth characteristics, and environmental impact. In fact, soybean yield in Hoa Binh in recent years tended to decrease. In Yen Thuy, the soybean yields over the years were 2.0 ton/ha (2011), 1.6 ton/ha (2013), 1.35 ton/ha (2014), 1.74 ton/ha (2015), 1.73 ton/ha (2016) [43]. In Lac Thuy, these yields in turn are 1.95 ton/ha (2011), 1.35 ton/ha (2014), and 1.4 ton/ha (2016) [82]. The locals have since converted their soybean plots to new, more profitable plant varieties (such as green skin pomelo, Dien grapefruit, tomato, and peas); consequently, the soybean area has declined. In addition, the effects of climate, the acidity of the rainwater, and the farming regime are important causes affecting yield. The negative effects of simulated acid rain on yield are also observed for other crops [83–85].

Overall, acid rain affects the growth and yield of soybean plants. The germination rate, the stem length, the number of basic branches, the leaf area index (LAI), and the chlorophyll content all decrease when the pH of acid rain decreases. Similarly, for the yield index and yield components of soybean, the lower the pH of the rainwater, the more the soybean yield declines with the quantity and quality of the yield components. The effects of acid rain on the growth and development of crops have been documented in studies on corn [24,86,87], soybean [64,77,88], tomato [83,84], and cassava [85]. The damage by acid rain includes chlorosis, necrosis, stunting, and early senescence [27,84]. More crops should be studied for their sensitivity to acid rain in relation to the increasing industrialization, urbanization, and intensification of agricultural activities in Vietnam. Furthermore, the growth of soybean in particular and of crops as a whole are also affected by other factors (e.g., salinity, contaminants, drought, etc.). Several evidences are reported in the literature. In summary, under exposure to antibiotics, root length and aboveground plant biomass were significantly inhibited by sulfonamides (SAs) whereas lateral roots exposed to sulfametoxydiazine (SMD) grew vigorously [68]. Fresh weight loss of two basil genotypes indicated that salinity causes significant decreases in the growth of these plants [70]. Moreover, decreasing the potential photosynthetic capacity due to water stress is one of the reasons reducing the plant quality of oriental *Lilies* (e.g., low plant height, flower length, flower diameter, and leaf area) [71]. Therefore, the simultaneous effects of acid rain and other factors as well as the changing secondary metabolites, which are produced by plants under environmental stress such as total phenol or proline, should be further studied.

This study calls for less acid rain, counteracting the adverse effects of burning fossil fuels, transport, and agricultural activities, which emit gases that result in acidic deposition. The high pressure from problems resulting from acid rain in Hoa Binh during 2000–2017 prompted integration of adaptations to the acid rain in agricultural policies and strategies at all levels: agricultural crop insurance, more training and information on acid rain, land management efficiency, and energy-saving policies in all fields should be deployed synchronously. Acid rain not only directly affects plants where rainwater is deposited on the leaves of plants but also indirectly affects the soil of the agricultural land [7,89]. Therefore, improving the management efficiency of arable land is important. The agricultural land area as of December 2018 accounts for 19.3% of arable land (equivalent to 88,400 hectares).

The managers of Hoa Binh province should develop agricultural land management plans and strategies addressing also the effects of acid rain. For agricultural land affected by acid rain, soil improvement measures are required. One of the cheapest solutions that are commonly applied by farmers is to apply lime on sour fields. Additionally, other approaches to reduce soil acidification should be promoted such as (i) sulfur-poor fertilizers such as nitrogen sulfate; (ii) fertilizing phosphorus (both providing nutrients for plants and effectively reducing the toxicity of alum) or organic fertilizer (loosening the soil porosity while reducing toxicity and reducing alum toxicity when combined with certain toxins present in the soil); (iii) in heavily acidified areas, the replacement of crops, i.e., selecting more acid-tolerant plants or changing land uses; (iv) changing the season and intensity of soybean cultivation; and (v) rehabilitating and building appropriate irrigation systems. In fact, changing cropping systems offer also a solution to achieve more efficiency in case the old plants are no longer suitable for the climate, soil, and water in the study area. In addition, the change in farming techniques such as intercropping, and rotation instead of monoculture should be considered. The intercropping method also results in high economic efficiency and saves production costs (i.e., soybeans intercropped with corn, and soybeans planted with sweet potatoes or other crops).

Implementing the above-listed improvements can increase the production and yield of soybeans, and economic development in Hoa Binh and in mountainous provinces in general. These adaptations can be applied to mountainous areas with similar conditions. However, depending on the environmental conditions and soybean varieties of each area, the solutions can be elaborated upon in more detail. In fact, adaptation to the effects of acid rain is still not receiving sufficient attention from managers, policymakers, and farmers. The effects of acid rain on crops are not clearly distinguished from those of climate change. Consequently, it is difficult to distinguish which impacts are from acid rain and which are due to climate change. Therefore, these adaptations should be combined with solutions to cope with climate change to achieve the best efficiency.

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