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Acidity Effect in Pollen Germination and Tube length of *Prunus amygdalus* Batsch and *Prunus domestica* L.

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Abstract

This present paper is aimed to examine effects of acidity on in-vitro polen germination and tube length of *Prunus amygdalus* Batsch and *Prunus domestica* L. plants. Pollens of *Prunus amygdalus* Batsch ve *Prunus domestica* L. blossoms are exposed the effect of acidic solutions in order to check pH 6.8 , 6.4, 6.0, 5.6, 5.2, 4.8, 4.4, 4.0, 3.6, 3.2, 2.8 For pollen germination, Brawbaker Kwack medium was facilitated. In accordance with the results gathered, the rate of germination and tube length of pollen for every plant has been affected as much as pH 6.4. The germination of pollen and the length of tube decreased at high rate with ascending acidity. The reduction of the rates were calculated % 93.43 for *Prunus amygladus Batsch* pollen germination and % 100 for tube length; %93.41 for *Prunus domestica L.* pollen germination and %99.63 for tube length at pH 2.8.

Keywords: Acidity, *prunus amygdalus* batsch, *prunus domestica* L., pollen germination, pollen tube growth

INTRODUCTION

The formation of acid precipitation is attributed to chemical reactions between nitrogen dioxide and sulfur dioxide gases released into the atmosphere as a result of industrialization and their absorption by water droplets and clouds in the atmosphere. These water droplets concentrated in the atmosphere fall on earth as rain and snow which lower the soil pH and disrupt the chemical balance of freshwater resources. Precipitation with a value of pH below 5.6 is considered acid rain because the pH value of rain formed in the typical diameter concentration in the air is approximately 5.6. However, the pH value of rain already ranges from 4.5 to 5.6 due to natural resources of acid, therefore, pH values below 5.0 can be considered a more accurate measure. Acid precipitation is the main cause of damage to forests in high altitudes and of pollution of rivers.

Acid precipitation affects both vegetative and generative parts of plants such as the male gametophyte (pollen grains) of sexually reproducing plants [1]. Studies show that pollen grains, which are bioindicators of environmental pollution, are very much affected by negative environmental factors [1,2]. Acid precipitation is one of the primary factors which adversely affect pollen germination and pollen tube growth [3].

A study on Camellia japonica (camellia) treated with hydrochloric acid (HCl), sulfuric acid (H2SO4) and nitric acid (HNO3) examines the effect of acidity of pollen germination and pollen tube growth. It is reported that the values of hydrochloric acid (HCl) above 0.6 mmol/l (pH<3.2) and sulfuric acid (H2SO4) 0.3 mmol/l (pH<3.2) prevent the pollen germination and pollen tube growth. It has also been shown that nitric acid (HNO3) affects the pollen grains more than hydrochloric acid and sulfuric acid [4].

Another study investigates the effect of acid rains on the pollen germination of Maize (Zea mays L.). The data indicate that acid rains create an unsuitable environment for the development of Maize pollen in surface germination. Maize can be grown efficiently in areas the acidity of which ranges from 5 to 8 pH [5]. It is reported that due to the physical and chemical modifications of the pollen surfaces, there is no increase in the germination when pollen surfaces which are contacted with pH 2.6 of acid concentration are then contacted with pH 5.6 of acid concentration [6].

The environmental factors not only change the morphology of pollen grains but also the ionic composition of the exine layer. However, the most important effect of the environmental factors is that they inhibit fruit formation [3].

The aims of this study are to determine the effect of low and high concentrations of acid on pollen grains of almond and plum plants, reveal the adverse effects of acid rains on the development of the pollen grains of these plants and assess the contribution of the results to the economic value of our country.

MATERIAL and METHOD

Research Area

The materials to be used in the study were selected from the villages of the surrounding districts of Elazığ.

Research Material

The research area and the plants to be used in the study were selected from Günev village of Sivrice district of Elazığ and from Obuz village of the Central district. Almond (*Prunus amygdalus* Batsch) plant pollen grains and plum (*Prunus domestica* L.) plant pollen grains used as material were brought from N38°24'-E38°95',1150m. and N38°50'-E39°38', 1260m. (Pioneer avic-F900BT model) GPS coordinates, respectively.

Medium

A Brewbaker Kwack medium was used for the germination of the pollen grains [7]. Due to the sucrose content, the prepared culture medium was sterilized in an autoclave (Eryigit, ERS2000D model) for 15 minutes at 121 °C under 1 atmosphere pressure in 50 ml Eppendorf tubes in order to withstand for long periods in the refrigerator at + 4 °C to prevent the growth of microorganisms.

Preparation of Pollen Preparates

Anthers of the flowers collected from the research area and labeled in polyethylene bags were brought to the laboratory and the anthers were opened with the help of needles under a stereo microscope. Using an Eppendorf Research model 10-100µl micropipette, 50µl BBC medium was dropped on the pollen grains removed from the anthers onto a slide.

For the two plants studied, the pollen grains removed from the anthers were sprinkled on three different slides. The slides were placed in glass rods on a previously dampened wet filter paper in petri containers. The petri containers were placed in an incubator (Heraus, B12 model) for the germination of the pollen grains. As germination concentration, pH 6.8 (control), pH 6.4, pH 6.0, pH 5.6, pH 5.2, pH 4.8, pH 4.4, pH 4.0, pH 3.6, pH 3.2, pH 2.8 acid values were set for the almond and plum plants. The oven was set to 24±1°C for the control group. For the fixation process, 10% 50µl ethanol solution was dropped on each medium on the slides in the peri containers removed from the incubator after 4 hours of germination [5]. The preparates closed with lamella for the measurements were investigated under a binocular microscope (Olympus, BX51 TF model). The measurements were made using an ocular micrometer at 10× magnification for the determination of the germination rates and at 10×, 20× and 40× magnifications for the determination of the tube lengths. The germination rates and the tube lengths were recorded according to the method described in Shivanna and Rangeswamy's study [7].

FINDINGS

Our study which determines the effects of acidity on pollen germination and pollen tube growth of the plum (*Prunus amygdalus* Batsch) and the almond (*Prunus domestica* L.) plants reveals that the germination rate and pollen tube length are least affected when pH value is 6.4 which is the closest value to that of the control group (pH 6.8). The results indicate that in the descending pH series (pH 6.0, pH 5.6, pH 5.2, pH 4.8, pH 4.4, pH 4.0, pH 3.6, pH 3.2, and pH 2.8) the germination rate and the tube growth decrease with a decline in the pH value. Figure 1, Figure 2, Figure 3 and Figure 4 show the pollen germination rates and the tube lengths for the almond and the plum plants.

Effect of Different Values of pH on Pollen Germination of Almond Plant

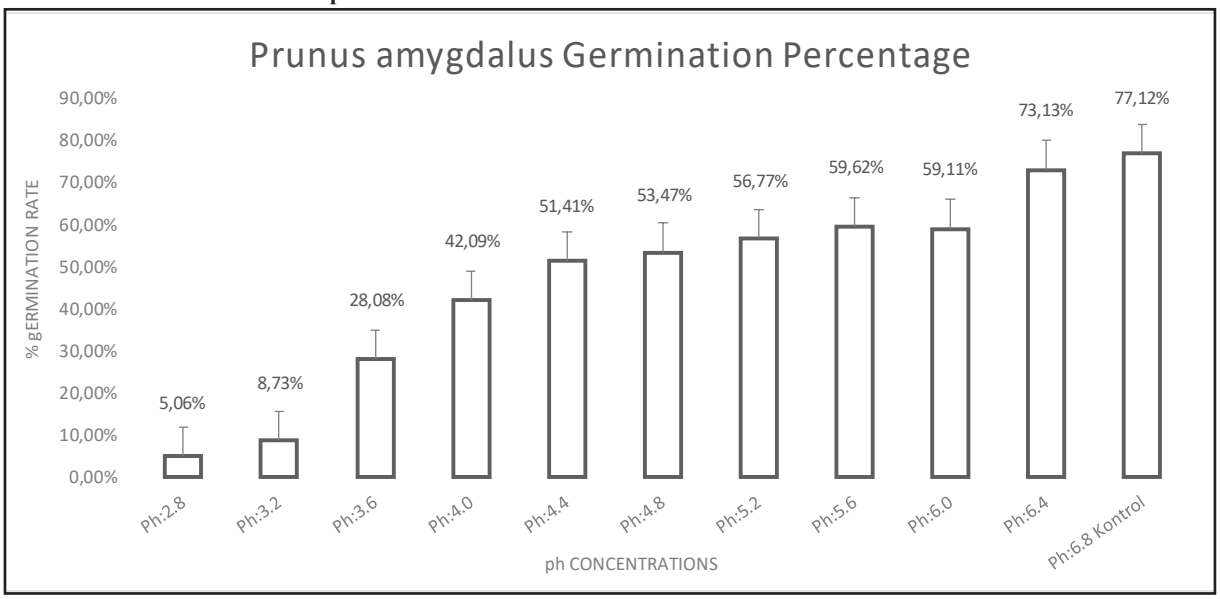

Figure 1. Effect of Different Values of pH on Pollen Germination of Almond Plant

Effect of Different Values of pH on Pollen Tube Length of Almond Plant

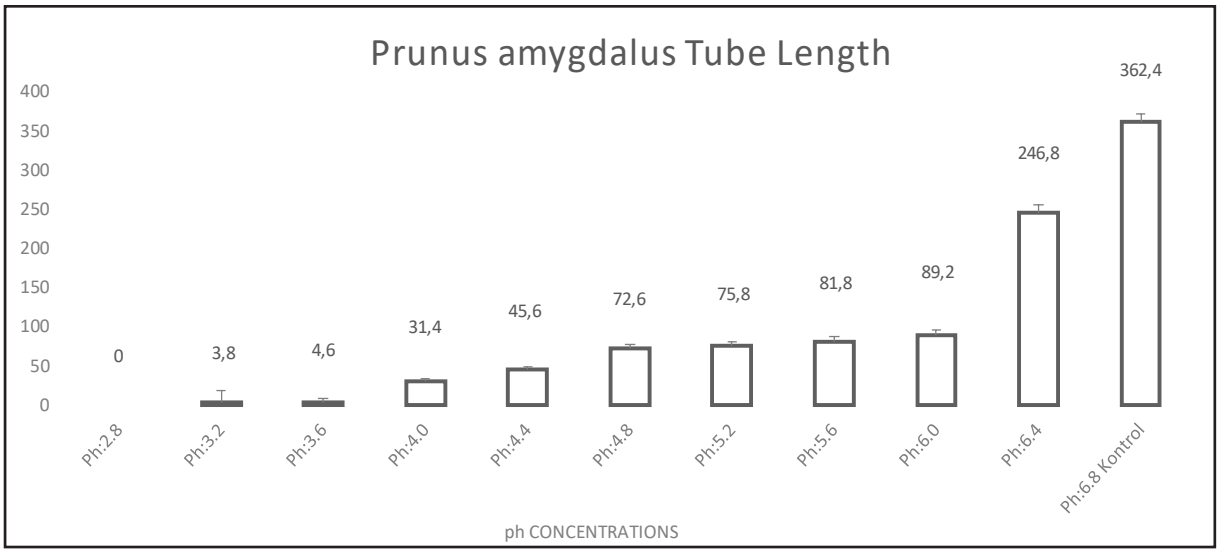

Figure 2. Effect of Different Values of pH on Pollen Tube Length of Almond Plant

Effect of Different Values of pH on Pollen Germination of Plum Plant

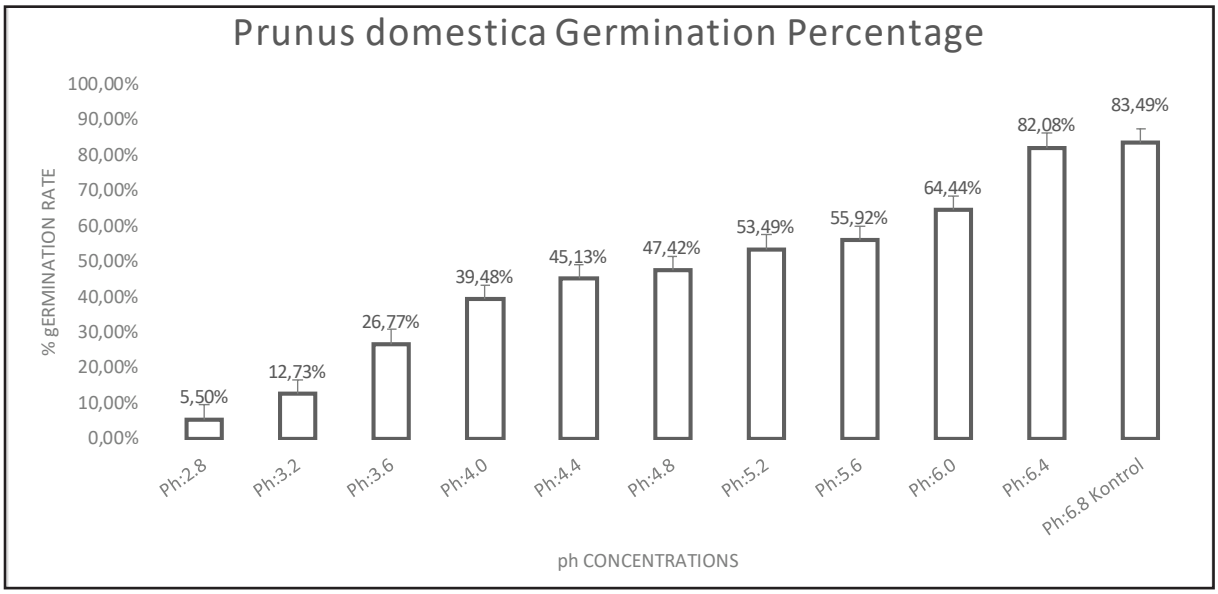

Figure 3. Effect of Different Values of pH on Pollen Germination of Plum Plant

Effect of Different Values of pH on Pollen Tube Length of Plum Plant

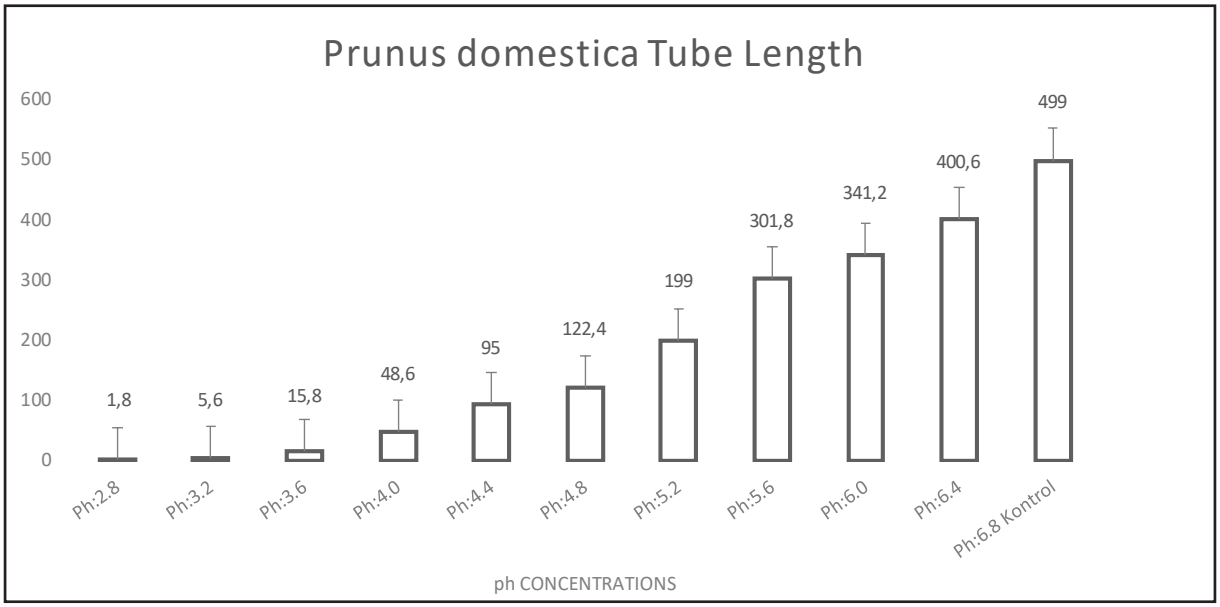

Figure 4. Effect of Different Values of pH on Pollen Tube Length of Plum Plant

RESULTS and DISCUSSION

The germination rate of the almond plant pollen grains in the control group (pH 6.8) is 77.12%. The germination rate at pH 6.4 is 73.13% which indicates that there is no serious decline in the germination rate. However, the germination rate has been found to be adversely affected by the decline in pH, therefore a steady decline has been observed in the germination rate. Although the decline seems stable, there is no statistically significant difference between pH 5.6 and pH 6.0 concentrations. Respectively, the germination rate declines at a stable rate in the concentrations with higher acidity. The germination rate is 56.77% at pH 5.22 while drops to 42.09% at pH 4.0 which shows the highest difference in reduction between two measurements. The germination rate is quite low (5.06) at the smallest pH value (2.8) prepared for the measurements. The comparison of the highest and the lowest acidity with the control groups indicates that there is a 93.43% and 5.17% decline at pH 2.8 and pH 6.4, respectively.

The examination of the tube length values of the almond plant indicates that it is 362.4 µm in the control group, drops to 246.8 µm at pH 6.4 and 89.2 µm at pH 6.0. These values were measured 3.8 µm at pH 3.2 while 0 µm at pH 2.8. The comparison of these values with those of the control group show that there is a 100 % decline in terms of tube length at pH 2.8 and 31.89% decline at pH 6.4.

The almond plant pollen grains which are adversely affected by the high concentration of acidity in terms of both tube length and germination rate show the highest performance at pH 6.4 which is the closest value to that of the control group.

The germination rate of the plum pollen grains at pH 6.8 is 83.49%. There is a steady decline in the germination rate with a decline in the pH. It is 82.08 at pH 6.4, 64.44% at pH 6.0 and 59.92% at pH 5.6. The comparison of the control group with the closest values to it shows that the germination rate decreases with a decline in the pH. The comparison indicates that there is an 84.75% decline in the germination rate which is 12.73% at pH 3.2 while there is a 93.41% decline in the germination rate which is 5.50% at pH 2.8.

The tube length of the plum plant at pH 6.8 (control group) is 499 µm. It is 400.6 µm at pH 6.4 while 1.8 µm at pH 2.8 which has the highest concentration of acid. The comparison of this tube length with that of the control group demonstrates that there is a 19.71% and 99.63% decline at pH 6.4 and pH 2.8, respectively. These results indicate that a reduction in the pH prevents the growth of the pollen tube of the plum plant and leads to a significant decline in its length.

This study reveals that the pollen grains of the plum and almond plants are very much affected by high concentrations of acid. The comparison of the effect of high concentrations of acid on these two plants in terms of germination rate shows that there is a 93.41% and 93.42% decline in the plum plant and the almond plant at pH 2.8, respectively which indicates that both plants are almost equally affected.

The comparison of the two plants in terms of tube length points out that there is a 99.63% and 100% decline in the plum plant and the almond plant, respectively, which point to the fact that the almond plant is entirely affected by high acidity and does not develop tubes.

The results show that the values observed for the pollen grains of the almond and the plum plants are not very different from each other and that the inhibition of the germination rate and the tube length as a result of an increase in the degree of acidity reveals similar results for both plants. It can be stated that both plants are almost equally affected by high acidity.

High acid can have harmful effects on all kinds of different plant species and in different parts of every species. These harmful effects are especially observed in the vegetative organs of plants.

High acid not only affects the seed and the vegetative parts but also the generative organs of plants. In particular, it considerably affects tube growth and germination of pollen grains which are male reproductive cells.

A study investigates the germination and tube length of the pollen grains of the Populus tremuloides Michx plant in vitro and compares them with a control group. The results indicate that there is a 10% decline at pH 5.6 compared to the control group and that pollen germination and tube growth is completely inhibited at pH 2.8. Our study shows significant declines in the tube lengths of both plants at pH

5.6, less decline in the germination rate compared to that in the tube lengths. It also reveals that the almond plant does not develop any tubes at pH 2.8 and the plum plant develops a tube of 1.8 um on average [8].

An artificial medium was prepared in laboratory environment in order to carry out a study on Maize (Zea mays) plant for pH 4.6 and pH 3.6 values which are below pH 5.6. The study reports that pollen grains develop and elongate at pH 4.6, however, no development or elongation is observed at pH 3.6 concentration. Similarly, our study shows that there are proportionally significant declines for both plants at pH 4.8 ad pH 3.6 [9].

The study on Betula pubescens ssp. Tortuosa plant carried out by Neuvonen et al to investigate the effects of acid rains reports 50% decline in the pollen germination rate and tube length between pH 5.6 and pH 4.6. Compared with our study, no significant decline has been observed between pH 5.6 and pH 4.8 concentrations for the two plants [10].

Klymenko investigates the effects of acidity on the viability of pollen grains of peach trees and compares them with a control group. The results point out that there is a 9-15% decline between pH 2 and pH 4. It is between 5% and 5.5% at pH 2.8 for both plants in terms of germination rate in our study [11].

Taking pH 6.4 as low acidity and pH 2.8 as high acidity, we observe that the pollen germination of the almond plant declines 5.17% at low acidity and 93.43% at high acidity. The tube length of the almond plant declines 31.89% at low acidity and 100% at high acidity. The pollen grains of the plum plant are affected more by the high acidity than by the low acidity in terms of pollen germination and pollen tube elongation.

Compared to the value of the control group, there is a 93.41% decline in the pollen germination of the plum plant at high acidity. There is a 19.71% decline at low acidity and a 99.63% decline at high acidity in the tube length values of the plum plant. Consequently, the pollen grains grown at low acidity show better improvement than the pollen grains grown at high acidity in terms of pollen germination and pollen tube length.

In line with the findings of this study, it is clear that air pollution has negative effects on plum and almond plants, which have great economic importance today, as well as various effects on many plant species grown in different regions. Increasing industrialization, unconscious consumption of chemical products by humans and constant increase in the number of motor vehicles result in high levels of air pollution and yield reduction especially due to damage to generative organs of plants. The world will inevitably be faced with the extinction of many species that it hosts in the future if its exposure to environmental pollutants continues in this way.

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