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ABSTRACT

Capsicum annuum var. *glabriusculum* known as "chiltepin" is a wild type chili pepper with an important consumption and market in Mexico. Usually it is produced in non-commercial agricultural systems and, therefore not improved varieties are available for farmers. Since chiltepin´s presents lack of germination, several research projects have been done in order to improve the percentage of germination and seedling emergence. The aim of this study was to determine the effect of four sulfuric acid concentrations (20, 40, 60 and 80%) and four soaking periods (1, 2, 3, and 4 hours) on seed germination and seedling vigor. An experiment was carry out under a completely randomized experimental design with four repetitions of 100 seeds each, including seeds soaked in distilled water as a control. A top of paper standard germination test was conducted in Seedburo® germination chamber for a period of 21 days, at 25 °C and 90% of relative humidity. The data was analysed by analysis of variance and Tukey's means test. Sulfuric acid affected seed coats and, as a consequence, seed imbibition and the germination process; however, the acid affected negatively the expression of seedling vigor. It is concluded that the use of sulfuric acid as a scarification method in *Capsicum annuum* var. *glabriusculum* seed is not recommended.

Keywords: germination, vigor, sulfuric acid, scarification.

Introduction

Chili pepper is a native plant form Mesoamerica and since pre-Hispanic times it has been essential to the Mexicans diet (Cano et al., 2015). Fruits are daily consumed as a fresh product, dry or cooked in different dishes, and then there is a wide diversity of chili, due to the exclusive preferences in every province of the country. For this reason, this species represents an important source for producers, who have found a significant market in United States as a result of the large amount of immigrants established in the States of California, New York, and Illinois.

Within the wide variability of chili pepper, 'chiltepin' (*Capsicum annuum* var. *glabriusculum*) has a particular market as a result of its flavour and pungency, and therefore represents a significant income for farmers since it reaches much higher prices than jalapeno, 'serrano' or sweet chili peppers. In recent decades 'chiltepin' is marketed with additional value, since it has been industrialized to be consumed as a candy, mixed with other species as food additive, or simply dried and grinded for typical Mexican dishes, consequently creating an excellent income opportunity for producers.

However, its domestication has not been strengthened, mainly because it requires particular conditions of soil, humidity and shade (Bañuelos, 2008), then its production is based on non-commercial cropping or commonly it is just gathered from wild plants, so it is common to confront problems of low seed germination and seedlings establishment (Cano et al., 2015).

In this context, several studies have been conducted to facilitate seed germination and seedling emergence using different levels of temperature, light and humidity (Hernández, 2004), treatments with gibberellic acid using 500 ppm (Hernández, 2004) and 400 ppm (Araiza et al., 2011), as well as soaking seeds in hot water (50°C) over six minutes (Mireles et al., 2015).

It has been observed that seeds show good germination once they have passed through birds tract (Bañuelos, 2008); suggests that the acid from their stomachs produces seed coat mechanical damage, so, it will be possible to improve imbibition using a corrosive substance that simulates what happens naturally; this hypothesis has been reinforced since Cano et al. (2015) concluded that the seed coat is an impediment for *Capsicum annuum* var. *glabriusculum* germination.

There are several chemicals that could produce seed coat damage and therefore promote the germination process, such as liquid nitrogen, phosphoric acid, and sulfuric acid (Cano et al., 2015; Kheloufi, 2017; Mavi, 2018). Thus, the present study intended to assess the use of sulphuric acid as an alternative to improve seed germination by applying the product at different concentrations and soaking periods.

Materials and Methods

Ripe fruits of intense red colour of 'chiltepin' were gathered at “Sierra Madre Occidental”, close to Obregon City, Mexico. The samples were sun dried during

five hours. A manual seed extraction was made and then they were dried at room temperature up to 11% of moisture content. The samples were classified by weight with a Dakota Seedburo® seed blower in order to remove empty seeds.

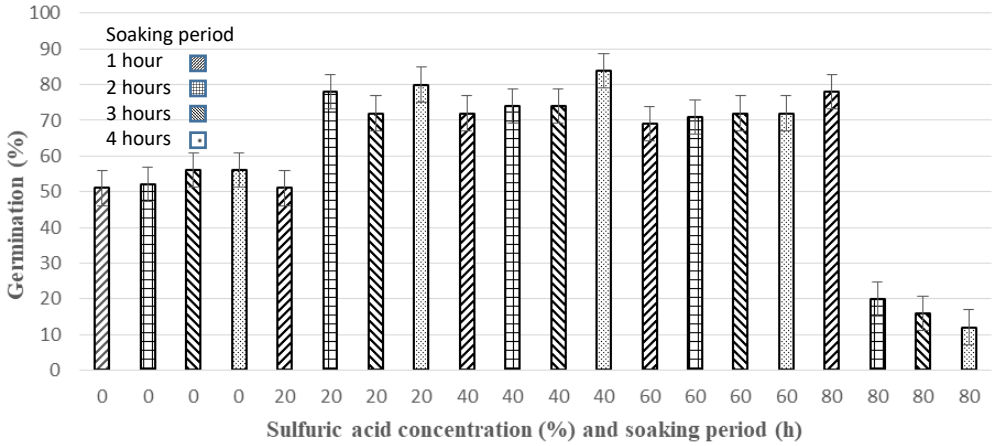
Scarification with sulfuric acid was performed using four concentrations (20, 40, 60 and 80%) over four soaking periods (1, 2, 3, and 4 hours); in addition, seeds soaked in distilled water were used as a control over the four periods previously mentioned. Once treated, seeds were washed with tap water for five minutes; afterwards, they were subjected to a top of paper germination test under a completely randomized experimental design with four replicates of 100 seeds each. The samples were sown in Petri dishes with filter paper moistened with distilled water, and finally they were introduced into a Seedburo® germination chamber at 25 °C and 90% relative humidity for 21 days. Watering was done every other day using distilled water.

From the 3rd and up to 21th day, counts of normal seedlings, abnormal seedlings as well as the number of non-germinated seeds were recorded. In addition, a sample of 10 seedlings was used to measured radicle length, seedling shoot length and seedling length. Data were subjected to an analysis of variance and a Tukey's ($p \leq 0.05$) means comparison test.

Results and Discussion

Sulphuric acid scarification treatments showed significant effect ($p \leq 0.05$) in the variables evaluated, showing an increase up to 20% in seed germination referred to the control (Figure 1), particularly under concentrations of 20, 40 and 60%. These results suggest that the seed coat was affected, and then seed imbibition and radicle emergence were improved. Then it is confirmed that seed coat is a physical impediment for seed germination in this species as reported by Cano et al. (2015); however, germination values decreased when seed were treated with sulphuric acid at 80%, showing the lowest values of the experiment. So, 80% concentration probably burned the embryo, as it has happened in other species (Kheloufi, 2017).

Figure 1. *Germination Percentage of 'Chiltepín' (Capsicum Annuum var. Glabriusculum) Seeds Scarified with Different Concentrations of Sulfuric Acid over Four Soaking Periods*



The percentage of non-germinated seeds confirm the hypothesis previously mentioned, since increase in acid concentration and in exposure period increases the percentage of non-germinated seeds, particularly those soaked with sulfuric acid at 80% for two, three and four hours. They showed an average 20% higher of non-germinated seeds than the control (Figure 2); then it could be supposed that the seed coat of chili seed is not as thick as some legumes and therefore many embryos could have died (Kheloufi, 2017).

Additionally, the highest percentage of normal seedlings was observed for the control treatment (Figure 3), while the lower values were observed in agreement to increases in sulfuric acid concentrations and length of soaking period. These results could indicate that the acid could also affect seedling performance. Then, the highest values of abnormal seedlings were found when sulfuric acid was applied (Figure 4); these agreed to previous studies where some pre sowing treatments with abrasive substances such as sulfuric, phosphoric and acetic acids adversely affected the seed and seedling physiology (Mavi, 2018).

Figure 2. *Non Germinated Seeds of 'Chiltepín' (Capsicum Annuum var. Glabriusculum) Scarified with Different Concentrations of Sulfuric Acid over Four Soaking Periods*

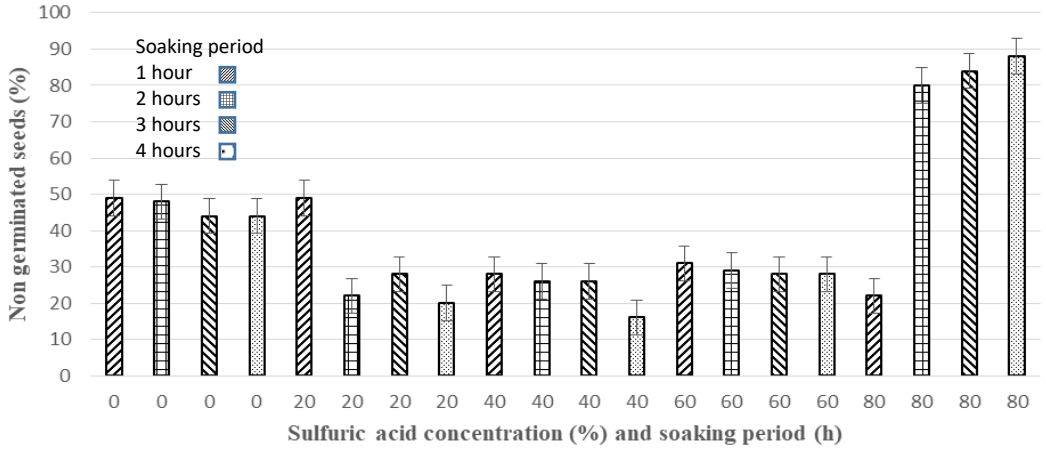


Figure 3. *Normal Seedlings Percentage of 'Chiltepín' (Capsicum Annuum var. Glabriusculum) Seeds Scarified with Different Concentrations of Sulfuric Acid over Four Soaking Periods*

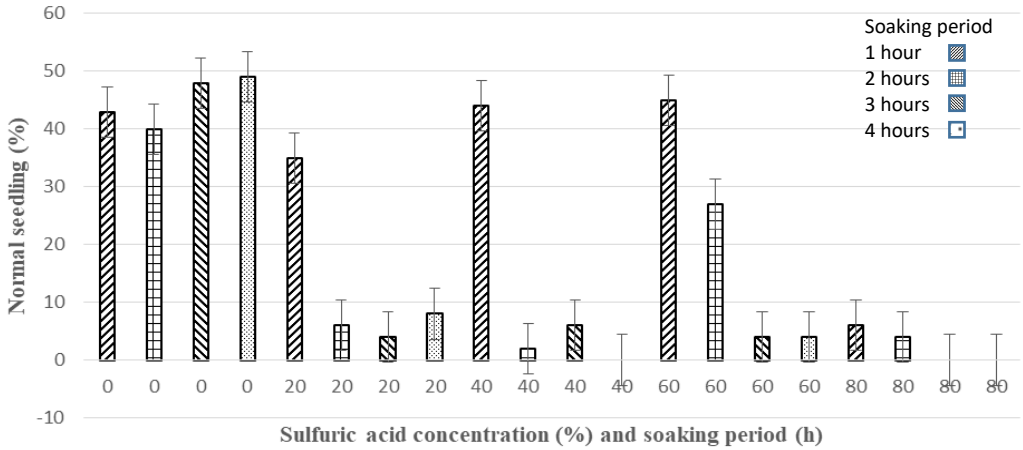
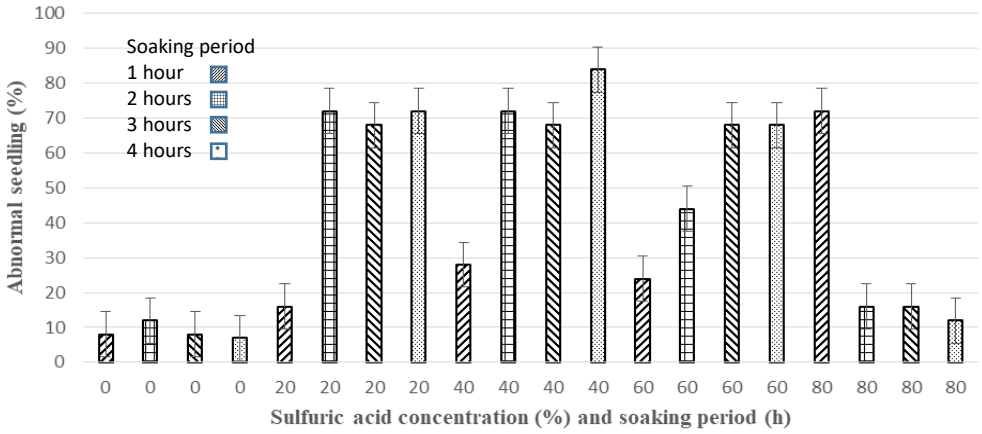
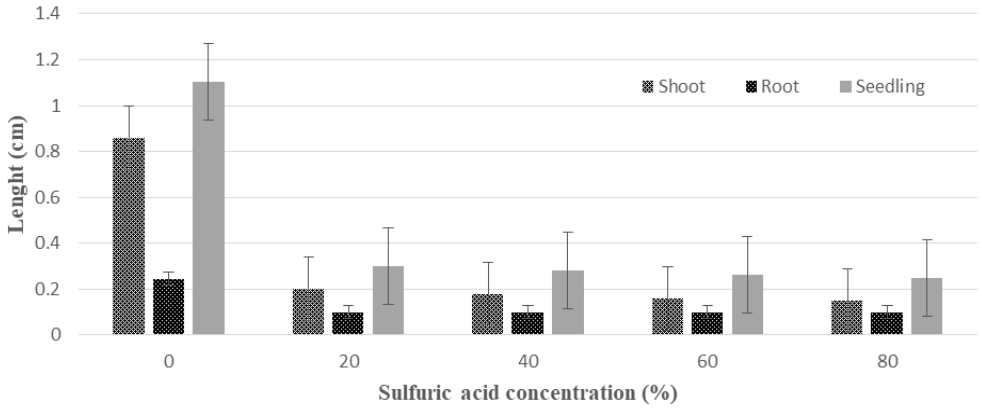


Figure 4. *Abnormal Seedlings Percentage of 'Chiltepín' (Capsicum Annuum var. Glabriusculum) Seeds Scarified with Different Concentrations of Sulfuric Acid over Four Soaking Periods*



Seedlings vigour originated from seeds soaked in sulphuric acid was negatively affected ($p \leq 0.05$) since a significant reduction of seedlings length was observed (Figure 5). However, the negative effect was slightly more perceptible in the plumule length; this result allowed supposing that this specie has rustic root and therefore it is tolerant to adverse factors such as salinity (Mireles et al., 2015).

Figure 5. *Seedling Vigour of 'Chiltepín' (Capsicum Annuum var. Glabriusculum) Seeds Scarified with Different Concentrations of Sulfuric Acid over Four Soaking Periods*



Based on the obtained results, it is concluded that seed immersion in water favoured germination, as it has been reported in other studies (Smarah et al., 2016). In addition, the use of sulfuric acid is not a good alternative for scarification of 'chiltepin' seeds, since vigor expression and the percentage of normal seedlings showed significant reductions particularly at 80% acid concentrations. These results suggest that although this product tends to deteriorate the seed coat, improving imbibition and therefore increasing the number of germinated seeds, at

the same time it promotes an irreversible effect on the seedling physiology affecting in a short period of time the vigor parameters as percentage of abnormal seedlings, which significantly increases according to the concentration of sulfuric acid used in the scarification process.

Conclusions

Sulfuric acid affected seed coats and consequently favoured the seed imbibition and the germination process, but it also affected negatively vigour expression of the emerged seedling. Seed immersion in sulphuric acid at 80% for scarification of 'chiltepin' seeds produced an irreversible damage to the embryo. The use of sulfuric acid as scarification method in *Capsicum annuum* var. *glabriusculum* seeds is not recommended.

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