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# Effect of harvesting *Jatropha curcas* L. seeds at different fruit maturity levels on germination, oil content and seed weight

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**ABSTRACT**

The physic nut is a plant species that produce seeds rich in oil that could be transformed into biodiesel; which was native of Central America and Mexico and now naturalized in Ethiopia at farm boundary and hedges. Owing to increasing demand for fossil fuel and its predicted decline, the importance of alternative energy has been increased in Ethiopia. However, research outcomes lack on the effect of fruit maturity levels of physic nut seed on oil content, seed weight and germination parameter. Therefore, this study aims to estimate the maturity level at which physic nut fruits should be harvested for high content of oil, seed weight and germination. Physic nut fruits harvested at different level maturity on mother tree young green fruit, yellow fruit, brown fruit and brown dried fruit dropped on the ground to determine oil content, germination and seed weight. Germination was tested in seed germination chamber in glass house and arranged in CRD with three replicates. In each treatment 100 seeds were sown and germination was recorded starting from 7th day of sowing to 35th day in seven day interval. Randomly 40 g of seed from each treatment were used to obtain oil by using solvent extraction method and 1000 seeds were also counted and weighted with three replicates. Variation in oil content, germination and its parameters and seed weight were analyzed using ANOVA and LSD at $P < 0.05$. The analysis shows that there is a relation between physic nut fruit maturity level with germination, oil content and seed weight. The germination (%), oil content and seed weight of physic nut increase from young green fruit, yellow fruit and brown fruit. But at late maturity mean that brown dried fruit dropped on the ground became reduced. The highest germination, oil and seed weight has been gotten from brown fruit on mother tree (90%, 34.59% and 592.1 g) and the lowest is from young green fruit (65.67%, 22.88% and 416.16 g), respectively. Weight and oil contents are positive relation: higher seed weight has higher oil content. Therefore, brown fruit on mother tree was ideal for higher germination, oil content and seed weight than yellow fruit and brown dried fruit dropped on the ground. This information will be important for the development of physic nut plantation in Ethiopia.

**Keywords:** *Jatropha curcas* L., maturity level, germination, germination parameter, oil content, seed weight.

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## INTRODUCTION

Physic nut (*Jatropha curcas* L.) is a plant which is native of Central America and Mexico and naturalized throughout the tropics and subtropics (Heller, 1996; Sarin et al., 2007; Achten et al., 2008). Therefore, *J. curcas* is grown in the continent of Africa, Latin America and Asia aiming to produce biodiesel (Ncube et al., 2012). The species has been characterized as a tall bush or a small tree (up to 6 m height), bearing seed that belongs to the botanic family Euphorbiaceae (Batin, 2011; Mohan et al., 2011). The genus Jatropha contains approximately 170 known species. It can survive with as little as 250 to 300 mm of rainfall but requires at least 600 mm to flower and to produce seed. The optimum temperatures for physic nut are between 20 to 28°C. The best soils for physic nut are aerated sands and loams of at least 45 cm depth. Physic nut is known for its ability to survive in very poor dry soils, in marginal conditions for agriculture. However, survival abilities do not mean that high productivity can be obtained under marginal environments. The genetic diversity of *J. curcas* is limited. Although, the plants are highly cross pollinated and can easily pollinate even with other species of Jatropha, even to-date superior hybrids of *J. curcas* are unavailable for large scale cultivation (Mohan et al., 2011). The seed production of physic nut ranged from approximately 0.4 to more than 12 t $ha^{-1}$ $y^{-1}$ after 5 years of growth (Achten et al., 2008).

Physic nut is believed to be spread from its center of origin via Cape Verde and Guinea Bissau to other countries in Africa and Asia. Due to its adaptation to wide environment tolerance to degraded land (Sarin et al., 2007), easy propagation and for its multiple purpose uses, it has been grown in wide areas in the world. In Eastern Africa, physic nut is cultivated as hedge, for erosion control, exclusion of animals from food crops and demarcation of properties particularly farm land. In Ethiopia, physic nut is grown traditionally for the same purposes and found abundantly in different areas, for example in Goffa and Mirab Abaya of Southern nations nationalities and people region, Shoa Robit and Bati of the Amhara region and Wolenchiti and Upper Awash in the Oromia Regions (Getinet, 2010).

Owing to declining petroleum reserves and the need for supplementing and replacing it, alternative renewable, non-toxic, and less polluted energy has attracted the interest of many countries (Worang et al., 2008; Moncaleano-Escandon et al., 2013). In this regard, Jatropha seed composition has high content of oil 25 to 40%, which has 73% of unsaturated fatty acids making the plant ideal for biodiesel industries (Kumar and Sharma, 2008; Moncaleano-Escandon et al., 2013). The Jatropha seed roughly comprises 47% crude fat, 25% crude protein and 8% carbohydrate (Ncube et al., 2012). In general, the interest of Jatropha as a biodiesel feedstock has increased due to environmental, energy security, economic and rural development factors.

For fossil fuel importing countries like Ethiopia, biodiesel is considered as alternative energy source for producing indigenous energy and reducing foreign currency. Currently, the Ethiopian government attempts to build green economy that can be supplemented by enhancing energy supply of biodiesel from non-edible oil. The Ethiopian government has been promoting expansion of *Jatropha curcas* L. plantation by providing significant area of land for investment. It also encourages the growing of this plant by individual farmers or cooperatives in degraded land striving for its dual purposes. Small and medium industry has been established for extracting oil from Jatropha seeds. In some area, such as Wello, marketing the seed of this plant is quite common.

In Ethiopian condition, small holders' production from hedges established for live stock barriers as well as property demarcations would be good source of Jatropha seed production. Farmers can harvest the physic nut seed from existing stands and deliver to the collection point usually at the farmer's cooperatives which will again deliver the bulk to the processors, Wello area can be taken as practical example. Such harvests incur little cost including harvesting and delivering. Once established, require little water and fertilizer, can survive on infertile soils, and is not browsed by cattle (Sarin et al., 2007) and making then suitable for cultivation on degraded soils (Achten et al., 2010).

The volume of oil that can be extracted from physic nut seed depends on the quantity and purity of its seeds. Escalating the production of seed depends on successful plantation which primarily requires, among other, viability of seed. The quality of physic nut seed depends on the degree of fruit maturity, appropriate container and storage condition, duration of storage, good viability and vigor of seeds (Worang et al., 2008). The proper time for harvesting physic nut seed is important for oil quality and quantity and viability of seed. Therefore, it is important to identify the proper fruit maturity stage for seed viability and quantity. This study aims to estimate the right stage of maturity at which physic nut fruit has to be harvested for higher germination, oil content and seed weight. This information will be important for the development of physic nut plantation in Ethiopia.

## MATERIALS AND METHODS

### Site description

*Jatropha curcas* L. fruits were harvested from vigorous mother tree at different fruit maturity level, via young green fruit, yellow fruit and brown on mother tree fruit and brown dried fruit dropped on the ground from Adami Tulu trial site in June, 2014 and air-dried. Damaged seeds were hand-picked out. For each fruit maturity level that showed in Figure 1A, B, C and D, 20 kg were collected. Each seed was dried in shadow room until 14% water content. Adami Tulu district is situated at latitude of 7° 19' N to 7° 40' N and '35 38° 30' E to 38° 53 'E and an altitude ranges from 1500 to 2000 m above sea level. The district is located at 160 km to the south of Addis Ababa the capital city of Ethiopia. The agro ecological zone of the district is semi-arid and sub-humid in which 90% of the area is low land while the remaining 10% is intermediate. The mean annual rain fall ranges from 750 to 1000 mm with the average minimum and maximum of temperature is 25 and 28°C, respectively. Rainfall distribution is highly variable between and within years (Adami Tulu Agriculture Research Center, 2014).

Germination experiment was conducted at Glass House in a growth chamber at Wondo Genet College of Forestry and Natural Resources in November, 2014. It is in Southern Nations, Nationalities and Peoples Regional State of Ethiopia. Wondo Genet located at 07° 192' N and 038° 382' E. It is 263 km far from Addis Ababa at south direction, altitude 1820 masl, total annual rainfall 1133mm, monthly maximum and minimum temperatures of 26.3 and 12.4°C (Fekadu, 2006). 300 seeds were randomly taken from each physic nut fruit maturity level and from each treatment 100 seeds were used for germination by using complete randomize design with three replications. Sandy, forest and compost soil in 1:2:1 ratio was used and filled to germination chamber to made nursery bed. Seeds were sown to each chamber by manual to the depth roughly equivalent to its size. Germination (emergency of plumbed to soil surface) was monitored for thirty five days. Seedling


**Figure 1.** Different maturity level of physic nut (A, young green fruit before maturity harvested on mother tree B, brown dried capsule dropped on the ground C, Yellow fruit harvested on mother tree, D, Brown dried fruit harvested on mother tree).

was started to germinate on fourth day (Figure 2A). It was counted every seven day at 9:00 am starting from seven day of its sowing. At 35th day, seeds that did not germinate were checked whether dead, live, or missing for unknown reason (Figure 2B). Seed germination percent in every seventh day was calculated.

Also from each maturity level of physic nut randomly 40 g of seed sample was taken and prepared for oil content analysis with three replications. Oil yield was obtained by using solvent extraction method at to Wondo Genet Agriculture Research Center, Natural products Laboratory Solvent extraction method procedures was explained as follow: Each sample was weighted and grounded and then added to tamper and closed by cotton. After that it added to soxhlet » equipment set up was made » hexane was added to soxhlet » flask and soxhlet was joined » and heated for four hours » rotary evaporation was made (using water base by heating by 40°C) to separate physic nut oil and hexane because of hexane is volatile and physic nut is non volatile. Oil content was calculated by:

$$Percentage\ of\ Oil\ content = \frac{\text{Oil yield obtained}}{\text{Sample weighted}} \times 100$$

Randomly 1000 seeds were taken, counted and weighted from each treatment with three replications. Experimental data was statistically analysis by analysis of variance ANOVA using (SAS Version 9.0) PROC GLM (2002) at $P < 0.05$. Differences between means were assessed using the least significance difference (LSD) test at $P < 0.05$. (Table 1).

**Seed germination**

The following seed germination associated parameters were calculated (Gairola et al., 2011) and the variation in those parameters was analyzed statistically using one way ANOVA and LSD.

***Speed of germination***

Speed of germination was calculated by the following formula given by Gairola et al. (2011).

Speed of germination= $n_1/d_1 + n_2/d_2 + n_3/d_3 + \ldots + n_m/d_m$

Where, n = number of germinated seeds; d = number of days; $_m$ = $m^{th}$ round count/days

***Mean germination time***

Mean germination time (MGT) was calculated by the formula given by Ellis and Roberts (1981):

MGT = $n_1 * d_1 + n_2 * d_2 + \ldots + n_m \times d_3$/ Total number of days

Where, n = number of germinated seed; d = number of days; $_m$ = $m^{th}$ round count/days

***Mean daily germination***

Mean daily germination (MDG) was calculated by the following

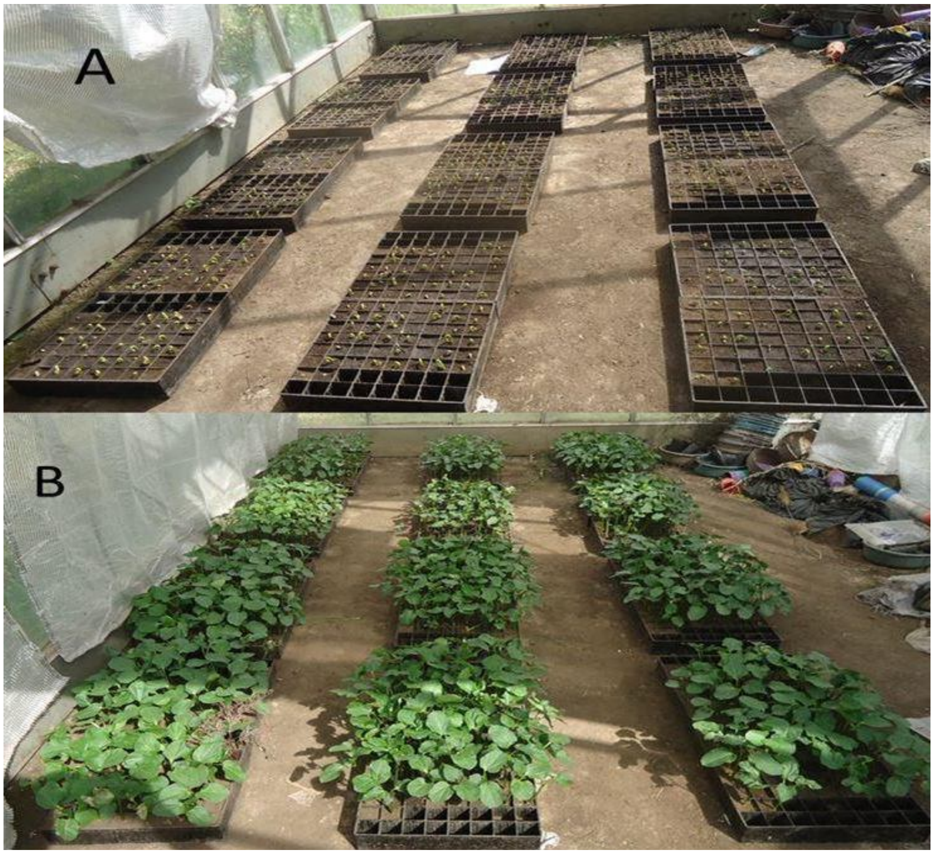


**Figure 2.** Seedlings of physic nut germinated in Wondo Genet College of Forestry and Natural Resources at Glass house.

**Table 1.** Treatment description.

| Treatment | Fruit maturity state |
|---|---|
| 1 | Green before maturity and harvested on mother tree |
| 2 | Yellow fruit harvested on mother tree |
| 3 | Brown dried harvested on mother tree |
| 4 | Brown dried fruit collected from the ground |

formula given by Czabator (1962):

MDG = Total number of germinated seeds / total number of days

#### *Peak value*

Peak value (PV) was calculated by the following formula given by Gairola et al. (2011):

PV = Highest seed germinated / Number of days

#### *Germination value*

Germination value (GV) was calculated by the following formula given by Czabator (1962):

GV = PV × MDG

## RESULT AND DISCUSSION

### Relation between fruit maturity level and germination

The different four maturity level of *Jatropha curcas* L. (physic nut) has been conducted to analysis germination percent. These are: young green, yellow and brown on mother tree and brown dried fruit dropped on the ground (Figure 1). The germination data has been taken starting from 7$^{th}$ days to 35$^{th}$ days for 5$^{th}$ times in 7$^{th}$ days intervals. The analysis of germination percentage from different fruit maturity level is found as follows; the highest germination was found from brown dried on mother tree (90%) and the lowest is found from young green (65.67%). The germination percentage is

**Table 2.** Different maturity level of physic nut seed effect on germination percent calculated every 7th days during 35th days.

| Treatment | Germination percentage (mean ± SE) | | | | |
|---|---|---|---|---|---|
| | **7th day** | **14th day** | **21st day** | **28th day** | **35th day** |
| 1 (Green) | 32.33 ± 5.51[a] | 64.67 ± 5.69[b] | 65.33 ± 6.81[c] | 65.67 ± 7.37[c] | 65.67 ± 7.37[c] |
| 2 (Yelow) | 42.67 ± 15.57[a] | 66.33 ± 8.96[b] | 67.67± 10.41[bc] | 74.33 ± 6.43[bc] | 76.0 ± 3.61[bc] |
| 3 (brown on tree) | 47.67 ± 7.64[a] | 86.67 ± 7.57[a] | 86.67 ± 7.57[a] | 89.33 ± 8.08[a] | 90.0 ± 6.93[a] |
| 4 (brown dropped) | 43.33 ± 4.93[a] | 78.00 ± 7.0[ab] | 80.67 ± 5.51[ab] | 81.67 ± 5.86[ab] | 82.0 ± 5.29[bc] |
| LSD 0.05 | 17.75 | 13.93 | 14.66 | 13.16 | 11.27 |
| CV | 22.71 | 10.01 | 10.37 | 8.99 | 7.63 |
| Mean | 41.5 | 73.92 | 75.08 | 77.75 | 78.42 |

Means with the same letter with in the same column are not statistically different at $P < 0.05$ according to least significant difference (LSD) test; *ns = Non* significant at $P < 0.05$.

increased from young green to brown dried on mother tree. But brown dried fruit dropped on the ground became lower (82%) (Table 2).

The detail discussion of different maturity level of physic nut seed effect on germination percent was calculated every 7th days during 35th days as follow (Table 2). The first seed germination was observed in fourth day from date of sowing (Figure 2A). The analysis at seventh day after sowing showed no significant variation in overall treatments comparison. Averagely, about 41.5% of seeds were germinated at seventh day (Table 2). Similar findings were reported by Wolka and Habte (2014) who reported "Effect of seed storage period and condition on viability of physic nut seed."

In the 14th date from sowing, brown dried on mother tree showed highest percent of germination and it was significantly higher than young green fruit, yellow and brown dried fruit dropped on the ground. The 14th date from sowing was the second with more number of seeds germinated (Table 2). About 74% of physic nut seeds were germinated at 14th days from date sowing. Young green fruit was no more germinated after 14th date from sowing and also shows the lowest germination percentage when compare to all treatments (Table 2).

Similarly, in the 21st day and 28st day of sowing, young green fruit is significantly lower than brown fruit on mother tree and brown dried fruit dropped on the ground at $P < 0.05$. Brown fruit on mother tree is also significantly higher ($P < 0.05$) than young green fruit and yellow fruit (Table 2).

On the 35th day from date of sowing, the highest germination percent was brown fruit on mother tree which 90% germinated, which is significantly higher ($P < 0.05$) than brown dried fruit dropped on the ground, yellow fruit and young green fruit which are germinated at 82, 76 and 65.67%, respectively.

Young green fruit is the lowest germinated this may be due to physiologically immaturity, dormancy problem and also easily decomposed by microorganisms in the soil during germination period. Therefore, brown fruit on mother tree is the good maturity stage which has the highest germination. At the end of the experiment, observed germination percentage being more than 88% is acceptable (Wolka and Habte, 2014).

In many experiments concerned with seed treatments, the pattern of germination, both in time and extent, is the key consideration. Not just the final germination percentage attained, but also the speed and distribution of this germination are often used to judge agronomic relevance of treatments (Kader, 1998). Various germination parameters measuring speed of germination in days, mean germination time, mean daily germination, peak value and germination value are evaluated in different four physic nut maturity levels as describe in Table 3.

Young green fruit has lowest speed of germination, which is significantly lower ($P < 0.05$) than brown fruit on mother tree and brown dried fruit dropped on the ground. The overall mean germination time and germination value did not show significant difference. Young green fruit has lowest mean daily germination, which is significantly lower than brown fruit on mother tree and brown dried fruit dropped on the ground. In addition, the yellow fruit has lowest peak value, which is significantly lower value than young green fruit at $P < 0.05$.

## Relation between fruit maturity and oil content

Different fruit maturity level is connected with the oil. The analysis of oil from different fruit maturity is described as follows; the highest concentration of oil is found from brown fruit on mother tree and the lowest is found from young green fruit. The oil content is increased from the young green to brown dried on mother tree. But brown dried fruit dropped on the ground is become lower (Table 4). Oil yield is the most important trait which will affect the overall commercial success of physic nut cultivation and its use as feedstock energy crop (Sushma, 2014). Oil yield results show that significant difference among treatments. brown fruit on mother tree has the highest oil content (34.59%) followed by brown dried fruit dropped on the ground, yellow fruit and young green fruit which oil

**Table 3.** Seed germination parameter of physic nut [speed of germination, mean germination time, mean daily germination, peak value and germination value (mean ± SE)].

| TRT | Speed of germination in days (mean ± SE) | Mean germination time (mean ± SE) | Mean daily germination (mean ± SE) | Peak value (mean ± SE) | Germination value (mean ± SE) |
|---|---|---|---|---|---|
| 1 (Green) | 12.85 ± 0.47[b] | 5.49 ± .40[a] | 1.87 ± 0.21[c] | 3.81± 1.02[a] | 7.01± 1.29 [a] |
| 2 (Yelow) | 15.19 ± 2.5[ab] | 5.33 ± 0.54[a] | 2.17 ± 0.20[bc] | 2.54 ± 0.42[b] | 5.55 ± 1.15[a] |
| 3 (brown on tree) | 17.87 ± 1.85[a] | 5.41 ± 0.18[a] | 2.57 ± 0.20[a] | 3.02 ± 0.59[ab] | 7.84 2.04[a] |
| 4 (brown dropped) | 16.31± 1.21[a] | 5.41 ± 0 .14[a] | 2.34 ± 0.15[ab] | 3.09 ± 0.15[ab] | 7.33 ± 2.68[a] |
| LSD0.05 | 3.28 | 0.55 | 0.39 | 1.23 | 3.07 |
| P value | 0.046 | 0.91 | 0.022 | 0.19 | 0.38 |
| Mean | 15.56 | 5.41 | 2.24 | 3.12 | 6.93 |
| CV | 10.55 | 5.05 | 8.62 | 19.69 | 22.17 |
| MS | 13.4 | 0.01 | 0.26 | 0.82 | 2.9 |

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test; *ns = Non* significant at $P < 0.05$. MS: Mean square.

**Table 4.** Effects of physic nut maturity stage on oil content and average weight of 1000 seeds.

| Treatment | Weight of 1000 seeds (g) | Oil content (w/w) % |
|---|---|---|
| 1 (Green) | 416.16[b] | 22.88[d] |
| 2 (Yelow) | 573.09[a] | 31.45[c] |
| 3 (Brown on tree) | 592.1[a] | 34.59[a] |
| 4 (Brown dropped) | 537.57[a] | 32.80[b] |
| Significance P-value (LSD) 0.05 | 102.8 | 0.48 |
| CV | 10.31 | 0.84 |
| Mean | 529.73 | 30.43 |

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test; *ns = Non* significant at $P < 0.05$.

content was 32.8, 31.45 and 22.88%, being significant different to each other (Table 4).

This research is found that the optimum oil production produced from the brown fruit on mother tree (34.59%). But other findings indicate that optimum oil production produced from the yellow fruit by the research (Saragih et al., 2007). Even if the optimum oil production produced from the yellow fruit, the oil content found by researcher below our results in both yellow fruit and brown fruit, that is, 24.40%. The difference could occur due to climate and soil factors. This physic nut grows in the rainfall of 750 to 1000 mm/year. The range of temperature available for physic nut is 25 and 28°C (Adami Tulu Agriculture Research Center, 1998). The too early and brown dried fruit dropped on the ground harvesting of physic nut the oil content is reduced. This finding is in line with the research by Stegar and loon (1941), Saragih et al. (2007), Yeyen et al. (2007) and Hambali (2006) that fruit maturity of physic nut is correlated with fruit maturity level, and if it collected too late the oil content will be reduced. This could happen due to the conversion and the oxidation of fat by enzyme (lipace) which could convert the fat to carbohydrate and protein as a result of Krebs cycle (Saragih et al., 2007).

Oil content of physic nut produced from the trial plot of Adami Tulu is higher than the one produced from Malinau (24.40%) and West Nusa Tenggara (29.38%). However, the Adami Tullu products is 34.59% oil content this lower concentration with this is also below the products from South America Santiago (59.78%), Fogo (52.83%) Sao Tome (46.72%) and Toubacouta (42.3%) (Heller, 1992). Climate and soil fertility is two major important aspects that have to be considered in the production of oil from physic nut (Saragih et al., 2007). Other reports indicate that the seeds contain between 25 to 40% (w/w) oil (Kumar and Sharma, 2008; Pompelli et al., 2010), with highest amount of unsaturated fatty acids (73%) (Kumar and Sharma, 2008), which makes it ideal for biodiesel industries (Pramanik, 2003).

### Relation between fruit maturity and seed weight

The relation between fruit maturity and seed weight of physic nut has been conducted and the following discussion will describe the relation between fruit maturity with the seed weight and oil content (Table 4). The analysis of oil from different fruit maturity is described as follows; the highest concentration of seed weight is found in brown fruit on mother tree (592.1 g) and the lowest is found in young green fruit (416.16 g). The seed weight is increased from the young green to brown dried on mother tree. But brown dried fruit dropped on the ground is reduced (Table 4).

Young green fruit has lowest seed weight per 1000 seeds (416.16 g), which is significantly lower than all the rest treatments. But other rest three treatment results 537.57 to 592.1 g interval that fit found by FACT Foundation (2006) that the seed weight per 1000 seeds was 556.9 g. Weight and oil contents are positive relation; higher seed weight has higher oil content.

## CONCLUSION AND RECOMMENDATION

The results show that there is a relation between physic nut fruit maturity level with germination, oil content and seed weight. The germination (%), oil content and seed weight of physic nut increase from early maturity to maturity. But late maturity means that brown dried fruit dropped on the ground will decrease. The highest germination, oil and seed weight were gotten from brown fruit on mother tree (90%, 34.59% and 592.1 g) and the lowest from young green fruit (65.67%, 22.88% and 416.16 g), respectively. Weight and oil contents are positive relation: higher seed weight has higher oil content. Therefore, it can be concluded that the brown fruit on mother tree was ideal for higher germination, oil content and seed weight. Farmer and/or user should harvest physic nut fruit at brown fruit on mother tree for higher germination, oil and weight at Adami Tullu and areas.

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