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Research Article

# Pre-germination treatments and application of soil amendments for Supa (*Sindora supa* Merr., Fabaceae)

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## Abstract

Through two experiments, this study aimed to determine the optimal pre-germination techniques and soil amendments for Supa. The first experiment tested four pre-treatment techniques: control, soaking in cold water for 12 and 24 hours, and soaking in hot water for 5 and 10 minutes. The second experiment applied different soil amendments to transplanted seedlings: garden soil/control, 2:1 garden soil and cow dung, 2:1 garden soil and chicken manure, 2:1 garden soil and vermicompost, and 2:1 garden soil and sawdust. Both experiments were arranged in a Completely Randomized Design. Results showed that Supa seeds soaked in hot water for 10 minutes (T4) had the highest percentage of germination and germinative energy, while seeds subjected to cold water for 24 hours (T2) had the earliest mean days of emergence. There was no significant difference among treatments for percent germination and germinative energy. Regarding soil amendments, Supa seedlings had a 100% survival rate in all treatments except for chicken manure (T2). The sawdust treatment resulted in the highest increase in root collar diameter, length, and number of secondary roots. In contrast, vermicompost resulted in the highest increase in shoot length, chlorophyll content, and root biomass. The untreated and cow dung treatments resulted in the highest shoot biomass. The different amendments significantly affected all parameters except for the number of secondary roots and root biomass.

**Keywords**: Germinative energy, percentage germination, percent survival, vermicompost

## Introduction

Today, there is a continuous demand for planting materials to support the different greening programs of the government, non-government organizations, and other groups and individuals. Planting materials may include seeds, seedlings, cuttings, and wildlings, but the increased demand calls for the intensified production of quality planting materials to support the government's effort for environmental services, enterprise development, and self-sufficiency not only for major forest products but also for fruit, ornamentals, and medicinal plants. Unfortunately, the quality, quantity, and availability of planting materials at this time are critical issues that need to be addressed to attain a successful massive greening program. To resolve these issues, there is a need to strengthen the production of planting materials, establish nurseries through the involvement of various stakeholders, and adopt appropriate technologies (Forest Management Bureau- Department of Environment and Natural Resources, 2007). Also, planting indigenous plants can help conserve biodiversity and address the problem of mitigating climate change. It is, therefore, important to study the botanical characteristics of plants as a basis for growing and domesticating plant species from the wild. Characterization of these plants can help address the need to grow plants that are resilient to climate change. These plants will be saved from being lost in future generations (Alicay and Balatico, 2018).

*Sindora supa* is a species of plant in the family Fabaceae that reaches a maximum height of 30 meters. It is endemic to the Philippines and thrives commonly in forests at low to medium altitudes and limestone ridges (Quisumbing, 1951). This species is an endangered Philippine endemic species based on the DENR Administrative Order No. 2017-11 and is widely distributed mostly in Aurora, Nueva Ecija, Pagbilao, and Atimonan in Quezon, Camarines, Albay, and Mindoro with an area of occupancy (AOO) of 72 km² and extent of occurrence (EOO) of less than 115,000 km². Confirmed occurrences are also found in Isabela (Energy Development Corporation, 2018), Ilocos Norte (Ayson *et al*., 2017), Quezon City (Gozon, 2014), and in Laguna (Razal *et al*., 2003). Despite the considerable extent of occurrence, *Sindora supa* still faces threats to its survival and future population proliferation due to consistent mining and quarrying activities in the limestone forests where it is naturally occurring in order to satisfy construction industries' needs (Energy Development Corporation, 2018). In line with the 1998 World Conservation Monitoring Centre assessment of the species, *Sindora supa* was globally assessed as vulnerable (VU) under the more detailed criteria based on the most recent available herbarium records, specimen collections, and literature. It was evaluated due to its less than 10 locations, the extent of occurrence (EOO), and the area of occupancy (AOO).

In the context of seed dormancy, many seeds have difficult germination due to hard seed coat, impermeability to water or physical resistance to embryo expansion, immature embryo, a period after ripening, and availability of light (Untalan, 1994). Hence, there is a need to apply different pre-sowing treatments for breaking seed dormancy (Yisau *et al.*, 2015). *Sindora supa* is a hard-seeded species (PROSEA Timbers, 2017), and the germination period takes longer. Therefore, this study was conducted to determine Supa's response to different pre-germination treatments to hasten its seed dormancy and the appropriate soil amendment needed to enhance its growth to produce quality and healthy seedlings, as this species has many commercial values and is considered vulnerable.

## Material and methods

### Study area and experimental design

The study was conducted at the plant nursery at Aurora State College of Technology, Baler, Aurora, Philippines.

Study 1 comprised of control and four treatments replicated four times with 20 experimental units per replication, summing up to 400 seeds. The treatment combinations applied in the Study 1 is as follows:

**Table 1**. The different treatment combinations applied in Study 1.

| Number | Treatments | Time |
|---|---|---|
| T0 | Control | No treatment |
| T1 | Cold water treatment (20 ˚C) | 12 hours soaking |
| T2 | Cold water treatment (20 ˚C) | 24 hours soaking |
| T3 | Hot water treatment (70 ˚C) | 5 minutes soaking |
| T4 | Hot water treatment (70 ˚C) | 10 minutes soaking |

Parameters were computed based on the following formula:

1. Percentage germination was determined using the formula below:

$$\%\ germination = \frac{Total\ number\ of\ seeds\ that\ germinated}{Total\ seed\ sown}\ x\ 100$$

2. Percentage germinative energy was determined using the formula below. The peak period was the day with highest number of germinants.

$$\% \, GE = \frac{Number \, of \; seed \, sown \, up \, to \, peak \, period}{Total \, seed \, sown} \; x \, 100$$

In Study 2, there were a control and four treatments, which were replicated four times with six experimental units, for 120 seedlings (Table 2). Both studies laid out The different treatment combinations in a Completely Randomized Design (CRD).

**Table 2.** The different treatment combinations applied in Study 2.

| Number | Treatments | Ratio |
|---|---|---|
| T0 | Control (garden soil) | |
| T1 | Garden soil: Cow dung | 2:1 by volume |
| T2 | Garden soil: Chicken manure | 2:1 by volume |
| T3 | Garden soil: Vermicompost | 2:1 by volume |
| T4 | Garden soil: Sawdust | 2:1 by volume |

**Preparation for Pre-germination Treatment**

Four hundred homogenous seeds collected from the Seed Production Area located at Dinadiawan, Dipaculao, Aurora and were subjected to different pre-germination treatments before sowing in the planting bed. For Treatments 1 and 2, seeds were submerged in 20˚C cold water in separate plastic containers and were placed in a refrigerator for 12 and 24 hours, respectively. For Treatments 3 and 4, seeds were soaked in hot water (70˚C) in stainless containers for 5 and 10 minutes, respectively. The seeds were removed immediately after soaking under specified times and placed in tap water to stop the heating action. Upon subjecting the seeds to different pre-germination treatments, seeds were sown in an improvised seed box filled with sterilized mixed garden soil and sand. The mixture was sterilized through cooking for 3 hours to eliminate microorganisms (Tacloy *et al.*, 2022). The seedbox was divided into five portions by partitions placed per treatment. The drill method was applied to sow the seeds. Rills of four rows per treatment replication were lined with twenty seeds each.

**Preparation of Propagation Media with Soil Amendments**

One hundred twenty germinants with a height of at least 5 centimeters, regardless of what treatment was applied in Study 1, were transplanted in individual polyethylene bags filled with sterilized garden soil and soil amendments. The garden soil was also sterilized following the same procedure done in Study 1. Propagation media were pulverized, screened thoroughly through a 2 mm wire mesh, and sun-dried for sterilization. Afterward, the garden soil was mixed with the soil enhancers with a 2:1 ratio. The garden soil served as the control.

**Propagation Media Analysis**

The propagation media was analyzed using the soil test kit from the Agricultural Systems Institute of the University of the Philippines. Based on the result of the chemical analysis (Table 3), supa seedlings can grow in soil with pH index levels of medium acidity (6.4) to very slight acidity (7.2) (Standard Methods of Analysis for Soil, Plant Tissue, Water, and Fertilizer, 1980). Soil analysis for potassium has a distinct cloudy yellowish layer. This means that all growing media used have sufficient potassium (0.19).

**Table 3.** Chemical analysis of the growing media

| Treatments | Soil Ph | Potassium (%) | Nitrogen (%) | Phosphorus (%) |
|---|---|---|---|---|
| T0 - garden soil/control | 6.4 | 0.19 | 0.1 | 9 |
| T1 - 2:1 garden soil: cow dung | 7.2 | 0.19 | 0.3 | 9 |
| T2 - 2:1 garden soil: chicken manure | 7.2 | 0.19 | 0.8 | 9 |
| T3 - 2:1 garden soil: vermicompost | 7.2 | 0.19 | 0.8 | 9 |
| T4 - 2:1 garden soil: sawdust | 7.2 | 0.19 | 0.8 | 9 |

**Termination of the Experiment and Data Collection**

The effects of the treatments on the test plants were evaluated using the following parameters:

*a. Percent survival.* The number of live seedlings was counted and recorded. This was computed using the formula below:

$$\% \, Survival = \frac{Number\ of\ surviving\ seedlings}{Total\ number\ of\ seedlings\ planted} x \ 100$$

*b. Root collar diameter.* It was measured using a digital tree caliper calibrated in millimeters from the base of the aerial part of the plant and to the top of the root system.

*c. Shoot length.* It was measured with a foot rule calibrated in centimeters from the point of origin up to the top.

*d. Root length.* It was determined by measuring the primary root with a foot rule calibrated in centimeters from the point of origin up to the tip of the elongated roots. Secondary roots were not measured.

*e. Number of roots.* This was done by manually counting the roots of each seedling per treatment.

*f. Chlorophyll content.* Shoots were submerged in a pail of water to wash off the dust and other particles. After that, the shoots were wiped individually with a clean towel. Only the top, middle,

and base leaves were inserted to the CCM-200 plus chlorophyll content index. After inserting, the chlorophyll content of each shoot was automatically measured.
*g. Root and shoot biomass.* Fresh weight was measured first before the roots and shoots were put inside the oven calibrated at 70 °C. After attaining the constant weight, dry weight was taken. Both fresh weight and dry weight were measured using a digital analytical balance.

Data analysis

All the data gathered were statistically assessed using Analysis of Variance (ANOVA). Significant results were subjected to test the significance between treatment means. Fisher's LSD post hoc analysis was employed.

## Results

### Study on pre-germination treatments

### Percent germination

Based on the observation, Supa seeds soaked in hot water for 10 minutes (T4) obtained the highest percent germination, while seeds soaked in cold water for 24 hours (T2) got the lowest.

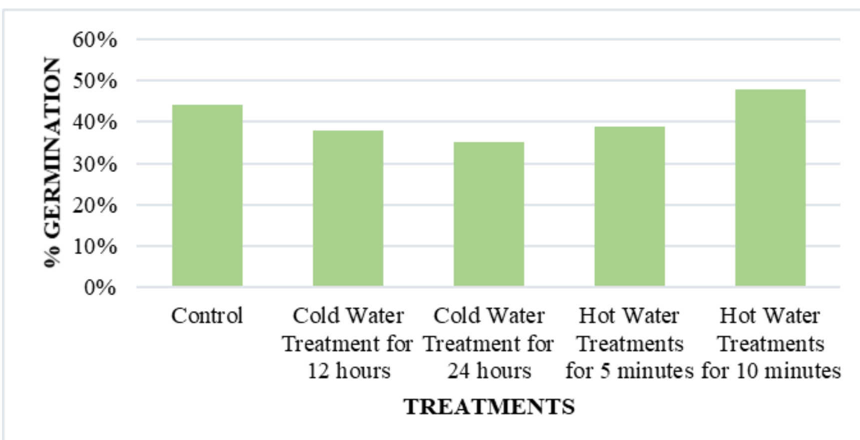


**Figure 1.** Percent germination of Supa seeds applied with different pre-germination treatments.

### Percentage germinative energy

As observed, Supa seeds soaked in hot water for 10 minutes (T4) gave the highest percentage of germinative energy at 32.50%, while the least was the control or untreated seed (T0) with 18.75%.

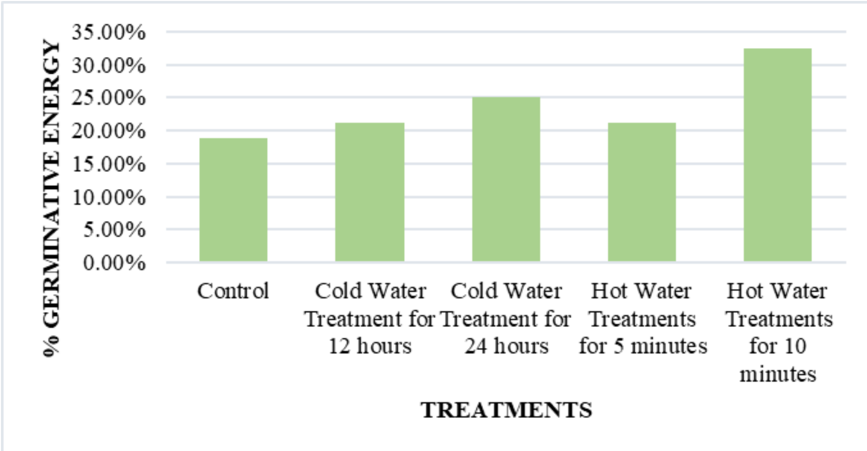


**Figure 2.** Percentage germinative energy of Supa seeds applied with different pre-germination treatments.

## Study on the application of soil amendments

### Percent Survival

After 3 months of observation, Supa seedlings had a 100% survival rate in garden soil alone/control and in garden soil applied with cow dung, vermicompost, and sawdust with a 2:1 ratio but lower (50%) survival when planted in garden soil mixed with chicken manure in 2:1 ratio.

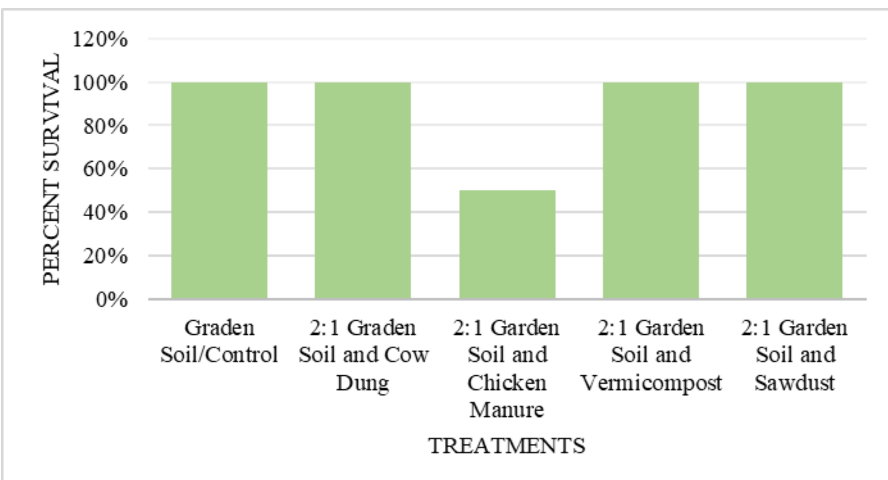


**Figure 3.** Mean percent survival of Supa seedlings in different treatments

## Growth Response of Supa

### Root Collar Diameter

The highest increase in root collar diameter was observed in Supa seedlings treated with sawdust. Meanwhile, the lowest increase in root collar diameter was observed in the control. The ANOVA test revealed significant differences among treatments regarding the increase in root collar diameter ($p < .001$).

### Shoot and Root Lengths

The highest increase in shoot length was observed in Supa seedlings applied with vermicompost, while seedlings treated with chicken manure got the shortest. ANOVA test found significant differences among treatments regarding the increase in shoot length ($p < .001$). Regarding root length, Supa seedlings applied with sawdust had the longest roots compared to other treatments. Thus, sawdust helps increase the root length of Supa. The ANOVA test revealed significant differences among treatments ($p = .002$).

### Number of secondary roots and Chlorophyll Content

The highest number of secondary roots was observed in Supa seedlings treated with sawdust, with a mean of 13.42, followed by control, with a mean of 10.50. On the other hand, the lowest number of secondary roots was exhibited by seedlings applied with cow dung, with a mean of 7.42. ANOVA test revealed no significant difference between treatments regarding the number of secondary roots ($p = .074$). The growth performance of Supa seedlings applied with vermicompost gained the highest chlorophyll content compared to other treatments with a mean of 19.781, followed by cow dung with a mean of 18.689. The least measured chlorophyll content was obtained by sawdust with 11.036. ANOVA test found significant differences among treatments regarding chlorophyll content ($p < .001$).

### Root and Shoot Biomasses

The highest root biomass was observed in seedlings treated with vermicompost, with a mean of 0.61 g. The seedlings with chicken manure followed it with a mean of 0.57 g. Meanwhile, the lowest root biomass was exhibited by seedlings applied with sawdust, with a mean of 0.45. The

ANOVA test revealed no significant difference among the treatments regarding root biomass (*p* = .442).

The highest shoot biomass was observed in untreated seedlings (control) and cow dung treatment, with an equal mean of 2.39 g and standard deviation values of 0.40 and 0.63, respectively. On the other hand, the least shoot biomass was exhibited by seedlings treated with sawdust and chicken manure with means of 1.90 g and 1.60 g, respectively. Additionally, untreated (control) and with the application of cow dung, Supa seedlings had bulky leaves compared with other treatments. ANOVA test revealed significant differences among treatments regarding shoot biomass ($p < .001$). Below is the summary of the Analysis of Variance (ANOVA) of the different parameters of the study on pre-germination treatments

**Table 4.** Summary of the Analysis of Variance (ANOVA) on percent survival, root collar diameter, shoot length, root length, number of secondary roots, chlorophyll content, root biomass, and shoot biomass of Supa seedlings applied with different soil amendments.

| **Treatments** | **% Survival** | **Root collar diameter (mm)** | **Shoot length (cm)** | **Root length (cm)** | **No. of Sec. Roots** | **Chlorophyll content** | **Root biomass (g)** | **Shoot biomass (g)** |
|---|---|---|---|---|---|---|---|---|
| T0 | 100 | 0.42[c] | 100.25[a] | 114.83[a] | 10.50 | 18.531[a] | 0.50 | 2.39[a] |
| T1 | 100 | 0.57[c] | 100.33[a] | 66.25[bc] | 7.42 | 18.689[a] | 0.49 | 2.39[a] |
| T2 | 50 | 0.59[bc] | 45.67[b] | 103.67[ab] | 8.83 | 12.811[b] | 0.57 | 1.60[b] |
| T3 | 100 | 0.78[ab] | 113.42[a] | 44.00[c] | 9.83 | 19.781[a] | 0.45 | 1.80[b] |
| T4 | 100 | 0.96[a] | 67.00[b] | 128.08[a] | 13.42 | 11.036[b] | 0.61 | 2.37[a] |
| *P* value | | < .001* | < .001* | 0.002* | 0.074 [ns] | < .001* | 0.951 [ns] | < .001* |

Note: * -significant, [ns] -not significant

# Discussion

## Study on pre-germination treatments

### Percentage germination

Possibly the reason for the low percentage of germination is due to the collection of the seeds from the ground, where there is a greater potential for insect attack and fungal infection. Though the ANOVA test revealed no significant difference among treatments in terms of percent germination ($p = .779$), soaking in hot water for 10 minutes (T4) is more effective because it enhances the absorption of seed coat permeability to water and maximizes the seed hydration that causes the seeds germinated effectively. The same result with the study of Azad *et al.* (2011) on seed germination of *Acacia auriculiformis* applied with different pre-sowing treatments, namely control, immersion in cold water, immersion in hot water, scarification with sandpaper, and immersion in concentrated $H_2SO_4$ wherein hot water treatment was recommended for this species for the reason that it gained the highest germination rate (83%). However, Ndor *et al.* (2012) concluded that seeds cannot germinate in boiled water due to the absence of oxygen.

**Percentage germinative energy**

Several studies confirmed that using hot water as a pre-germination treatment can improve the germination rate. Saikou *et al.* (2008) claimed that pre-treatment of *Acacia senegal* seeds soaked in hot water for 10 minutes increased its growth potential. This is also attributed to the *study on Acacia pilispina* seeds immersed in hot water for 1 minute gave significantly higher percent germination (97%) than all the other treatments used in the study of Teketay (1998) in consistent with study of Egharevba *et al.*, (2005) and Asiedu *et al.* (2011). This implies that subjecting seeds to hot water for a short period improved the germination rate since this affects the seed coat permeability, as revealed by the results of this study's percentage germination (48%) and percentage germinative energy (32.50%). Thus, prolonged contact with boiled water could kill the seed embryo, as mentioned by Amusa in 2011 and supported by Diamante and Vallesteros (2022) in their study on Supa seeds soaked in hot water for 24 hours.

Seeds soaked in cold water for 24 hours had the lowest percentage of germination (35%). The same was the report of Danthu *et al.* (1995) and Rasebeka (2014), who acclaimed that soaking in cold water was generally ineffective and showed the lowest germination percentage in Acacia species, namely *A. tortilis, A. erioloba*, and *A. nigrescen*s. This is affirmed by the findings of Amusa (2011) on the germination of *Afzelia africana* seeds due to oxygen deficiency.

## Study on the application of soil amendments

### Percent Survival

The high ratio of chicken manure is possibly the reason for the mortality rate of Supa seedlings. This is supported by Dikinya and Mufwanzala (2010), who state that using chicken manure improves the concentration of water-soluble salts in the soil. Thus, plants absorb nutrients through soluble salts, but excessive accumulation can suppress plant growth. Wong *et al.* (1983) also found that the acidity due to chicken manure addition severely affects root growth and seed germination. However, if chicken manure is applied correctly, it is a good soil amendment and/or fertilizer (Agbede *et al*., 2008).

### Growth Response of Supa

### Root Collar Diameter

The result implies that the application of sawdust positively affected the average root collar diameter of Supa seedlings due to its high porosity, high water retention, moderate drainage, and high bacterial tolerance. The result is in agreement with the study of Osmar (2020) on cacao seedlings with the application of compost composition of sawdust and manure with 1-2 ratios, wherein it was found that sawdust increases the root collar diameter. Moreover, Ashiono *et al.* (2017) concluded that sawdust and cow dung mixtures applied in Blue Gum (*Eucalyptus saligna*) had the highest height, root collar diameter, and biomass measurements at week 20.

### Shoot and Root Lengths

The effect on shoot length conforms with the study of Lazcano *et al.* (2010) wherein they observed positive effects of using vermicompost in forestry species such as acacia, eucalyptus and pine tree regarding shoot and root development. Similar to the study on the mango (*Mangifera indica* L.) cultivars applied with different growing mixtures wherein it was found that soil + sand + vermicompost (1:1:2) is the most effective for better germination of mango seedlings in terms of seedling height (Kaur, 2017). Regarding root length, the same findings were obtained to *Gongronema latifolia* wherein sawdust and soil gave a better performance in terms of root length (Agbo and Omaliko, 2006). Meanwhile, the lowest increase was observed in the seedlings treated with vermicompost, with a mean of 44 cm (SD = 33.85). This conflicts with the conclusion of Alvarez and Grigera (2005) that vermicompost promotes the rooting process and improves nutritional absorption as well as the growth and development of plants.

**Number of secondary roots**

The result was similar to the study of Fagbenro *et al.* (2013) on the response of *Moringa oleifera* Lam. seedlings to sawdust biochar and NPK 15:15:15 inorganic fertilizer amendments wherein they concluded that the said tree species responded positively to the sole application of either inorganic fertilizer or sawdust biochar over the non-treated control. Possibly, the elongation of secondary roots in Supa was greatly promoted by the increase of air-filled soil porosity, i.e., oxygen availability. According to Hardgrave and Harrisman (1995), sawdust improves the porosity and water retention of the growing media as compared with garden soil alone.

**Chlorophyll Content**

This is similar to the study of Ashiono *et al.* (2017) on Blue Gum (*Eucalyptus saligna*) seedlings wherein sawdust alone did not favor seedlings growth. As Garner (2014) reported, seedling growth is poor growth of seedling applied with sawdust because of its low nutrient content. Meanwhile, applying vermicompost to plants increased seed germination, stem height, number of leaves, leaf area, leaf dry weight, root length, root number, and chlorophyll content (Joshi *et al.*, 2015).

**Root Biomass**

The result is attributed to the study on *Carica papaya* seedlings applied with soil + vermicompost + vermiculite (1:1:1) found to be the best and significant medium for the growth of the said species with the highest growth parameters in terms of stem girth (5.90 mm), number of roots (16.06), root length (9.07 cm), and shoot and root biomass (4.66 g and 1.04 g, respectively) (Meena *et al.*, 2017). It also goes with the study of Bhardwaj (2014) wherein the mixture of vermicompost + soil + pond soil (1:1:1) significantly reduces papaya seedling mortality and produces maximum healthy seedlings (93.15%). This implies that vermicompost as growing media provides sufficient levels of oxygen to roots and adequate storage of water and nutrients for plants (Chaudhary *et al*., 2020).

### Shoot Biomass

As asserted by Ashiono *et al.* (2017) on the growth characteristics of *Eucalyptus saligna* seedlings wherein sawdust and cow dung mixtures had the highest measurements for height, root collar diameter and shoot biomass at week 20 of observation. The same study with Singh *et al.* (2020) on seed germination and seedling growth of *Carica papaya*.

## Conclusion

Based on the result of the study on pre-germination treatments, although there is no significant difference in the values for percentage germination and percent germinative energy, soaking in hot water for 10 minutes gave the highest percentage for both percentage germination and percent germinative energy and can, therefore, be favored all the other treatments. On the other hand, vermicompost is the best soil amendment for Supa seedlings, improving shoot length, chlorophyll content, and root biomass. Meanwhile, sawdust gave the highest mean in terms of root collar diameter, root length, and number of secondary roots. Therefore, these mixtures are recommended for Supa seedlings.

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