



## **The Effects of Pre-germination Treatments on Seed Germination and Growth of Wild Guavas in the Kingdom of Eswatini, Southern Africa**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author TS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KAN, MTM and PKW managed the analyses of the study. Authors KAN and MGZ managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Dormancy is a condition where seeds will not germinate even when the environmental conditions (water, temperature and aeration) are permissive for germination. Wild guavas (*Psidium guajava* L.) are very popular in all agro-ecological zones of Eswatini. Farmers have shown an interest towards guava cultivation but have to cope with the shortage of quality propagation material. The demand is not fulfilled because of unavailability of superior seedling rootstocks, which might be due to poor seed germination and seedling growth. Nevertheless, it has been reported that guava seeds exhibited seed dormancy, which affects their growth and development. The experiment was carried out to study effects of different pre-germination methods on seed germination of guava. The study was conducted at the University of Eswatini, Luyengo Campus. The objective of the study was to get maximum germination of guava seeds in as short a time as possible. Four methods were used i.e., soaking in distilled water for five days at room temperature, soaking in hot water at 80°C for three minutes, subjecting seeds to heat at 80°C in oven for six minutes and

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soaking in 20% dilute sulphuric acid for three minutes. All these treatments significantly decreased days to germination of seeds compared with the control. Among the methods, treatment of guava seeds with 20% dilute sulphuric acid for three minutes was judged best with maximum germination percentage (93.3%); lowest germination mean time was observed in seeds soaked in distilled water (31 days). Highest plant height (44 mm) and highest stem girth of (3.37 mm) were recorded from seeds soaked in sulphuric acid after 150 days of sowing. Seeds without any pre-germination treatment showed poor germination (26.7%). On the basis of the findings, it can be recommended that propagators use sulphuric acid in seed priming for higher germination, growth and development.

*Keywords: Seed-dormancy; priming; wild-guava seeds; uniform germination; root-stock.*

## 1. INTRODUCTION

Guavas (*Psidium guajava* L.) are common tropical fruits cultivated and enjoyed in many tropical and sub-tropical regions [1]. Guava is a small tree in the family Myrtaceae, native to Central America particularly Mexico [2]. Guavas are of interest to home growers in sub-tropical areas as one of the few tropical fruits that can grow to fruiting size in pots indoors. When grown from seeds, guavas bear fruit as soon as two years and as long as 40 years [2,3]. In order for guava seeds to germinate, their dormancy should be broken. Seed dormancy is one of the most important mechanisms of viability in plants. Generally, seed dormancy is seen little in plants that are domesticated from ancient times compared to wild and native species. Water absorption, enzymatic activity, embryo growth, seed coat rupture and plant growth are important steps of germination [4-6]. Each desert plant species has its own set of mechanisms that allow it to start under a broad variety of conditions [7].

A dormant seed is one that is unable to germinate in specified period of time under a combination of environmental factors that are normally suitable for the germination of a non-dominant seed [4,6,8]. An understanding of dormancy mechanisms is of ecological and economic importance [2,9,10]. Many species of plants have seeds that delay germination for many months or years, and some seeds can remain in the soil bank for more than 50 years before germination, hence that is a big issue in availability and distribution of that species. Although some seeds have a very long viability period, the oldest documented germinating seed was nearly 2000 years old based on radiocarbon [3-6].

Although guava is popular in Eswatini and other countries, little has been done to improve the

production of wild guavas mainly because the seeds have high degree of dormancy. Appropriate and low cost techniques have not yet been found to assist research farmers who wish to produce the fruit both at subsistence and commercial farming level. Seed dormancy is the main issue in the germination and distribution of guavas, but there is no information about breaking seed dormancy of guava in the country. Seed dormancy is the core limiting factor for plant propagation in nurseries especially of guava. Seeds with hard seed coats have delayed and non-uniform germination causing 40% loss of genetic resources if non-effective seed dormancy treatment is employed. Mechanical, thermal and chemical treatments are the main techniques used to make hard seed coat permeable to water or oxygen [6,11-15].

There is a need to determine the effect of different dormancy breaking methods and identify the best method in order to promote germination of guava seeds in the Kingdom of Eswatini. This will help to achieve a stable supply of guava tree seedlings and increased farm income in order to achieve food security.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site

The experiment was conducted at the Seed Science Laboratory of the Horticulture Department, University of Eswatini, Luyengo Campus, Southern Africa. The other phase of the experiment was conducted in the lath house which is also located at the University of Eswatini, Luyengo Campus under the Horticulture Department Farm. The average temperatures of the location is 21°C and annual precipitation of about 800 mm. Luyengo has latitude between 26°34' S and longitude 31°12' E at 750 m above sea level [16].

## 2.2 Plant Materials

Guava seeds were collected from a mature guava fruits. Immediately after collection seeds were separated, and then stored in sealed paper bags after drying for 1 week in the shade under normal room temperature 25-30°C. Only uniformly-sized seeds were used in various seed germination experiments. Sixty days after sowing, the plants were transplanted into 2 liter polyethylene bags filled with loam soil, for ease growth, so that parameters like plant height and stem girth could further be assessed.

## 2.3 Experimental Design

The experiment was laid out in Randomised Complete Block Design (RCBD), with three replicates and 10 seeds per Petri dish. The treatments were 20% sulphuric acid, 80° hot water treatment,

## 2.4 Seed Sterilization

Seeds were surface sterilized by soaking in 70% alcohol for one minute, then immediately soaked in 2.5% Sodium hypochlorite (NaOCl) solution for three minutes and subsequently rinsed five times with sterilized water before all the various dormancy breaking treatments were applied.

### 2.4.1 Sulphuric acid

Ten seeds of guava were immersed in a beaker with sulphuric acid (20%) for three minutes. Then the seeds were rinsed using distilled water thoroughly to reduce the corrosiveness of the acid which can destroy the endosperm hence may result in poor or no germination. Then 10 seeds were placed into a sterilized labeled petri dish and watered to keep them moist. Three-ply Whatman No. 598 (Whatman plc, Maidstone, United Kingdom) were placed in the Petri dishes, the filter paper was moistened with water and this helped conserve the moisture and temperature which was required for seed germination. All petri-dishes were covered to reduce water loss and placed at 25°C in a germination chamber room. Filter paper at the bottom of petri dish was double layered [17].

### 2.4.2 Hot water treatment

Water was heated until it reached (80°C) and 10 guava seeds were put into the water for one minute and then removed. After the required soaking period, the seeds were removed and cooled then placed in a labeled petri dish and

moistened. Three-ply Whatman No. 598 filter paper which was moistened with water was placed at the bottom of the Petri dishes and this helped conserve the moisture and temperature which was required for seed germination. All Petri dishes were covered to reduce water loss and placed at 25°C in a germination chamber room. Filter paper at the bottom of petri dish was double layered [17].

### 2.4.3 Oven heat

Guava seeds were placed in sterilized petri dish and kept in the oven at 80°C for six minutes. The seeds were then removed, cooled and placed in a sterilized labeled Petri dish with moistened three-ply Whatman No. 598 filter paper. All petri-dishes were covered to reduce water loss and placed at 25°C in a germination chamber. Filter paper at the bottom of Petri dish was double layered [17].

### 2.4.4 Soaking in distilled water

Guava seeds were soaked in distilled water for five days. The seeds were then removed and placed in a sterilized labeled Petri dish with moistened three-ply Whatman No. 598 filter paper. All petri dishes were covered to reduce water loss and placed at 25°C in a germinator. Filter paper at the bottom of petri dish was double layered [17].

## 2.5 Data Collection

A seed was considered germinated when the tip of the radicle (2 cm) had grown free of the seed coat. Data was first recorded on the 4<sup>th</sup> week after sowing and germination counts were made every day for 5 weeks. Data regarding germination index (GI), germination percentage (GP), mean germination time (MGT), germination rate index (GRI), plant height (H) and stem girth (SG) were collected and computed.

### 2.5.1 Germination index

The germination index (GI) was calculated as described by Ellis and Roberts [18] using the following formula:

$$GI = (\text{No. of germinated seeds/Days of first count}) + \dots + (\text{No. of germinated seeds/Days of final count}).$$

### 2.5.2 Mean germination time

Mean germination time (MGT) was calculated according to the [19] equation:

$$MGT = \frac{\sum (Dn)}{\sum n}$$

Where n is the number of germinated seeds or emerged seedlings on day D and D is the total number of days counted from the beginning of germination.

### 2.5.3 Germination percentage

The germination percentage (%) was determined using the equation;

$$\text{Germination \%} = G/N \times 100$$

Where

G = Total number of seeds that germinated.  
N = Total number of seeds in the Petri dish.

### 2.5.4 Germination rate index

Germination rate index was calculated using the formula;

$$GRI = G1/1 + G2/2 + \dots + Gx/x$$

Where

GRI = Germination rate index  
G1/1 = Germination rate in day 1  
G1/2 = Germination rate index in day 2  
Gx/x = Germination rate index per number of days

### 2.5.5 Plant height

Shoots were measured after seven days using a string and a 30 cm ruler.

### 2.5.6 Stem girth

The stem girth was measured at the base of the plant using a pair of Vernier caliper (Pierre Vernier, 1637, Ornas, France) at 1 cm above the ground.

## 2.6 Data Analysis

Data collected was subjected to analysis of variance using GenStat Statistical Package [20]. Where significant differences were detected, mean separation was performed using Duncan's New Multiple Range Test (DNMRT) at 5% probability level [21].

## 3. RESULTS

### 3.1 Germination Percentage

There were significant ( $P < 0.05$ ) differences observed on germination percent of guava seeds

at 40 days after sowing (DAS). Seeds pre-treated with sulphuric acid showed the highest (93.3%) germination percentage. The second highest germination percentage was observed from seeds treated with hot water and the lowest (26.7%) seed germination percentage was observed from the control seeds (Fig. 1).

### 3.2 Germination Index

There were significant ( $P < 0.05$ ) differences observed on the germination index of guava seeds. The highest germination index (273.3) was observed from seeds treated with sulphuric acid. The second highest germination index was observed from seeds treated with distilled water and the control seeds had the lowest (58.3) (Fig. 2).

### 3.3 Mean Germination Time

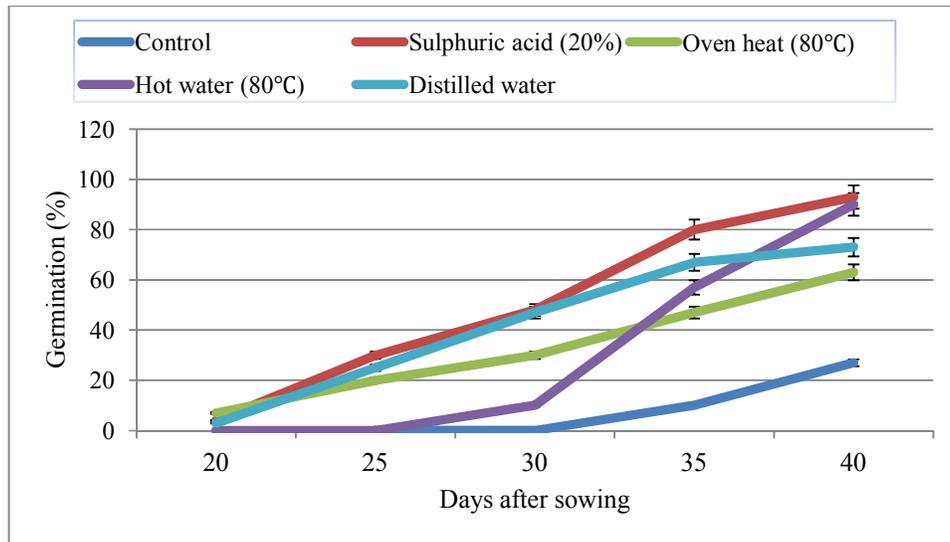
There were significant ( $P < 0.05$ ) differences in mean germination time among the pre-germination treatments (Fig. 3). The highest mean germination time (39 days) was obtained from control seeds while the lowest (31 days) was observed from seeds treated with distilled water, however it is not significantly different from sulphuric acid. The second highest mean germination time was obtained from seeds treated with hot water (36 days) (Fig. 3).

### 3.4 Germination Rate Index

There were significant ( $P < 0.05$ ) differences in the germination rate index among the pre-germination treatments. The highest germination rate index (0.3148) was obtained from seeds treated with sulphuric acid while the lowest (0.0702) was observed from the control seeds. The second highest germination rate index was obtained from seeds treated with distilled water (Fig. 4).

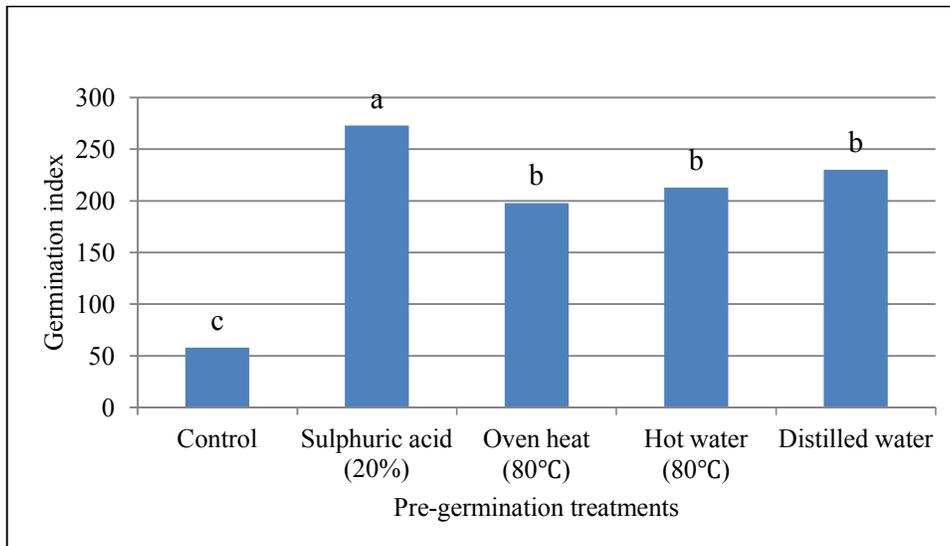
### 3.5 Plant Height

There were significant ( $P < 0.05$ ) differences observed in plant height at (150 DAS). Seeds pre-treated with sulphuric acid had the highest plant height at (44 cm) and the lowest (33.77 cm) was observed from the control seeds. The second highest plant height (41.67 cm) was observed from seeds treated with oven heat (Fig. 5).



**Fig. 1. The effects of different dormancy treatments on the germination percentage of guava seeds**

Vertical bars represent standard error (SE) below and above means



**Fig. 2. The effect of different dormancy treatment on the germination index of guava seeds**  
**Bars with the same letter are not significantly different from one another**

Mean separation by DNMRT at  $P=0.05$

### 3.6 Stem Girth

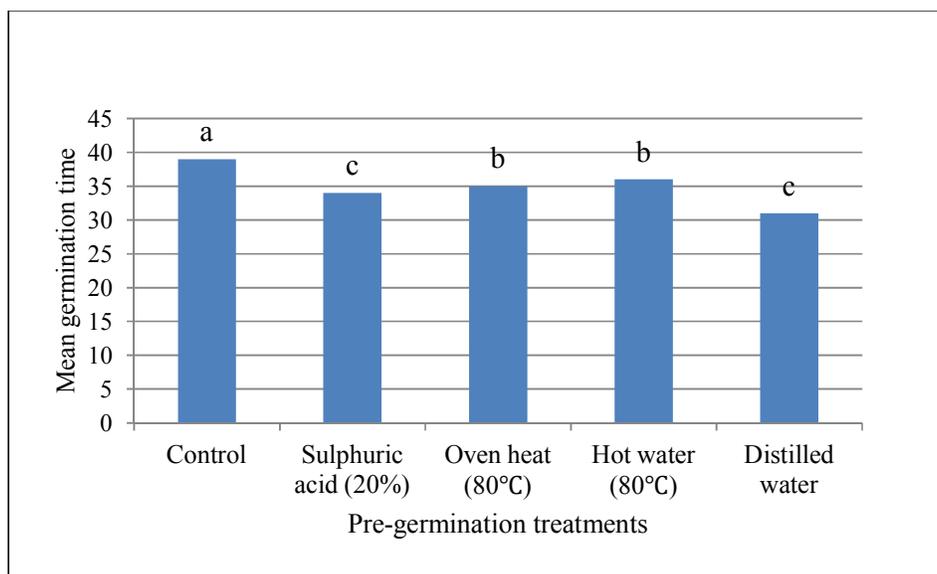
There were significant ( $P<0.05$ ) differences observed on stem girth of guava seedlings at 150 DAS. The highest stem girth (3.73 cm) was obtained from seeds pre-treated with sulphuric acid while the control seed had the lowest (3.10 cm) stem girth. The second highest (3.57 cm) stem girth was observed from seeds treated with the oven heating (Fig. 6).

### 4. DISCUSSION

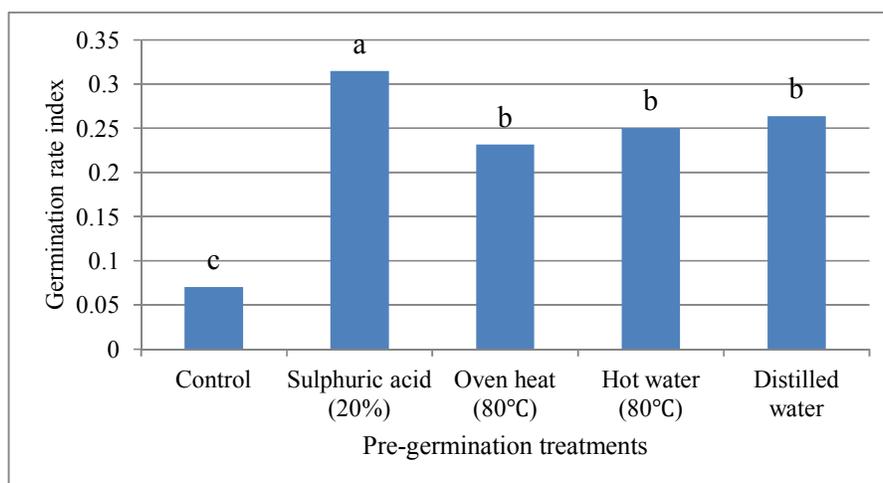
Seed treatment with sulphuric acid at 20% for three minutes was the most effective treatment for enhancing germination over a short period. Acids are often used to break down especially hard, thick impermeable seed coats [22]. It was noted that dilute sulphuric acid gets in contact with the seed coat; it triggers cracking of the seed coat, without interfering with the

micropyle. The cracks therefore allow the entrance of water into the endosperm thus initiating germination. Ellis and Roberts [18,19], reported similar results on okra seeds that once the inhibitors of seed germination were leached out inhibitors from the seeds, germination is emphasised. These results are close conformity with finding of Brijwal et al. and Egley [23,24] as they reported similar experimental results e.g., hydrochloric acid (10%) for 2 minutes treatment.

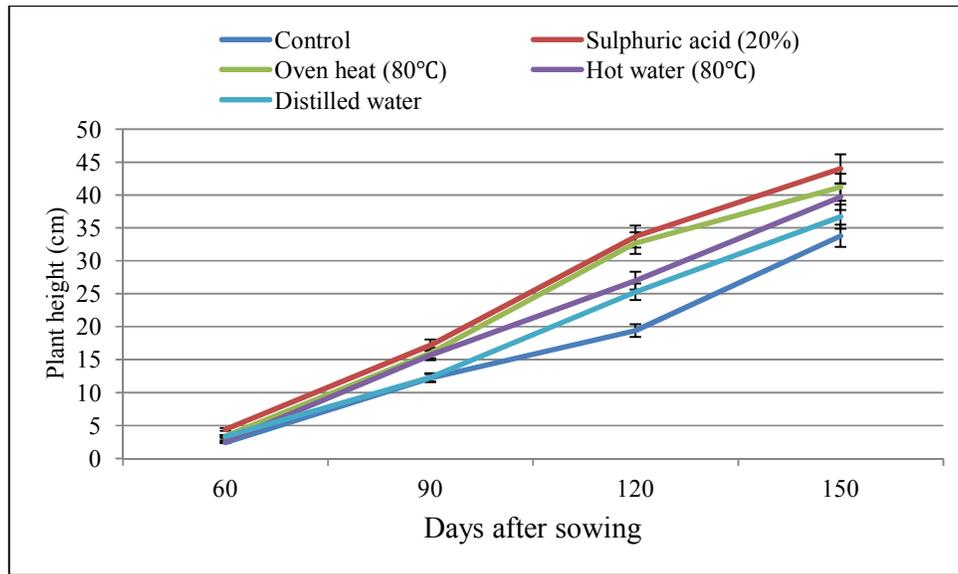
In this experiment, seeds treated with hot water (80°C) recorded the second highest germination percentage. The effectiveness of boiling water treatment in enhancing germination has been attributed to the release of physical dormancy from hard seeded species by causing ruptures in the seed wall thereby allowing imbibition, oxygen uptake, diffusion and germination to occur [10, 25]. When hot water gets in contact with the seed coat, it triggers cracking of the seed coat, without interfering with the micropyle though [22].



**Fig. 3. The effects of different dormancy treatments on mean germination time of guava Seeds**  
**Bars with the same letter are not significantly different from one another**  
*Mean separation by DNMRT at P=0.05*

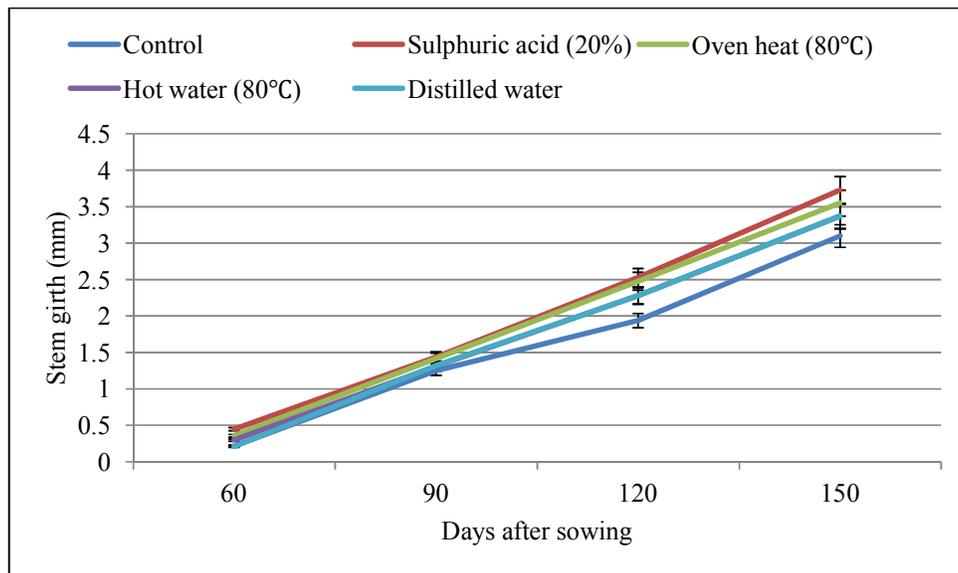


**Fig. 4. The effects of different dormancy treatment methods germination rate index of germination of guava seeds**  
**Bars with the same letters are not significantly different from one another. Mean separation by DNMRT at P=0.05**



**Fig. 5. The effects of plants height at different dormancy treatment methods on seedlings height on guava seeds**

*Vertical bars represent standard error (SE) below and above the means*



**Fig. 6. The effects of different dormancy treatments methods on the stem girth of guava seeds**

*Vertical bars represent standard error (SE) below and above means*

In this experiment, soaking of seeds in distilled water also improved germination. Soaking seeds in distilled water promotes germination by softening the hard seed coat, activating the enzymes and minimizing the effects of inhibitors. However, Reisman-Berman, et al. [26] reported 90% of germination of seeds by soaking in water for 36 hours before sowing. Reisman-Berman, et

al. [26], Oladiran [27], reported that decrease in germination percent after 72 hours water soaking may be attributed to water trapped in tissue between the embryo and seed coat creating an oxygen barrier.

Other treatment used in the experiment was oven heat at 80°C of seeds for six minutes. The

results obtained showed significant effect in dormancy breaking of a guava seed. This method enhances germination and these results also agree with the findings by Baskin and Baskin and Emongor et al. [8,17]. Untreated seeds showed a poor germination percentage. This explains the facts that the seeds had dormancy.

Plant height and stem girth rapidly increased as the plants were growing. It was observed that seeds treated with sulphuric acid and oven heated had rapid growth soon after germination. Plants treated with sulphuric acid recorded the highest plant height. This result is in agreement with the report by Baskin and Baskin and Oladiran [8,28] who stated that breaking seed dormancy in jute using sulphuric acid significantly improved seed germination and growth. They indicated that seeds which germinated earlier than others recorded higher plant heights. This is usually possible where the conditions for growth are favourable to all plants under the same conditions. Distilled water and hot water treatments in this experiment together with control recorded the lowest plant heights because the seeds germinated later.

Priming seeds with sulphuric acid (20%) was the most effective method observed in the experiment. A germination rate of 90% was recorded over a shorter period. Acid are often used to break down especially hard, thick impermeable seed coats according to several authors [8,10,22]. An experiment conducted by Soliman and Abbas [29], indicated that the maximum germination percentages were noticed in seeds treated with hot water at 80°C and sulphuric acid (98%) which gave 91% and 88% respectively without significant difference in first and second season. Deeping the seeds in the sulphuric acid for more than 10 minutes decrease germination capacity in that as acid being corrosive might damage the embryos of the seeds [8,10,17].

## 5. CONCLUSION

Pre-germination treatments using sulphuric acid 20% for three minutes was the effective method for breaking seed dormancy and promoting growth and development of guava seeds. The highest germination percentage was obtained from seeds treated with sulphuric acid. The lowest germination percentage was observed in control seeds. It is recommended that guava seeds be treated with diluted sulphuric acid prior

to sowing to obtain great performance on growth and development. Hot water can be also used as second best alternative. It is also recommended that a study to determine yield quantity and quality should be carried on.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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