Research Journal of Forestry

ISSN 1819-3439

Academic Journals Inc.

www.academicjournals.com

Research Journal of Forestry 8 (2): 56-63, 2014
ISSN 1819-3439 / DOI: 10.3923/rjf.2014.56.63

Effect of Seed Storage Period and Condition on Viability of *Jatropha curcas* L. Seed

Kebede Wolka and Yidnekachew Habte
Wondo Genet College of Forestry and Natural Resources, Hawassa University, P.O. Box 128, Shashemene, Ethiopia

Corresponding Author: Kebede Wolka, Wondo Genet College of Forestry and Natural Resources, Hawassa University, P.O. Box 128, Shashemene, Ethiopia

ABSTRACT

The *Jatropha curcas* L. is a biodiesel rich plant species native to Central America and Mexico and is now naturalized in Ethiopia at farm boundaries 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 period and condition of storing seed of this plant before propagation. This study aims to assess the viability of *Jatropha curcas* seed when stored in high, middle and low elevation sites with varying packing material. Matured seeds were collected from vigorous mother trees and air-dried. Seeds were stored at rooms in cool, medium and hot climatic conditions three different packing media in terms of air circulation (sisal, sack and sack with plastic bag) were used. The seed was stored for 450 days in each place. The three climatic categories and three packing media combination created 9 treatments. Germination was tested in a seed germination chamber filled with common soil for nursery seedbed and placed in a glass house. The 9 treatments were arranged in random block design with 3 replications. In each treatment and replication 128 seeds were properly sown. Starting from 4th day of sowing, germination was recorded until day 35. Variation in germination percent, speed of germination, mean germination time, mean daily germination, peak value and germination value was analyzed using ANOVA and LSD. For all parameters, there was no overall significant variation (at $p<0.05$). However, when mean of each treatment was compared, treatment 8 has the lowest value. Each treatment showed satisfactory germination (even the lowest was 88%). Thus, we conclude that the seed of *Jatropha curcas* can be stored for 450 days at rooms in three climatic categories with varying packing material for propagation purposes.

Key words: Climate category, Ethiopia, seed germination, packing material, seed viability

INTRODUCTION

Physic nut (*Jatropha curcas* L.) is a plant species native to Central America and Mexico and is now naturalized throughout the tropics and subtropics (Heller, 1996; Sarin *et al*., 2007; Achten *et al*., 2008). In the world, *Jatropha curcas* is grown in Africa, Latin America and Asia as an aim 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 and belongs to the Euphorbiaceae family (Batin, 2011; Mohan *et al*., 2011). The genus *Jatropha* contains approximately 170 known species. It can survive with as little as 250-300 mm of rain fall but requires at least 600 mm to flower and to produce seed. Optimum temperatures for physic nut are between 20-28°C and the best soils are aerated sands and loams of at least 45 cm deep. Physic nut is known for its ability to survive in very poor dry soils, in conditions marginal 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 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 ranges 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. Owing to its adaptation to wide ecology, tolerance to degraded land (Sarin *et al*., 2007), easy for propagation and multiple purposes, it has been grown in a wide array of areas across the globe.

In Eastern Africa, physic nut is cultivated as a hedge, an erosion control, used to exclude animals from food crops and as a demarcation of properties (particularly farm lands). In Ethiopia, physic nut is grown traditionally for the above mentioned purpose and found abundantly in different areas (i.e., Goffa and Mirab Abaya of Southern Nations Nationalities and People Region (SNNPRS), Shoa Robit and Bati of the Amhara region and Wolenchiti and Upper Awash in the Oromia Region (Alemaw, 2010). Large amounts of physic nut seed can be harvested from existing plantations for various purposes.

Owing to declining petroleum deposits and the need for supplementing and replacing it, alternative fuel sources that are renewable, non-toxic and less polluting to the air has attracted the interest of many countries (Worang *et al*., 2008; Moncaleano-Escandon *et al*., 2013). In this regard, the seed of *Jatropha* has been identified for its high content of biodiesel, 25-40% oil from which about 73% is unsaturated fatty acid 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, interest on biodiesel of *Jatropha* increased due to environmental, energy security, economic and rural development factors.

For fossil fuel-importing countries like Ethiopia, it is considered as alternative energy source for producing indigenous energy and reducing foreign currency. Currently, the Ethiopian government is attempting to build a greener economy that can be supplemented by enhancing energy supply of biodiesel from inedible oil. The Ethiopian government has been promoting the expansion of this plant species by providing significant areas 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 industries have been established for extracting oil from its seed. In some areas, such as Wello area of Amhara Region, marketing the seed of this plant is common.

In Ethiopian conditions, small holders' production from hedges established for live stock barriers as well as property demarcations will be a good source of *Jatropha* seed harvesting. 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; the Wello area can be taken as practical example. Such harvests incur little cost including harvesting and delivering. Once established, the physic nut requires little/no cultivation, fertilizing or watering implying minimum production cost. This will benefit resource poor farmers, women and youth as a source of income.

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 plantations, which primarily requires viability of seed. The quality of *Jatropha* seed depend on the degree of fruit maturity, appropriate container and storage conditions, duration of storage, good viability and vigor of seeds (Worang *et al*., 2008). During seed storage, however, fungal infections can occur, which causes a decrease in viability, discolouration and various biochemical changes (Worang *et al*., 2008). However, little attention has been given to the processes of seed storage (Moncaleano-Escandon *et al*., 2013), which can influence storage life, seed viability and oil quantity and quality. Although propagation is done through vegetative and seed (direct sowing or seedling rose in polyethylene pots), the best method remains to be the planting of seedlings from polyethylene pots.

Today, in Ethiopian conditions, research outcomes lack on seed viability of *Jatropha* when it is stored under different environmental conditions with practically common storage media for most cultivators of the physic nut.

This study aims to assess the viability of *Jatropha curcas* seed when stored in high, middle and low elevation sites with varying media of storage.

MATERIALS AND METHODS

Site description: For this experiment, seed was stored in Holeta, Wondo Genet and Melka Werer Agriculture Research Center. The germination test was conducted at Wondo Genet College of Forestry and Natural Resources. Thus, these sites have the following characteristics:

Melka werer agriculture research center: It is in Afar National Regional State (ANRS), about 260 km North East of Addis Ababa, altitude 780 m above sea level, mean annual rainfall of 564 mm, total annual evapo-transpiration with values between 1400 and 2700 mm, maximum and minimum temperature of 18.9 and 38°C, respectively (Radesse, 2006).

Wondo Genet College of Forestry and Natural Resources and Wondo Genet Agriculture Research Center are in SNNPRS at about 263 km South of Addis Ababa, altitude 1820 m above sea level, mean annual rainfall 1133 mm, monthly maximum and minimum temperatures of 26.3 and 12.4°C, respectively (Gemechu, 2006).

Holeta agriculture research center: It is in Ormia Regional State, about 63 km North West of Addis Ababa, altitude 2400 m above sea level, mean annual rainfall 1100 mm, maximum and minimum temperature 22.2 and 6.1°C, respectively.

Methodology: Matured seeds were collected from vigorous mother tree and air-dried. Damaged seeds were hand-picked and eliminated. Equal weight of seed was stored at Holeta Agriculture Research Center (highland-representing cool area), Wondo Genet Agriculture Research Center (representing medium altitude) and Melka Were Agriculture Research Center (low elevation-representing hot climate) for 450 days. At each storage place, the seed was stored in a room packed in three media: sisal ('joniya'), sack ('madaberia') and sack with plastic bags ('madaberia' with polythene bags). The first media, sisal, in which seed was packed allows for relatively better air circulation. The second material is comparatively poor in air circulation and the third, the sack with polythene bag, has no opening on its surface.

Table 1: Treatment description

Treatment	Description
1	Packed in sack (madaberia) and stored in Holeta
2	Packed in sack (madaberia) and stored in Wondo Genet
3	Packed in sack (madaberia) and stored in Melka Were
4	Packed in sisal (joniya) and stored in Holeta
5	Packed in sisal (joniya) and stored in Wondo Genet
6	Packed in sisal (joniya) and stored in Melka Were
7	Packed in sack with plastic bag (madaberiya be lastic) and stored in Holeta
8	Packed in sack with plastic bag (madaberiya be lastic) and stored in Wondo Genet
9	Packed in sack with plastic bag (madaberiya be lastic) and stored in Melka Were

Air temperature and humidity of the three storage places was monitored (measured at 08:00 am in morning and 02:00 pm in afternoon) for 450 days. After 450 days from the date of storing, a germination experiment was conducted in a glass house in a seed germination chamber at Wondo Genet College of Forestry and Natural Resources. Seeds were randomly taken from each storage media and place. The 9 combinations of treatments (Table 1) in 3 replications each containing 128 seeds were practiced. The treatments were in random block design. The tree seed germination chamber, which is commonly used for seed germination, was filled with soil for nursery beds. Seeds were sown in each seed germination chamber manually to a depth roughly equivalent to the seed size.

Germination (emergency of plumule soil surface) was monitored for 35 days. Seedlings started to germinate on the day 4. It was counted every day at 10:00 am starting from the 4th day of its sowing. By day 35, seeds that did not germinate were checked for whether they were dead, alive or missing for unknown reasons.

Seed germination percent on every 7th day was computed and a one-way ANOVA was employed to compare the significance of variation between treatments. To identify the significantly varying mean of one treatment to another, LSD was used.

In addition, 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):

$$\text{Speed of germination} = n_1/d_1+n_2/d_2+n_3/d_3+....+ n_m/d_m$$

where, n: No. of germinated seeds, d: No. of days, m: mth round count/day.

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

$$\text{MGT} = n_1{*}d_1+n_2{*}d_2+....\ n_m\times d_3/\text{Total No. of days}$$

where, n: No. of germinated seed, d: No. of days, m: mth round count/day.

Mean Daily Germination (MDG): Mean daily germination can be calculated by the following formula given by Czabator (1962):

$$MDG = \text{Total No. of germinated seeds/Total No. of days}$$

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

$$PV = \text{Highest seed germinated/No. of days}$$

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

$$GV = PV \times MDG$$

RESULTS AND DISCUSSION

In this experiment, the first seed germination was observed on the fourth day from the date of sowing (day 0). The analysis on the 7th day from the date of sowing showed no significant variation in overall the comparisons of treatments (Table 2). However, when the mean of one treatment was compared to another, only three treatments showed a significant difference to each other on the 7th day from sowing. In treatment 1 (Table 1, 2), a significantly higher percentage ($p<0.1$) of seeds germinated compared to treatment 8 and in treatment 6 higher percentage ($p<0.1$) of seeds germinated compared to treatment 8. In general, on the 7th day from sowing, treatment 8 (seed packed in sack with plastic bag at Wondo Genet for medium altitude) showed the lowest germination percentage. This might be related to the dormancy created by plastic bags that allow little air circulation.

On the 14th day from sowing, treatment 8 showed the lowest germination percentage and it was significantly lower ($p<0.1$) than treatment 9. On the 21st day from sowing, treatment 5 was significantly lower than treatment 3 ($p<0.1$) and treatment 9 ($p<0.05$). Treatment 9 was also significantly higher ($p<0.1$) than treatment 8. On the 28th day from sowing, treatment 5 showed

Table 2: *Jatropha curcas* seed germination percent calculated on every 7th day for 35 days

Treatment	Germination percent (Mean±SE) in every 7th day from date of sowing (days)				
	7th	14th	21st	28th	35th
1	42.71±19.7^{a}	80.99±7.29	83.59±5.47	87.76±4.43	93.23±0.69^{a}
2	20.05±18.1	77.60±2.08	82.81±2.07	82.81±2.07^{a}	91.93±0.69
3	32.29±9.74	77.86±6.99	86.72±2.26^{a}	91.67±0.26^{b}	92.97±0.45
4	35.42±14.71	82.03±2.82	84.64±3.17	87.76±2.93	91.93±1.45
5	13.80±5.87	69.01±10.50	71.09±9.89^{a*}	77.08±8.20^{abc}	90.63±4.35
6	40.10±17.67^{b}	79.69±5.76	83.07±4.97	90.63±2.74	93.23±0.52
7	14.58±4.78	76.04±11.45	79.95±9.72	85.67±8.09	91.67±2.48
8	5.47±2.07^{ab}	68.49±10.42^{a}	74.48±8.33^{b}	85.68±1.45	88.28±2.34^{ab}
9	36.72±18.92	87.50±4.77^{a}	90.89±1.88^{*b}	94.53±0.45^{c}	93.75±0.90^{b}
p-value (overall)	0.52	0.74	0.49	0.31	0.66
Significant p-value (LSD)	0.1	0.1	Letters (0.1), *(0.05)	0.05	0.1

Table 3: *Jatropha curcas* seed germination (speed of germination, mean germination time, mean daily germination, peak value and germination value (Mean±SE)

	Mean±SE				
Treatment	Speed of germination	Mean germination time	Mean daily germination	Peak value	Germination value
1	145.68±29.95^{a}	1421.90±100.48	3.51±0.03^{a}	3.58±0.06^{a}	12.57±0.29^{a}
2	118.63±21.00	1371.90±41.94	3.46±0.03	3.46±0.03	11.98±0.18
3	127.79±13.91	1422.50±36.24	3.50±0.02	3.61±0.02^{b}	12.62±0.12^{b}
4	136.87±20.06	1403.10±63.74	3.46±0.05	3.50±0.07	12.12±0.43
5	98.79±14.52^{b}	1232.20±124.13^{a}	3.41±0.16	3.43±0.17	11.77±1.09
6	141.49±26.38	1423.60±80.57	3.51±0.02^{b}	3.56±0.06*	12.48±0.26^{c}
7	106.82±16.94	1324.30±127.55	3.45±0.09	3.49±0.12	12.05±0.72
8	91.65±6.09^{ac}	1256.50±70.40^{b}	3.32±0.09^{abc}	3.32±0.09^{ab*c}	11.06±0.58^{ab*c}
9	149.89±24.02^{bc}	1506.70±50.36^{ab}	3.53±0.03^{c}	3.64±0.03^{c}	12.84±0.25*
p-value (overall)	0.41	0.40	0.66	0.29	0.43
Significant p-value (LSD)	0.1	0.05	0.1	Letters (0.05), *(0.1)	Letters (0.1), *(0.05)

Fig. 1(a-b): Seedling of *Jatropha curcas* germinated in (a) Wondo Genet College of Forestry and (b) Natural Resources at glass house

the lowest germination percentage and it was significantly lower (p<0.1) than treatment 2, 3 and 9. During the 35th day from sowing, the lowest germination percentage was 88.28±2.34, which was significantly lower (p<0.1) than treatment 1 (in which 93.23% germinated) and treatment 9 (in which 93.75% germinated) (Fig. 1).

The overall speed of germination did not show any significant differences. However, treatment 8 showed the lowest speed and it was significantly lower ($p<0.1$) than treatment 1 and 9. Treatment 5 was also significantly lower ($p<0.1$) than treatment 9 (Table 3).

In general treatment 9 had highest mean germination time, which was significantly higher ($p<0.05$) than treatment 5 and 8. Treatment 8 had lowest mean daily germination rate, which was significantly lower ($p<0.1$) than treatment 1, 6 and 9. In addition, treatment 8 was the lowest peak value, which was a significantly lower value than treatment 1, 3 and 9 at $p<0.05$ and treatment 6 at $p<0.1$. Furthermore, treatment 8 was lowest germination value with a significantly lower value ($p<0.1$) compared to treatment 1, 3, 6 (at $p<0.1$) and 9 (at $p<0.05$).

Result of this study is not concurrent with report of Moncaleano-Escandon *et al*. (2013), which indicated germination of only 7 and 2% for *Jatropha* seeds in paper bags and stored, respectively in room temperature and refrigerator for 12 months. Literatures related to our experiment are less available. But agricultural literatures report that reduces viability through time due to loss of reserves (Rice and Dyer, 2001), loss of organic solutes as result of respiration (Booth and Showa, 2001), deterioration of biological membranes etc. In contrary to some other findings, the storage of *Jatropha* seed for 450 days did not show high viability. The range of temperature in Wondo Genet room was 20-24°C and the humidity ranges from 67-77.5%. The range of temperature in Holeta room was 17.4-19.7°C and the humidity ranges from 60.9-61.4%. The range of temperature in Melka Werer was 26.2-32.7°C and the humidity ranges from 55.2-60%. The lower germination in treatment 8 might be related to comparatively higher percent and also fluctuation of humidity coupled with poorly air-circulating material at Wondo Genet.

CONCLUSION

In general, the germination percent of the *Jatropha curcas* seed stored for 450 days in room at cool, moderate and hot climates in the three packing materials that have different air circulation (high, medium and poor) showed no significant variation in the considered period. However, treatment 8 (seeds packed in sacks with plastic bags and stored at Wondo Genet Agriculture Research Center) showed the lowest germination percent for almost all of the considered period.

Similarly, the overall speed of germination, mean germination time, mean daily germination, peak value and germination value was lowest for treatment 8 and this treatment had the lowest figure that significantly varied with some treatments on those parameters.

The seed of *Jatropha curcas* can maintain its viability for long 450 days after its harvest. In this study, we conclude that seeds of this plant can be stored for 450 days at room in areas experiencing cool, medium and hot climatic conditions using packing material that has poor, good or high air circulation possibility. Treatment 8 can be avoided if the percent of germination should be more than 89% after 450 days, which still requires further investigation. The three commonly used seed packing material, which are practically used for packing grain etc, can also be used for packing *Jatropha* seed. The result has great importance in avoiding confusion of storing place and selection of packing materials. Further study is required on trend of viability after storing longer period in those materials and places.

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