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Effects of intermittent acid rain on proline and antioxidant content on medicinal plant "*Pereskia bleo*"

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Abstract. Global warming due to CO_2 and other greenhouse gas emissions from human activities have led to climate change and environmental degradation. The acid rain, with the pH of rainwater below 5.6, is a serious environmental problem. Arising from air pollution and potentially harmful to health, it can damage old buildings and distract the growth and physiological metabolism of sensitive plants. How does the influence of climate change on medicinal plants such as *Pereskia bleo*? The leaf of *Pereskia bleo* (Kunth) DC. contains high antioxidants with benefits for anti-cancer, anti-tumor, anti-rheumatic, and anti-inflammatory. This research aims to investigate the influence of acid rain on the proline level and antioxidant content of *Pereskia bleo*. Having been carried out from June to August in Jogjakarta, this study was conducted through the use of artificial acid rain with pH 5.8, 4.9, 3.7 and 2.9, by adding sulfate acid (H_2SO_4) to rainwater. The interval of intermittent watering acid rain to the plants is once a day, twice a day, and once in three days with three replications for six weeks. The results showed that Acid rain with a pH less than 4.9 and the intermittent interval of acid rain twice a day and once in three days significantly suppresses growth and chlorophyll content. In contrast, it increases the proline and antioxidant levels as a tolerant action of the plant.

1. Introduction

Climate change is largely the result of human activity. Climate change is largely the result of human activity. Growth Industrialization has increased pollutants into the environment. Increased levels of global warming due to CO_2 and other greenhouse gas emissions from human activities have led to climate change and environmental degradation. Acid rain is the most serious environmental problem arising from air pollution. Natural rainwater is slightly acidic, with a pH value ranging from 5.6 to 7.0. As a result of the presence of CO_2 in the atmosphere, it dissolves in the rain when it falls. In addition, the advent of industrialization and transportation also plays a role in raising the acidity of rain. This increase in pH is not only caused by CO_2 emissions but also SO_2 and NOx as pollutant gases. SO_2 and NOx that reacts with water molecules in the atmosphere produce acid as H_2SO_4 and HNO_3 that is quickly dissolved in clouds and water. This acid rain potentially damages old buildings, health, water and plant environments [1,2,3]. Acid rain has already occurred in several Indonesian cities [4]. The pH of rain during the period 2001-2013 ranged from 4.3 to 5.6 in Bandung, Serpong, Jakarta, Kototabang, and Maros [5].

Acid rain is a serious problem for environmental balance in the world that interferes with the life and development of plants, aquatic life, socio-economic development, and human health. Acid rain has a direct or indirect impact on plants, among others chlorosis on leaves, inhibition of photosynthesis and disruption of nutrient and mineral absorption [6,7,8]. There are several indirect effects of acid rain on soil fertility, such as the increase of soil acidity and the release of toxic minerals that would endanger the development of plant roots [2] and result in the disruption of plant growth and its development. Acid rain has a significant effect on wheat growth. The increasing rain acidity, with $pH \leq 4.5$, can decrease total free amino acids and protein content [9].

Plants that are exposed to water stress will reduce mass significantly [10]. Water is an essential production factor for plants not only the quantity but also the quality. It is also considered as the most limiting factor for growing most plants and disrupting plant metabolism. Water quality, among others, is determined by acidity (pH), salinity and mineral content. Acid rain, with a pH of less than 5.6 has the potential to suppress plant growth [11] and against its metabolism [12].

Pereskia bleo is a medicinal plant that contains high antioxidants. It belongs to primitive cactus (Cactaceae) plants, which are leafy [13]. This plant contains high antioxidants [14], which are useful as anti-cancer [15], antimicrobial [16] and anti-inflammatory [17]. Drought stress reduces the efficiency of photosynthesis and decreases root dry weight and potential matric on the root zone. However, since the leaf area and leaf number are not affected, it can be concluded that Pereskia is tolerant of drought stress [18].

Proline is an amino acid that can increase plant tolerance to abiotic stress by lowering osmotic potential and maintaining cell turgor [19,20]. High proline content may be a good source of components that will help increase the overall antioxidant capacity of an organism and protect it from lipid peroxidation [21]. Plants under pressure produce both enzymatic and nonenzymatic defense mechanisms in chloroplasts and mitochondria [22]. Hernandez *et al.* [23] stated that the antioxidant is a component tolerance mechanisms in peas. Although there has been a lot of research on acid rain effect on plant growth, research of acid rain to *Pereskia bleo* has not been conducted yet. Therefore, this research aims to study the impact of acid rain on proline and antioxidant dynamics of *Pereskia bleo*.

2. Materials and methods

The research had been carried out from June to August, in Sleman, Jogjakarta. The age of *Pereskia bleo* plant material derived from shoots cuttings was eight months. This research was conducted through the use of artificial acid rain. The preparation of acid rain using rainwater collected in April 2017 at Sleman Yogyakarta was 7,720 S, 110.36 E. Elevation 242 m. The pH of rain obtained was 5.8 (Control). After the addition of 2 ml of 10% H_2SO_4 in 600 ml, 850 ml, and 1050 ml, the pH obtained were 2.91 ± 0.12; 3.7 ± 0.17 and 4.9 ± 0.11, respectively. The interval of intermittent watering acid rain to the plants is 1x1 day, 1x2 days, and 1x3 days, with three replications for six weeks. The comparison of soil medium and organic base fertilizer is 2:1, with the weight of 5 kg per polybag. In this research, media is placed in water saturated condition before treatment. Watering on intermittent is done using mini sprayer by adjusting nozzles and passing to leaves until soil media is saturated within 5 minutes with a volume of 1000 ml per plant. Parameter variables were used to plant height, a number of leaves, root volume; chlorophyll a, b and total, proline, and antioxidants. Chlorophyll was then analyzed by a spectrophotometer method whereas proline was analyzed by a ninhydrin method [25]. Meanwhile, antioxidants were analyzed by the DPPH method. The experimental design for both experiments was factorial randomized complete block design. The data were subjected to analysis of variance, and treatment means were compared using the least significant difference test ($p = 0.05$).

2.1. Extraction and estimation of chlorophyll

One gram of finely cut leaf sample was weighed and grounded with acetone. It was then centrifuged. The supernatant is transferred. The absorbance of the solution was measured at 645 nm against the solvent (80% acetone) as blank. The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were then calculated using the following equation:

Chlorophyll a (mg/g) = 12.72(OD 663)-2.59(OD 645) (1)

Chlorophyll b (mg/g) = 22.9(OD 645)-(OD 663) (2)

Total Chlorophyll (mg/g) = 20.31(OD 645) + 8.05(OD 663) (3)

2.2. Proline analysis

Proline analysis was performed according to Bates, 1973 [24]. Diethyl ether extract was exempted from this test since proline is practically insoluble in diethyl ether. Briefly, 50 mg of extract were homogenized in 10 ml sulphosalicylic acid (3% w/v) and filtrated through filter paper. Two milliliters of the filtrate was mixed with 2 ml of acid ninhydrin solution (1.25 g ninhydrin + 30 ml glacial acetic acid + 20 ml 6 M H_3PO_4) and 2 ml of glacial acetic acid and kept at $\pm 100^0$ C for one hour. Then, the reaction was stopped by transferring the mixture to an ice bath. Four milliliters of toluene was added to the mixture and vortexed for 15–20 s. The toluene phase was aspirated, and the absorbance at 520 nm was measured using pure toluene as a reference. A calibration curve was prepared with pure proline. Results were expressed as µg proline/ gram extract.

2.3. DPPH radical scavenging assay

Radical scavenging activity of plant extracts against 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined spectrophotometrically [25]. The principle of the assay is based on the color change of the DPPH solution from purple to yellow as the radical is quenched by the antioxidant. Briefly, 1 ml of 0.1 mM DPPH in ethanol was mixed with 3 ml of extract solution with differing concentrations (5–250 lg/ml). Then, the mixture was vortexed. The samples were kept in the dark for 30 minutes at room temperature. Afterwards, the decrease in absorbance at 517 nm was measured. The absorbance of DPPH solution in the absence of plant extract was measured as the control. Ascorbic acid, BHT, and rutin were used as positive controls. DPPH radical scavenging activity was expressed using the formula: % DPPH radical scavenging activity = [(A0 _ A1)/A0] _ 100 where A0 was the absorbance of the control and A1 was the absorbance of the sample.

3. Result and discussion

3.1. Growth component

The influence of acid rain and intermittent on plant growth and development showed that leaf color change started at week 4, especially at the treatment of pH 3.7-2.9 with intermittent 1x3 days. Direct contact of low pH acid rain contains H_2SO_4 to the leaves, causing chlorosis spotting. The impact of treatment on intermittent 1x3 days also causes the plant to be not fresh. Analysis of growth component yielded at 6th week shows that there is an interaction between acid rain and intermittent that tends to suppress plant height at pH below 5.8 (control), (Figure 1). The number of leaves and root volume of the acid rain and intermittent treatment of each one is significant (Table 1 and Table 2). Acid rain has the potential to suppress plant height and damage the leaf surface. Acid rain also causes necrosis in G. Americana [14] and seriously damages the leaf surface [7], but this depends on the sensitivity of leaf surfaces of various plants [26]. The net photosynthetic rate of *P. massoniana* exposed to acid rain with pH 4 and 2.5 having decreases of 20% and 34% has shown that *P. massoniana* is susceptible to exposure to acid rain [27].

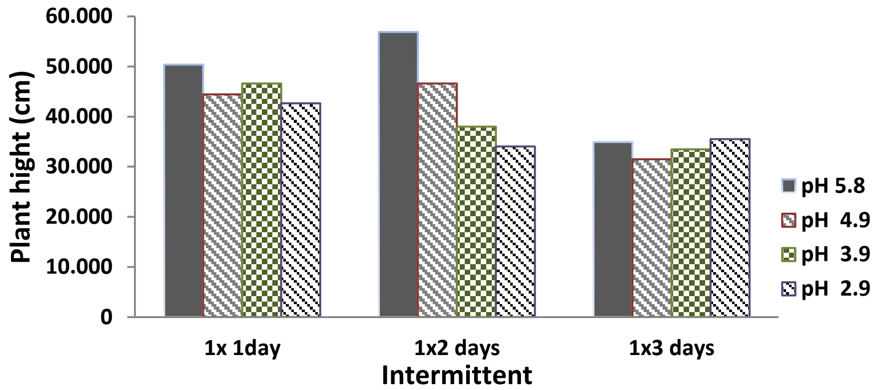

Figure 1. Interaction of acid rain and intermittent on plant hight.

Table 1. The effect of acid rain and intermittent on the volume of the root.

	pH acid rain				
Intermittent	5.8 (control)	4.9	3.7	2.9	Average
1x 1 day	29.33	26.33	26.00	19.00	25.17 b
1x 2 days	29.6	29.67	35.33	26.00	30.17 a
1 x 3 days	35.67	25.33	21.33	18.33	25.17 b
average	31.56 A	27.11 B	27.56 B	21.11 C	

Note: The same letter in the column or row is not significantly different at p = 0.05.

Table 2. The effect of acid rain and intermittent on the number of leaves.

	pH acid rain				
Intermittent	5.8 (control)	4.9	3.7	2.9	Average
1x 1 day	32.00	27.00	20.33	15.33	23.67 b
1x 2 days	33.00	30.33	26.00	22.00	27.83 a
1 x 3 days	33.00	28.67	19.00	17.00	24.42 b
average	32.67 A	28.67 AB	21.78 C	18.11 C	

Note: The same letter in the column or row is not significantly different at p = 0.05.

3.2. Chlorophyll content

There is a decrease of chlorophyll content (a, b and total) at pH≤ 4.9, except acid rain pH 2.5 with intermittent 1x3 days showed higher chlorophyll content than acid rain to pH 4.9. (Figure 2) The decrease in chlorophyll content is due to the leaf surface damage caused by chlorosis since its formation also requires water [28]. Acid rain with pH 4 and pH 3 decreases chlorophyll a and b significantly, with increasing acidity level (pH ≤ 5.0) [29]. Previous studies showed that acid rain decreases leaf chlorophyll content to 67% per pH of plant units in mainland China [30,31]. It suggests that simulated acid rain at pH 4.5, 3.5 and 2.5 significantly decreased the chlorophyll content than with control.

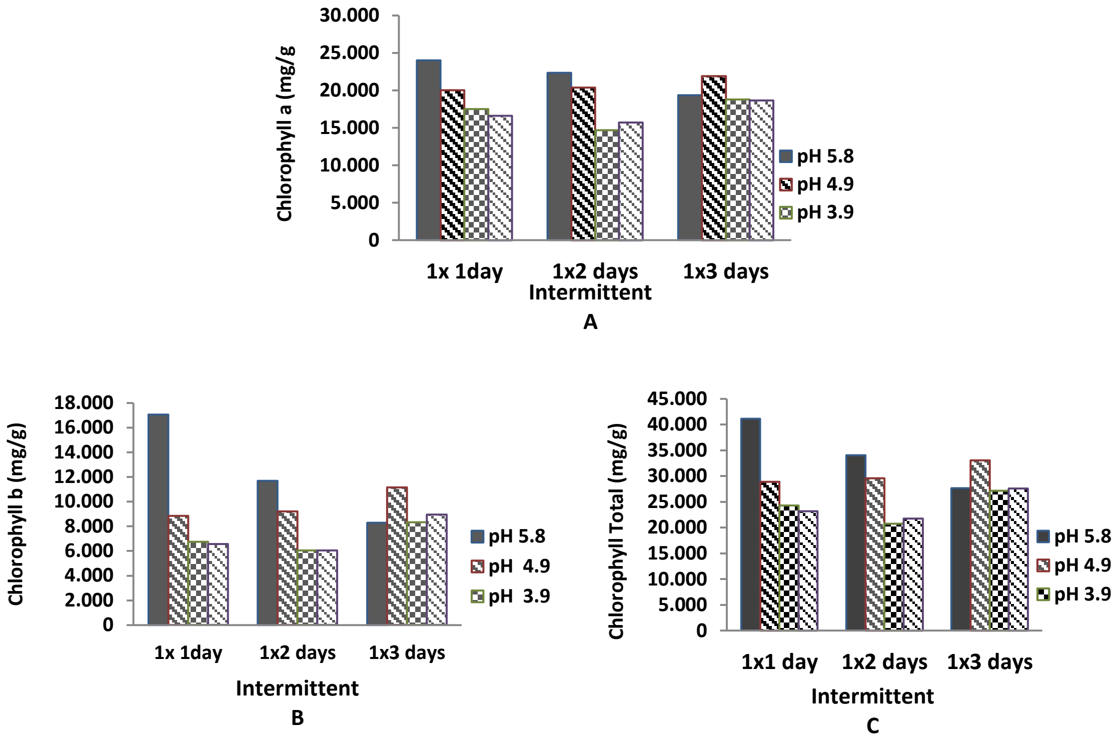

Figure 2. Interaction of acid rain and intermittent on Chlorophyll an (A), b(B) and total (C).

3.3. Proline and antioxidant content

In this research, acid rain and intermittent treatment had a significant effect on proline level and there was an interaction (Figure 3a). Acid rain with pH ≤ 4.9 -2.9 increases proline levels at all intermittent levels. Proline metabolism is coordinated in chloroplasts, cytoplasm, and mitochondria. Increased metabolism in stress-conditioned plants has been noted by some researchers [31]. It has been shown to be a molecular mechanism of defense against stress with accumulated proline [32,33,34]. A significant increase in proline content under stress is associated with increased rates of synthesis, reduced catabolism levels and loss of enzyme inhibitors on proline biosynthesis P5CS [35]. Abiotic and biotic stresses in plants are responded by increasing levels of proline. An increased level of proline due to stress is an indication of plant resistance to stress [36].

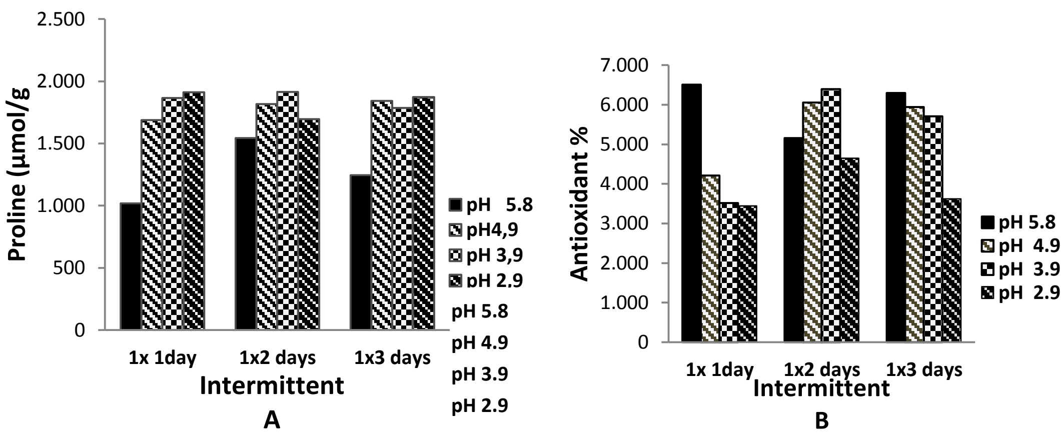

Figure 3. Interaction of acid rain and intermittent on proline content (A) and Antioxidant (B)

The increase of proline level is followed by an antioxidant percentage increase, at pH 3.7 - 2.9 with intermittent 1x2 days and 1x3 days (Figure 3b). Jahantigh *et al.* [37] stated that *Hyssopos officinalis* plant tolerance to salinity stress is characterized by the increase of proline and antioxidants. The antioxidant activity of catalase enzyme, peroxidase, and superoxide dismutase in drought-tolerant varieties increased significantly, observed during the different accumulation of proline on upland rice varieties. It can be concluded that antioxidant enzymes and accumulation of proline associated plant tolerance to drought stress [38].

4. Conclusion

Climate change has affected the activity of living organisms, such as the occurrence of acid rain on medicinal plants. The importance of medicinal plants is the content of secondary metabolites or their active ingredients. The content of active ingredients from medicinal plants is highly dependent on environmental factors. Environmental stresses can have a negative or positive impact on this active ingredient and depend on the activity of tolerance of each type of medicinal plant.

The results of this study indicate that acid rain with pH less than 4.9 and the intermittent interval of acid rain of one day, and once in three days significantly suppresses growth and chlorophyll content. In contrast, it increases the proline and antioxidant levels as the act of tolerance of the plant.

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