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Full Length Research Article

Effects of Storage-Induced Moisture Loss on Germination Performance and Oil Yield of *Pongamia pinnata*

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ABSTRACT

Pongamia (*Pongamia pinnata*) is a non-food plant that has the potential to be developed as a biofuel because its seeds' oil content is up to 40%. Therefore, it is necessary to study the characteristics of seeds for germination in relation to mass cultivation and information on oil content as a production consideration. This paper aimed to describe the sensitivity of pongamia seed moisture content to germination and its oil content and also to understand the seed category. Variations in moisture content were obtained from storing seeds at ambient temperature for up to 5 months. Moisture content, morphological characters, oil content, and germination rate were examined for each storage period. Data were analyzed using analysis of variance to determine the treatment effect. The results showed that storing seeds at ambient temperature caused a decrease in moisture content. A decrease in moisture content of 29.36% in the first month's storage significantly affected the reduction of morphological characters and seed weight. Meanwhile, during the 2–5 months storage period, the decrease in moisture content was only 2–5% and did not affect seed length. Reducing moisture content (MC) increased oil recovery but was not statistically significant. The best germination rate was 84.67%, obtained from fresh seeds (MC of 46.64%); the germination rate decreased as the moisture content decreased during storage. Pongamia should be categorized as an intermediate seed based on the seed moisture content characteristics and germination test results.

1. Introduction

Oil energy consumption in Indonesia is predominantly provided by fossil fuels (reaching 98%), while the rest can be supplied by natural gas and biodiesel (Suharyati et al. 2019). Fossil oil is a non-renewable source, and its production tends to decrease; on the other hand, energy demand is projected to increase (Suharyati et al. 2019). Energy from renewable sources is expected to be able to contribute to the supply of national energy. Renewable energy sources must be consistently developed to achieve self-sufficiency and reduce dependence on imports, often trapped in price turbulence. Indonesia has challenges and the potential to produce biofuels from several non-food crops, including pongamia (*Pongamia pinnata*).

In Indonesia, pongamia spread across in Sumatera, Java, Madura, Bali, Nusa Tenggara, and Kalimantan (Leksono et al. 2018). This species grows at an altitude of 0–1,200 m asl. Pongamia can self-fix nitrogen (Biswas and Gresshoff 2014) and tolerate various land conditions, including marginal land (Islam et al. 2021). This capability would contribute to the mass development of the species, which would provide commercial raw materials on degraded lands of at least 140,000 ha (Jaung et al. 2018). This action was taken to minimize the utilization of productive areas in the agriculture-plantation sector while contributing to land rehabilitation in Indonesia.

Pongamia oil content varies between 20–40% (Pavithra et al. 2013; Sharma et al. 2016). The oil is produced from seeds that can be processed into biodiesel (Cox et al. 2014; Dwivedi and Sharma 2014; Rengasamy et al. 2014) or for jet fuel (Shahriar and Khanal 2022). Some methods can extract oil from the seeds (Nde and Foncha 2020). Several methods have been used in extracting pongamia oil, namely pressing the seeds mechanically (Anand et al. 2019), using solvents (Sharma and Kumar 2021), microwave (Kumar et al. 2018) and ultrasound techniques (Sharma and Kumar 2021). Each extraction technique will provide different yield results.

Whatever method is used or a combination of both, seeds can play a role in influencing oil yield. The high seed oil content is important information because it considers economic feasibility. Fresh, mature seeds contain more oil than young seeds (Pavithra et al. 2012). Different provenance and family-influenced oil content (Jayusman and Pudjiono 2019) and family (Krisnawati et al. 2024).

After harvesting, seed quality will deteriorate gradually during storage (Kumar et al. 2011). As a recalcitrant seed, a decrease in moisture content will reduce germination ability (Aminah and Syamsuwida 2013). In addition, seed storage periods might be shorter for several weeks before germination (Suita et al. 2023). On the other hand, storing seeds will reduce the oil content, which will be significant after 24 months of storage (Deswal et al. 2015). The use of seeds for oil production will differ from the use of seeds for propagation. This research determined seed moisture content sensitivity by storing pongamia seeds on seed morphology characters, oil yield gain, and possible germination after storage.

2. Materials and Methods

2.1. Research Sites

Pongamia pods were collected from Batukaras. Administratively, it belongs to Cijulang District, Pangandaran Regency, West Java. Geographically, it is located at 07° 45' 8" South Latitude and 108° 30' 10" East Longitude (**Fig 1**). The pods were harvested in December 2022 at Batukaras and brought to Bogor to be processed. Oil extraction was conducted at the Seed Laboratory in Bogor, and germination was conducted in a greenhouse at Nagrak Sukaraja Bogor. Both facilities are managed by the Center for Implementation of Environmental and Forestry Standards and Instruments, Bogor. The research was conducted for 7 months, from December 2022 to June 2023.

2.2. Seed Handling

Ripe pods were collected and selected because of their excellent visual appearance and because they were free from pests and mechanical injuries (Supriyanto et al. 2017). Pods were divided into six parts, placed in six open polynets separately, and then stored for six periods (0, 1, 2, 3, 4, and 5 months). During storage, the polynets were placed at ambient temperature of 28–30°C and relative humidity of 77–84%). Pod extraction was done manually by opening the pods using scissors or knives to get the seeds. Seeds without storage treatment were immediately measured.

Fig. 1. Collection site of the *Pongamia pinnata*.

2.3. Morphology Characters

A total of 200 seeds divided into four replications were measured for their morphology characters, such as length, width, thickness and weight. Length, width, and thickness were measured using a vernier caliper (Mitutoyo) with an accuracy of 0.1 mm. Seed weight was gained using a digital electronic balance with an accuracy of 0.1g. Seed volume, eccentricity index (EI) and flatness index (FI) were calculated using Equation 1-3 (Cervantes et al. 2016; Lestari and Pratiwi 2022).

$$\text{Volume} = \text{seed length x seed width x seed thickness} \quad (1)$$

$$\text{Eccentricity index (EI)} = \frac{\text{Seed length}}{\text{Seed width}} \quad (2)$$

$$\text{Flatness index (FI)} = \frac{\text{Seed length+seed width}}{\text{2 x seed thickness}} \quad (3)$$

2.4. Oil Extraction

The oil content of the seeds was estimated using a mechanical method. The oil was extracted using a hot screw press machine. A total of 450g of seeds that had been separated based on fresh weight were divided into three replications. The seeds were cut in half following the manual instruction from the press machine. The temperature of the press tool was positioned at 200°C. Pressing was done gradually for each iteration, followed by the next iteration. Tool cleaning was carried out during the pressing period between repetitions. The oil content value was calculated by dividing the weight of the oil (g) by the weight of the seeds (g).

2.5. Moisture Content

Measurement of moisture content followed the oven-dry method (ISTA 2012). A total of 5 g of seeds were withdrawn as a sample for measuring the moisture content at the time of oil extraction and seed germination. Seeds were dried using an oven at 103 ± 2°C for 17 hours. Moisture content was calculated using Equation 4.

$$MC = \frac{(WCBd - WCAd)}{(WCBd)} \times 100\% \qquad (4)$$

where *MC* is moisture content (%), *WCBd* is the weight of the container and seeds before drying (g), and *WCAd* is the weight of the container, cover, and seeds after drying (g).

2.6. Germination of Seeds

Pongamia seed germination refers to (ISTA 2012). Germination was conducted in a plastic bed of 30 cm × 20 cm × 15 cm, containing media soil: sand (v/v = 1:1). A total of 20 seeds with three replications were sown for germination. Plastic beds were then placed in a greenhouse with fog-colling system facilities. Observation of seed growth begins when the seed germinates, viz., shoots emerge and ends after 2 leaves appear. The observed parameters were germination capacity, rate, mean germination time, and value (Sudrajat et al. 2017a).

2.7. Data Analysis

The observed data were analyzed for variance (ANOVA) in a completely randomized design to test the effect of storage on the observed parameters. Percentage data is transformed into arcsin before ANOVA is performed. Pearson correlation analysis was conducted to determine the relationship between the parameters. All statistics were performed using Minitab (version 20.3) at $p < 0.05$ significant level.

3. Results and Discussion

3.1. Morphological Characteristics

The mean value of pongamia seed size at different storage periods is presented in **Table 1**. Fresh seeds have the highest moisture content and significantly differ from seeds after storage. The value of fresh seeds' moisture content obtained was lower than moisture content of pongamia seeds from several other regions in Indonesia such as Banyuwangi-East Java 51.37% (Aminah and Syamsuwida 2013), Carita-Banten 57.05–63.39% (Suita et al. 2023; Suita and Syamsuwida 2015), Ciamis-West Java 63.39% (Suita and Syamsuwida 2016), Ujung Kulon National Park-Banten 54.9% (Jayusman 2012) and also seeds source from other country such as Sri Lanka 51% (Jayasuriya et al. 2013). The average moisture content of fresh seeds was 46.64%, and the maximum decrease (29.36%) occurred in just 1 month of storage. In the 2-month storage period, the seed moisture content decreased to 5.18%; in the subsequent period, it only reduced by less than 2%. The Moisture content was significantly different in each storage period.

A decrease also followed the seeds' moisture content reduction during storage in the seeds' dimensions. Fresh seeds with the highest moisture content show the largest dimensions (length, width and thickness) compared to seeds with lower moisture content after storage. The decrease in moisture content after 1 month of storage was 29.36%, followed by shrinkage in the dimensions of length (2.04 mm), width (2.25 mm) and thickness (1.89 mm). The difference in values between fresh seeds and seeds after storage is statistically significant ($p < 0.05$). However, the dimensional shrinkage during a storage period of 1–5 months showed a narrow range of values and was not significantly different. In general, seed dimensions decreased along with decreasing moisture content with the same decreasing pattern for all characters (**Fig 2**).

Table 1. Variation of pongamia seed size at different moisture content by storage

Storage period (months)	Moisture content (%)	Length (mm)	Width (mm)	Thickness (mm)	Weight (g)
0	46.64 ± 0.76 a	19.52 ± 2.76 a	16.80 ± 1.31 a	11.69 ± 0.89 a	2.43 ± 0.53 a
1	17.28 ± 0.12 b	17.48 ± 2.7 b	14.55 ± 1.61 bc	9.80 ± 1.69 b	1.49 ± 0.34 b
2	12.10 ± 0.04 c	17.08 ± 2.7 b	14.17 ± 1.8 cd	9.32 ± 1.57 cd	1.18 ± 0.4 d
3	11.50 ± 0.02 d	17.5 ± 2.78 b	14.73 ± 1.71 b	9.67 ± 1.49 bc	1.32 ± 0.38 c
4	10.79 ± 0.02 e	17.01 ± 2.65 b	14.00 ± 1.79 d	9.10 ± 1.66 d	1.3 ± 0.36 c
5	9.11 ± 0.19 f	17.63 ± 2.68 b	14.32 ± 1.34 bcd	9.50 ± 1.42 bcd	1.36 ± 0.34 c

Notes: Values in the same columns followed by different letters (a-f) are significant ($p < 0.05$).

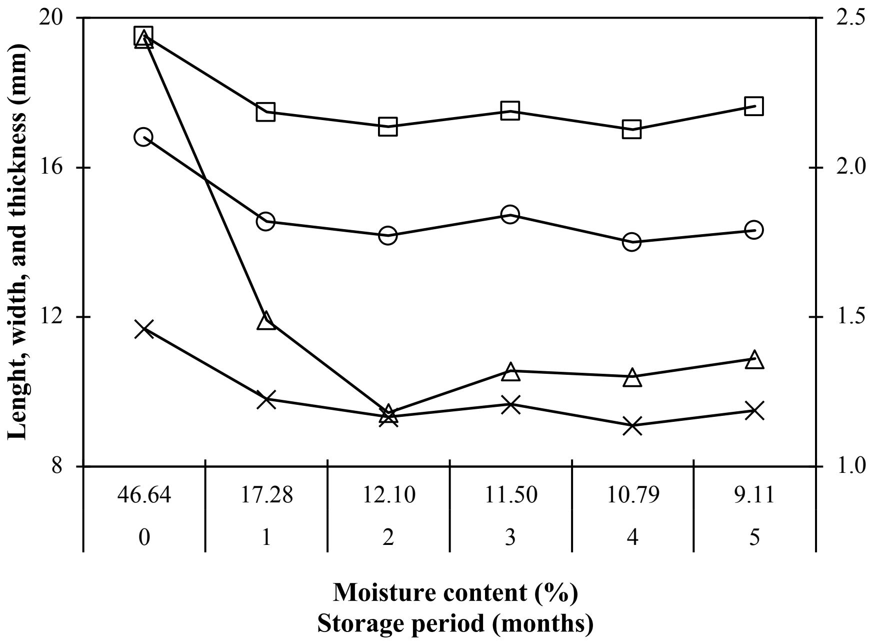
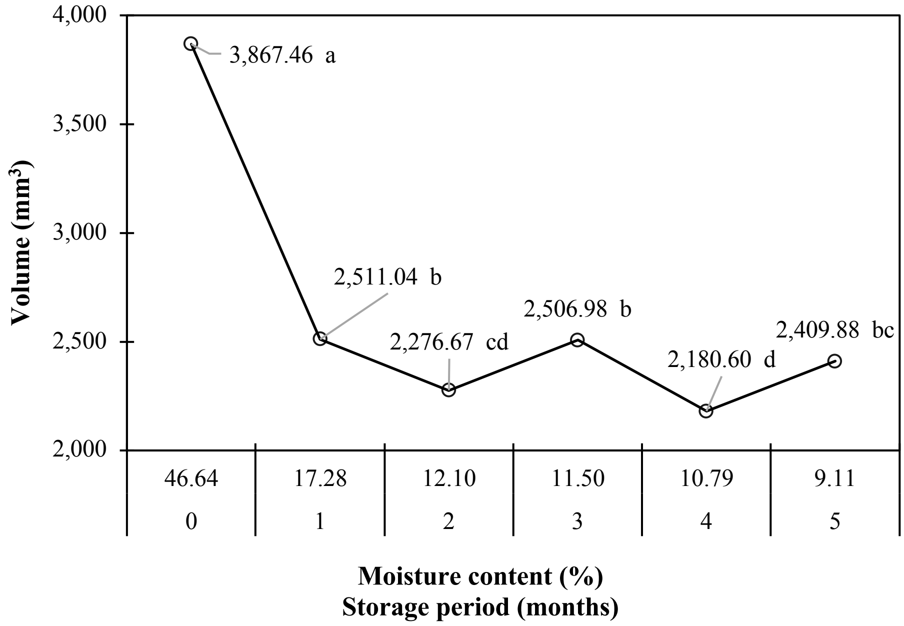
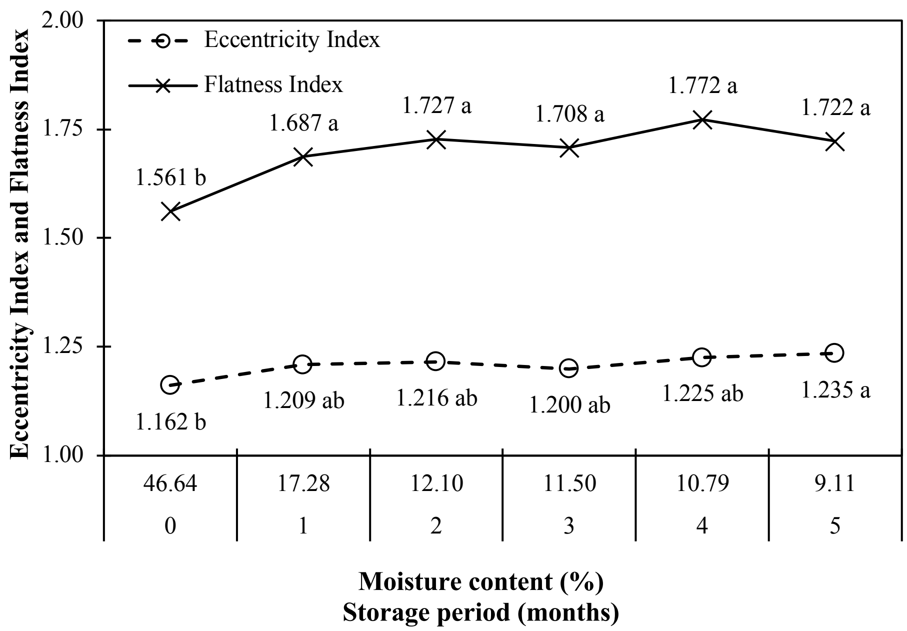


Fig. 2. Variation of seed size characters with moisture content after storage (□) length; (○) width; (×) thickness; (Δ) weight.

A decrease in moisture content also causes a reduction in seed weight (**Fig 2**). The weight of fresh seeds compared to seeds after storage is significantly different. The moisture content decrease of fresh seeds after 1 month was 29.36%, followed by a reduction in seed weight of 0.94 gr. A decrease in moisture content of >5% significantly impacted reducing seed weight. The seed weight change was insignificant below this value (**Table 1**).



(a)



(b)

Fig. 3. Variation of seeds shape with moisture content after storage (a) volume; (b) eccentricity index and flatness index.

There were changes in the shape of fresh seeds and seeds after storage, as seen from the volume, eccentricity index and flatness index (**Fig. 3**). The volume of seeds decreased along with the storage period (**Fig. 3a**). The maximum volume shrinkage was achieved in 1 month after storage (1,356.42 mm^3). Statistically, the volume of fresh seeds was significantly different than after storage. During the storage period, there was a fluctuation in the volume of seeds, but in general, the seeds experienced a decrease in volume at the end of the observation and looked wrinkled but not damaged/rooted. Estimation of seed shape based on EI showed that EI values increase until the observation's end. Significant differences only occurred in fresh seeds compared to seeds after 5 months of storage (**Fig. 3b**). Seed shape estimation based on EI shows that the EI value increases until the end of the observation. The only real difference occurs in fresh seeds compared to seeds after 5 months of storage (**Fig. 3b**). Similar to EI, the FI value also increases (**Fig. 3b**). However, fresh seeds are significantly different from all seeds after storage.

3.2. Oil Content

Oil extraction at several seed moisture contents resulting from storage treatment showed increased oil content (**Fig 4**). An increase occurred in the range of 3.4%, which was from 18.98% fresh seeds to the highest amount of 22.38% at the end of the storage period. Statistically, seed storage up to 5 months did not affect the oil content.

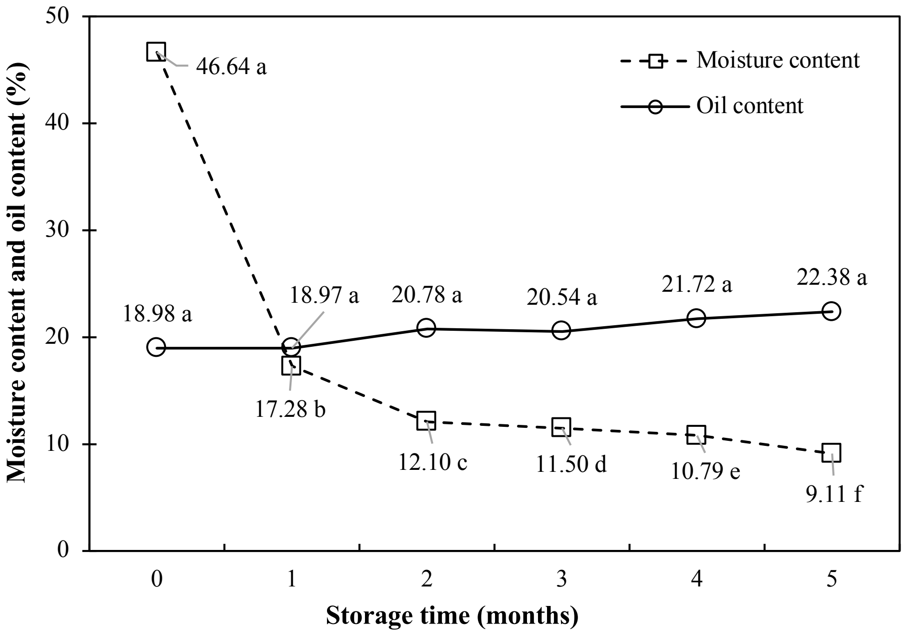


Fig 4. Oil content yield and moisture content based on the storage period.

The mechanical extraction process using a hot screw press machine is thought to evaporate seeds' moisture content and extract oil simultaneously, even from fresh seeds containing 46% moisture content. Using this method, the heat in the machine evaporates the water contained in the seeds, and then the press machine extracts the oil with a maximum yield of 18.98%. There was no pre-treatment of the seeds, such as drying or heating, to reach a specific moisture content or repeated pressing so the seeds could release oil immediately after being hot pressed. The amount of oil obtained was more significant than that of conventional mechanical presses 13.13–15.59% (Jayusman and Pudjiono 2019) and 15.92–19.6% (Hasnah et al. 2020). Higher results can be obtained if other extraction methods, such as solvents, are used (Krisnawati et al. 2024)

Based on these results, maximum oil content can be obtained using a combination of seed drying and hot screw press machine. The difference in oil yield reached 3.4% compared to without seeds drying treatment. Reducing seeds' moisture content may make extraction easier (Krisnawati et al. 2024). Preparing seeds in optimal dry conditions has also been carried out for several species to obtain maximum oil yield, such as *Callophyllum inophyllum* (Fadhlullah et al. 2015), *Pongamia pinata* (Krisnawati et al. 2024) and *Moringa oleifera* (Adejumo et al. 2013).

3.3. Seed Germination

Seed storage influenced all germination observation parameters. Fresh seeds gave the best germination response and significantly differed from other treatments (**Table 2**), while storage for 5 months resulted in zero germination. Generally, the longer the seeds are stored, the lower the moisture content, germination percentage, germination rate, average germination time, and germination value gained (**Fig. 5**).

Table 2. Germination average of pongamia seeds based on storage treatment

Storage period (month)	Moisture content (%)	Germination (%)	Germination rate (%/day)	Mean germination time (day)	Germination value
0	46.64 ± 0.76 a	84.67 ± 0.03 a	2.62 ± 0.62 a	23.49 ± 4.29 a	7.65 ± 0.75 a
1	17.28 ± 0.12 b	65 ± 0.05 b	0.71 ± 0.06 b	7.92 ± 0.66 b	4.08 ± 0.46 b
2	12.10 ± 0.04 c	50 ± 0.05 c	0.59 ± 0.05 bc	8.14 ± 0.97 b	3.15 ± 0.63 bc
3	11.50 ± 0.02 d	40 ± 0.05 c	0.46 ± 0.06 bc	6.68 ± 0.86 bc	2.03 ± 0.5 c
4	10.79 ± 0.02 e	13.33 ± 0.03 d	0.11 ± 0.03 bc	2.31 ± 0.46 cd	0.17 ± 0.06 d
5	9.11 ± 0.19 f	0 e	0 c	0 d	0 d

Notes: Values in the same columns followed by different letters (a-f) are significant ($p < 0.05$).

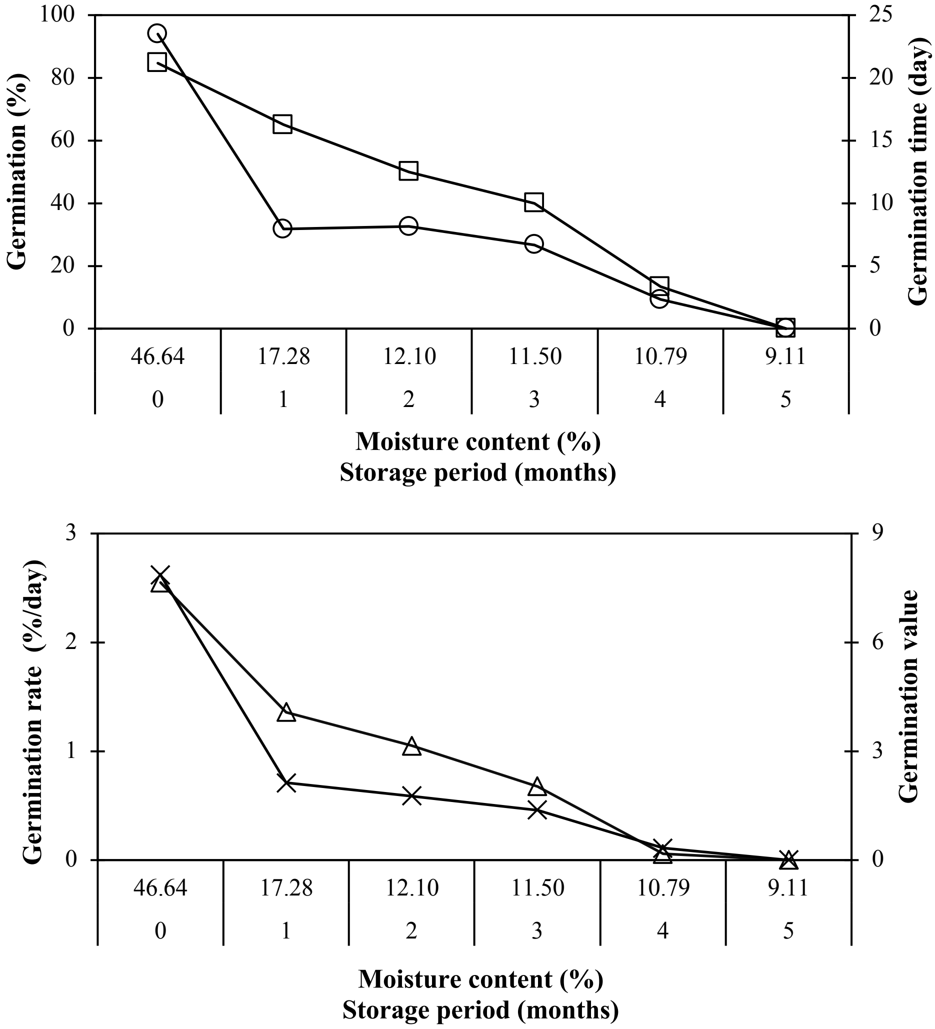


Fig. 5. Variation of germination of seeds with moisture content after storage
(□) germination; (○) mean germination time; (×) germination rate; (Δ) germination value.

Pongamia seeds have a high initial moisture content (46.64%). Seeds that have a moisture content when ripe of 30–70% are recalcitrant (Schmidt 2000; Sinery et al. 2024). Recalcitrant seeds cannot be stored for long periods due to their high moisture content (Triani 2021). Recalcitrant seeds are not drought-resistant, cannot be stored for long periods, and have a moisture content of > 30% (Sudrajat et al. 2017b; Yuniarti et al. 2016). In contrast to malapari seeds, although the moisture content at physiological maturity is high (46.64%), after being stored for 4 months until the moisture content is 10.79%, they still germinate (13.33%) (**Table 2**). The moisture content can even be reduced to 6–9% (Deswal et al. 2015). Pongamia has been classified as a recalcitrant (Aminah and Syamsuwida 2013; Jayasuriya et al. 2013). Referring to Hong and Ellis (1996) regarding the determination of seed character, malapari seeds should be categorized as intermediate seeds, namely seeds that have a character between orthodox and recalcitrant. Their moisture content can be reduced to 8–12% and stored for less than 1 year (Sudrajat et al. 2024). The character of the seed is similar to the character of other seeds with high oil content, namely *Sterculia foetida*, which has a high initial moisture content (42.4%) but can still be dried to a moisture content of 9–12% (Sudrajat et al. 2011).

The correlation analysis (**Table 3**) showed that all seed morphology characters (length, width, thickness) and weight were positively and significantly correlated. Moisture content, which affects the seed morphology, also showed a positive and significant correlation. The germination positively and significantly correlated to other parameters except seed length. Only oil content was not influenced by all parameters. Even though the seeds sourced from several parent trees of provenance showed differences in seed size, this did not determine the pongamia oil content (Arpiwi et al. 2013; Danu et al. 2024).

Table 3. Correlation between seed morphology, moisture content, oil content and germination value

	Length	Width	Thickness	Weight	Oil content	Moisture content
Width	0.983 **					
Thick	0.986 **	0.994 **				
Weight	0.977 **	0.972 **	0.98 **			
Oil content	-0.557 ns	-0.653 ns	-0.682 ns	-0.621ns		
Moisture content	0.949 **	0.968 **	0.975 **	0.982**	-0.697ns	
Germination value	0.798 ns	0.862 *	0.881*	0.827*	-0.893*	0.904*

The best germination was obtained from fresh seeds without storage, which also occurs in *Cananga odorata* (Nurhayani and Wulandari 2019) and *Pithecellobim lobatum* (Manurung et al. 2016). The moisture content of fresh seeds in this study was 46.64%, on average. This value was lower than fresh seeds reported by other studies (Aminah and Syamsuwida 2013; Jayusman 2012; Nurdin et al. 2022; Suita et al. 2023; Suita and Syamsuwida 2015, 2016). It was suspected due to slight evaporation by transportation from the source (Ciamis) to the laboratory (Bogor) and loss of moisture during the seed extraction process. The maximum germination (84.67%) was also lower than in some other studies, which can reach 100% without any seed treatment (Jayasuriya et al. 2013). The longer the seeds were stored, the germination parameter decreased. No germination was found at the end of storage (5 months, 9.11% moisture content).

There was a positive correlation between moisture content and germination (**Table 3**). The higher the moisture content of fresh seeds will give the maximum response to germination. This condition occurred in several experiments on pongamia seed germination and other types (Jayasuriya et al. 2013). For germination purposes, seeds can be stored at ambient temperature for 12 months (Deswal et al. 2015). The rest requires specific storage techniques and methods to be stored longer while maintaining viability, such as storing seeds in a particular condition (Sandeep and Saswat 2015), different seed moisture content, temperature and types of containers (Deswal et al. 2015) or using additional media (Suita et al. 2023). The results of this study indicated that pongamia seeds could still germinate after 4 months of storage at ambient temperature, even though the germination rate was meager (13.33%).

4. Conclusions

Storing pongamia seeds at ambient temperature decreases the moisture content of the seed. Moisture content changes affect the morphological characters, oil content, and germination ability. Decreasing moisture content significantly reduces the dimensions and seed weight in terms of morphological characters. The first month's storage significantly reduced the moisture content by 29.36% and reduced seed dimensions and weight. After that, moisture content decreased from 2–5% per month and did not affect the reduction in seed length but affected other seed dimensions. A decrease in moisture content increased oil yield. Seeds with a moisture content of 9.11% produced 22.38% oil using the hot screw press extraction method. A decrease in moisture content reduced the ability of seeds to germinate. The best germination rate has resulted from fresh seeds. Storing seeds at ambient temperature for up to 5 months resulted in a moisture content of 9.11% and no germination. Moisture content correlates closely with dimensions (length, width, thickness), seed weight, and germination value but not with oil content. Based on the seed moisture content characteristics and germination test results, pongamia seeds should be categorized as intermediate.

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