# Seed Dormancy Breaking and Germination Rate Improvement in Mucuna (*Mucuna bracteata*) Seeds using Mechanical and Fungicide Treatments

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**Abstract.** Mucuna (*Mucuna bracteata*) is a cover crop for young plants in oil palm plantations. However, it has a very low germination rate due to its hard outer shell and fungi infestation after germination. Thus, this study was conducted to determine the effectivity of mechanical treatment combined with 15-min of Dithane M-45 treatment to improve the germination rate of Mucuna's seed and its seedling growth. The experiment was performed with the combination of two factors: mechanical treatment and Dithane M-45 (0.00 g/L, 0.05 g/Lg, 0.10 g/L and 0.15 g/L) in pentaplicate for each group. The seeds were sowed on wet cotton, and the data was analyzed using ANOVA ($\alpha = 0.05$) followed by DMRT ($\alpha = 0.05$). The shell nicking seed followed by a 0.10 g/L of Dithane M-45 treatment resulted in the best germination rate (88 ± 9.1%), the fastest germination speed (6.29 ± 0.65 %), the shortest mean germination time (4.25 ± 0.87 days), the longest shoot, and the highest of fresh and dry biomass for both shoot and root of the seedlings. Therefore, the shell nicking and fungicide immersion before sowing the seeds could improve Mucuna's germination and seedling growth.

## 1 Background

Oil palm is considered to have a high economic value in the international market [1, 2]. Thus, it has recently become one of the strategic commodities in the plantation sector [2, 3]. Indonesia, the world's largest palm oil producer, has transformed the palm oil industry into a major contributor to foreign exchange earnings and a significant contributor to national economic development [1]. Oil palm plantation also provides employment opportunities, employing approximately 16 million workers, both directly and indirectly [4-6].

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An effective management in plantation practice plays a pivotal role in determining the optimal productivity of the oil palm[7-9]. Not only is it conducted on the mature oil palm, but it is also practiced on the seedlings (immature plants). Sustainable management overall stage of the plants significantly impacts the yield in an oil plant plantation [8]. This management also indirectly avoids a reduction of the land quality and even protects from land erosion [10].

Growing cover crops is one of the critical management practices in oil palm plantations. This practice is mandatory, especially during the early stage of oil palm cultivation when the topsoil is uncovered due to the narrow trunk coverage of the oil plant's seedling. Uncovered topsoil would significantly increase water evaporation, aside from being prone to land erosion [11,12]. Thus, it impacts the water availability for the young plants and puts the plants at a high risk of undergoing long- and short-term physiological damages, including reduction of chlorophyll content, lower photosynthesis rate, and prevention of the development phase of the young plants [13,14].

Mucuna (*Mucuna bracteata*) is a legume cover crop (LCC) species widely used in oil palm plantations. Mucuna is essential to support the optimal growth and development of oil palm seedlings, particularly in creating a more favorable microenvironment. This microenvironment encompasses soil conditions and the surrounding climate for the seedlings [15]. The study indicated that Mucuna could reduce surface runoff and soil leaching during wet sessions, besides could prevent water evaporation from the uncovered ground during dry sessions [11]. Further, Mucuna prevents the uncontrollable rapid growth of weeds through allelopathic compounds without considerably affecting the oil palm seedlings [16]. In addition, the phenolic compounds of the Mucuna are also unfavorable for general grassers and reduce the potential infestation of pest *Oryctes rhinoceros* in oil palm seedlings [17]. As a legume, Mucuna supports nitrogen fixation and enriches the soil nutrient contents [18].

However, difficult propagation of Mucuna becomes a challenge, especially through the generative method. The seed of Mucuna is covered by a hard outer shell that protects the bud and hinders germination for a particular period. This seed dormancy will be altered after outer factors break the hard outer shell and permit water imbibition that triggers the seed germination.

Naturally, the outer shell hardly breaks without mechanical force treatments. Farmers commonly used several methods, such as shell nicking and scarification, to scrap the outer layer [19]. Nevertheless, other obstacles interfere with the germination, including fungi infestation during the early germination stage. Our previous study indicated that the germination rate without appropriate treatments is considerably low, about 12% [20,21]. Hence, it requires solutions to improve the germination rate of the seed and avoid seedling death at the early growth stage.

On the other hand, a possible solution to combat fungi is by using fungicides. Dithane M-45 is a protective contact fungicide in the form of a yellowish-grey powder. It prevents the formation of spores in fungi to prevent their spread. Thus, this research aimed to investigate the effectiveness of the treatments to break dormancy combined with Dithane M-45 fungicide application to improve the Mucuna seed germination and the growth of its seedling.

## 2 Materials and Method

### 2.1 Location and Duration

The research was conducted at the Sinarmas Oil Palm Plantation, PT. Ivo Mas Tunggal, at Riau, Indonesia. The research was carried out from March to April 2023 at 101 meters above sea level.

## 2.2 Germination Responds to the Treatments

This study applied two factors of treatment. The first factor was mechanical treatments: shell nicking, shell scarification, and hot water immersion. The shell nicking treatment involved the ablation of the edge of the shell seed near its embryo using a clipper. The shell scarification was performed by rubbing the seeds on the sand scrapper. The seed immersion was done by soaking the seed in hot water (± 60°C) for ± 2 minutes. Following those treatments, the seeds were immersed in the Dithane M-45 solution for 5 minutes. The concentrations of Dithane M-45 solution used in this study were 0 g/L, 0.05 g/L, 0.10 g/L, and 0.15 g/L. Each treatment employed 20 seeds, and each group was replicated four times. The treated seeds were sowed on the humid cotton and placed under a shading area. The germination of the seeds was monitored for two weeks. The complete germination was indicated by the emergence of the hypocotyls.

## 2.3 The effect of the mechanical and fungicide treatments of biomass of *Mucuna bracteata*

The germinated seeds were planted in the polybag using the top soil of yellow podzolic soil as the media. The seedlings were grown for two weeks and harvested for its shoot and root. The length of the shoot was measured, and the biomasses of the wet shoot and root were then weighted. Afterward, the shoot and the root were dried using a desiccator oven and re-weighted for the dry biomasses (Table 1).

## 2.4 Data Analysis

The germination rate (GR), the speed of the germination, and the mean germination time (MGT) were calculated for the primary data of this study (Table 1). Data collected from the experiment was analyzed using two-way ANOVA (α = 0.05). Further, the post hoc test was performed (α = 0.05) to monitor the significant difference between treatments. The calculation was conducted in python 3.12 employing anova_lm and pairwise_tukeyhsd function from statsmodels library. Eventually, the data was visualized in the line graphs to compare the responses from the treatments using Ms. Excel.

**Table 1.** The method of calculating parameter of growth of *Mucuna bracteata*

| Parameters | Calculation Formula | Measurement Unit | Reference |
|---|---|---|---|
| Germination Rate (GR) | $GP = \frac{Ni}{N} x100$ | % | [22] |
| Speed of Germination (SR) | $SR = \sum_{i=1}^{d} \frac{ni}{Di}$ | %/day | [22] |
| Mean Germination Time (MGT) | $MGT = \sum \frac{ni \cdot Di}{Ni}$ | day | [22] |
| Shoot Length | Ruler | cm | [22] |
| Wet shoot biomass | scale | g | [22] |
| Wet root biomass | scale | g | [22] |
| Dry shoot biomass | scale | g | [22] |
| Dry root biomass | scale | g | [22] |

**Note:** N (the total number of seed), Ni (the total number of germinated seeds at the end of observation), Di (counting day), ni (germinated seeds per day)

# 3 Results and Discussion

## 3.1 Results

### *3.1.1 Germination Responds to the Treatments*

The result of the study indicated a significant effect of mechanical treatments combined with Dithane M-45 fungicide treatment on the germination rate, germination speed, and average days to germination of Mucuna seeds (p-value < 0.05 for all parameters). The best germination rate was achieved from the combination of shell nicking treatment with the 0.10 g/L of Dithane M-45 that, obtained 88 ± 9.1% within 14 days (**Fig. 1A**). Although insignificant, a higher concentration of the fungicide showed a slight reduction in germination rate of about 85 ± 6.1 %. A lower concentration revealed a lower germination by less than 60%. This trend was followed by other mechanical treatments (shell scarification and hot water immersion), where the higher fungicide concentration resulted in a higher germination rate.

In addition, the germination speed also displayed a similar pattern to the germination rate, in which the combination of shell nicking treatment with 0.10 g/L of Dithane M-45 resulted in the best record (**Fig. 1B**). On average, it was about 6.29 ± 0.65 % of seeds germinated per day. A lower concentration revealed a lower germination speed. A slight drop in germination speed was also monitored when the concentration of Dithane M-45 exceeded 0.10 g/L, yet it was statistically insignificant.

Further, the shell nicking treatment also showed the most effective treatment to foster seed germination compared to the other treatments (**Fig. 1C**). The effectivity of shell nicking to shorten the germination time increased when combined with 0.10 g/L of Dithane M-45 treatment, which showed the fastest germination time with an average of only 4.25 ± 0.87 days. If treated using other methods, the seeds needed more than 4.5 days to germinate.

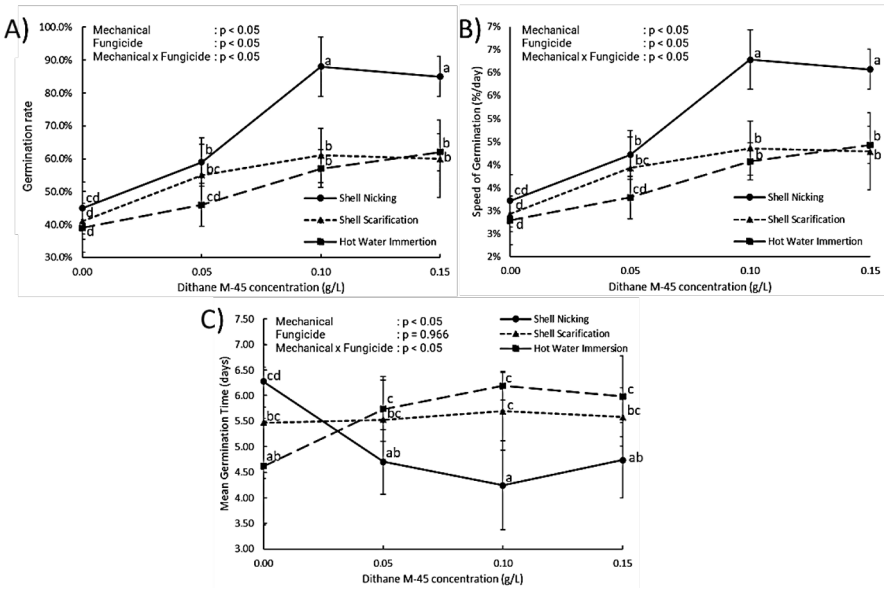


**Fig. 1.** The effect of mechanical treatments combined with Dithane M-45 application in the germination rate (A), the speed of germination (B), and the mean germination time (C) of *Mucuna bracteata* seeds. Note: [a,b,c,d] means followed by the same letter within the same species and different temperature are not significantly different at p-value < 0.05.

### *3.1.2 Assessment of Plant Growth Parameters*

**Fig. 2** indicated that the treatment combination between the shell nicking and 0.10 g/L of Dithane M-45 immersion positively impacted all parameters. After undergoing shell nicking and 0.10 g/L of Dithane M-45 treatment, the seedling achieved the longest shoot after 14 days with an average of 130.6 ± 4.88 cm. The treatment also affected the improvement of wet and dry biomasses of both shoot and root with 15.00 ± 1.58 gram and 6.00 ± 1.00 gram, approximately. The group threatened by the combination of shell nicking and 0.10 g/L of Dithane M-45 immersion also achieved the highest dry biomass for both shoot and root. The treatment group achieved 1.30 ± 0.16 gram and 0.54 ± 0.11 gram, respectively. However, the group undergoing the shell nicking and the higher concentration of Dithane M-45 (0.15 g/L) recorded almost similar results. Although the average records were slightly lower, the latter treatment group was statistically indistinctive. The statistical difference was only achieved when the groups were threatened by mechanical treatment other than shell nicking and the Dithane M-45 concentration of less than 0.10 g/L.

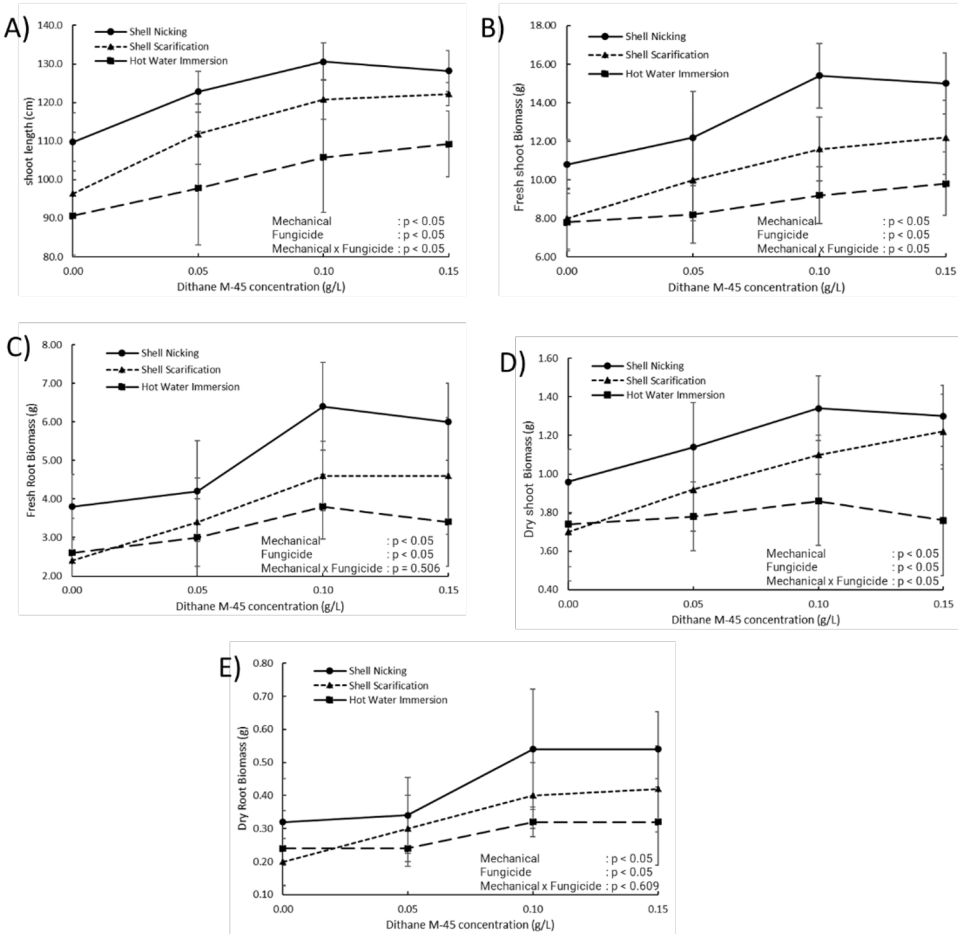


**Fig. 2.** The effect of mechanical treatments combined with Dithane M-45 application on the shoot length of *Mucuna bracteata* measured (A). The effect of the treatments on the fresh biomass (B & C) and dry biomass (D & E) of *Mucuna bracteata* shoot and root.

### 3.2 DISCUSSION

Seed germination is one of the earliest stages of growth that almost all plants should pass to conserve their species. The ability of seeds to germinate depends on various internal and external factors. The internal factors include the availability of energy and compounds for synthesizing seedling organs, including roots and shoots. The higher the availability of these compounds, the greater the seed's ability to germinate, indicating a high germination capacity and promoting the growth of essential plant parts such as shoots, leaves, and roots. At the same time, the external factors involve factors surrounding the seed. It could be biotic, such as pathogens or abiotic, such as water availability, mechanical stress, or temperature stress.

Mechanical stress is commonly beneficial for plants with a hard outer layer seed. A hard shell can protect the seed from animal predation, yet it can inhibit water penetration into the embryo and prevent germination. According to Siregar *et al*., the germination rate of untreated Mucuna seed was less than 20%. Others even reported only 0.91% [24]. Thus, the outer layer needs to be removed.

In this study, the shell nicking treatment, which opened up the outer layer of Mucuna seeds, resulted in the highest records for all parameters monitored. It means that the method is the best to allow water absorption and air penetration into the seed, facilitating the imbibition process and starting the germination process. According to [25, 26], shell nicking and shell scarification are the most common pre-treatment method applied to seeds with a hard outer shell, aimed at breaking dormancy and accelerating uniform seed germination. Naturally, the seed's outer shell abrades mechanically and chemically in the gastrointestinal tract of an animal [27, 28]. Some species also need to experience extreme heat or freeze to trigger germination [29].

On the other hand, pathogens also easily grow on the seed during or after the germination stage. It can hinder the growth of the seedlings or kill them at a high rate [30]. The most common pathogen is fungi that grow easily when the sowing medium is wet. The fungi usually come from contaminated sowing medium or from the seed itself [31]. Study by Bhat *et al*., indicated that various species isolated from Mucuna seed. Consequently, its apparently normal if the fungi are able to kill the seedling or induce growth faltering. The risk is even higher if the showing medium and the soil are contaminated with the naturally-occurred fungi. Thus, prevention should be taken from the beginning to avoid failure during seed sowing.

The fungicide application was proven to handle fungi infestation and avoid seedling death [30, 31]. The active compound in fungicide, Mancozeb, could interfere with the biochemical process within the cell cytoplasm and the mitochondria of the pathogenic fungi [32]. However, an excessive concentration of Dithane M-45 fungicide is suspected to inhibit the activity of enzymes in the seeds [32]. Thus, a slight declining effectivity was monitored at a higher concentration for all parameters.

The germination rate also showed linearity with the shoot growth and the increase of the seedling biomass. The successful germination and the avoidance of the seedling death by pathogens support the sustainable development of the seedling. The fungicide application reflected that pathogen probably plays an important role in seedling growth. Thus, the growth of the seedling was significantly better when the fungicide was applied. In other plants, the inhibition of fungal growth could be performed by co-culture with mutualism bacteria, cold atmospheric plasma (CAP), and various synthesis or natural fungicides [30-34].

## 4 CONCLUSION

According to the study, the application of mechanical treatment combined with Dithane M-45 treatment effectively improved the germination rate, germination speed, and mean

germination time of *Mucuna bracteata* seed. The seed nicking followed by 5-minute immersion in 0.10 g/L of Dithane M-45 solution revealed the best effect of the treatments. These treatments combination also positively affected the increasing length of the Mucuna shoot besides gaining more both wet and dry biomass of Mucuna shoot and root. Therefore, the pre-treatment of *Mucuna bracteata* seed prior to its plantation in oil palm plantation with the combination of shell nicking and 5-minute immersion in 0.10 g/L of Dithane M-45 solution seems to be promising to apply.

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