

## Physiological changes in *Jatropha curcas* L. seeds during storage<sup>1</sup>

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**ABSTRACT** – Oil seeds, such as *J. curcas*, are more prone to deterioration and consequently to loss of quality during storage. In this context, adequate techniques for its preservation are of great importance. This study aimed to evaluate the effects of different environments and packaging for the conservation of *J. curcas* seeds during twelve months of storage. The seeds were placed in three different types of packaging: a multiwall paper bag (or Kraft paper bag); a cloth bag; and a high-density plastic bag. After this, the seeds were stored in three different conditions: a laboratory ( $23 \pm 3$  °C;  $64 \pm 11\%$  of RH); a refrigerated room ( $20 \pm 2$  °C;  $55 \pm 5\%$  of RH) and a cold chamber ( $10 \pm 2$  °C;  $55 \pm 5\%$  of RH). Initially and thereafter every three months, the physiological qualities (germination and vigor) of the seeds were evaluated. *J. curcas* seeds packed in the plastic bags and stored in a cold chamber maintained their germination potential during twelve months. There was a decline in the physiological quality of the seeds stored in the laboratory conditions, independent of the packaging used. It was concluded that the most suitable condition for the storage of *J. curcas* seeds was packing them in plastic bags, placed in a cold chamber ( $10 \pm 2$  °C;  $55 \pm 5\%$  of RH).

Index terms: oil seed, physiological quality, conservation.

## Alterações fisiológicas em sementes de *Jatropha curcas* L. durante o armazenamento

**RESUMO** - Sementes de oleaginosas, como as de *J. curcas*, são mais propensas à deterioração e consequente perda de qualidade durante o armazenamento. Nesse contexto, técnicas adequadas para a sua conservação tornam-se importantes. Nesse estudo, objetivou-se avaliar diferentes ambientes e embalagens na conservação de sementes de *J. curcas* ao longo de doze meses de armazenamento. As sementes foram acondicionadas em diferentes embalagens: saco de papel multifoliado; saco de pano e saco plástico de alta densidade. Em seguida, foram armazenadas em diferentes condições: laboratório ( $23 \pm 3$  °C;  $64 \pm 11\%$  de UR); sala refrigerada ( $20 \pm 2$  °C;  $55 \pm 5\%$  de UR) e câmara fria ( $10 \pm 2$  °C;  $55 \pm 5\%$  de UR). Inicialmente, e a cada três meses, foram realizadas avaliações da qualidade fisiológica (germinação e vigor) das sementes. Sementes de *J. curcas* acondicionadas em saco plástico e armazenadas em câmara fria mantiveram seu potencial germinativo por doze meses. Houve redução da qualidade fisiológica das sementes mantidas em ambiente de laboratório independente da embalagem utilizada. Verificou-se que a condição mais adequada para o armazenamento das sementes de *J. curcas* foi o acondicionamento em saco plástico em câmara fria ( $10 \pm 2$  °C;  $55 \pm 5\%$  de UR).

Termos para indexação: sementes oleaginosas, qualidade fisiológica, conservação.

### Introduction

The *Jatropha curcas* L. species, known as jatropha (or physic nut), is an oilseed belonging to the Euphorbiaceae family. It's widely distributed in tropical and subtropical areas and has potential for biofuel production. The oil content in the seeds varies between 30 and 40% (Dias, 2011). The oil has little significant variations of acidity, besides having better oxidation stability than soy and palm oil. It has good viscosity when

compared to oil from castor beans (Tapanes et al., 2008).

Oilseeds are more prone to deterioration during storage. This process involves a series of physiological, biochemical and physical changes that cause progressive and irreversible decline in the quality, ending in their death (Delouche and Baskin, 1973). Thus, the intensity and speed of the seed deterioration process may be related to their chemical composition, especially to their oil content. However, the longevity of seed during storage depends, besides on the

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chemical composition, on factors such as water content, environmental conditions, packaging, microorganism activity, among others (Marcos-Filho, 2015).

The deterioration process of the seeds can be reduced when the seeds are kept under appropriate storage conditions. The temperature and relative humidity are the main physical factors that directly affect the speed of deterioration. The relative humidity is considered more important, given its direct relationship with the seed water content, since an increase in the water content of seed raises their metabolic activity. However, the temperature also contributes significantly affecting the rate of the biochemical processes (Delouche and Baskin, 1973). The type of packaging used for the storage of the seeds is also relevant to its longevity.

Thus, the deterioration of seeds can be delayed by adopting appropriate technologies for storage. However, there isn't conclusive information relating the deterioration process to storage conditions of *J. curcas* seeds.

Studies about the storage of *J. curcas* seeds indicate that they maintain their viability for 12 months when stored at ambient conditions without control of temperature and relative humidity (Joker and Jepsen, 2003). In turn, Ratee (2004) found that seed germination decreased from 90% to 43% after 112 days of storage at environmental conditions.

For Guzman and Aquino (2009), the water content had more influence on the quality of stored seeds than the temperature; seeds with water content between 4 and 5% in waterproof containers can be stored for one year with slight reduction in germination. Worang et al. (2008) observed that water content between 7.9 and 8.4% was considered safe for seed storage under ambient condition. These authors also found that seeds held in plastic containers under environmental conditions had reduced germination from 89% to 75% after one month of storage, declining to 53% after six months. Some authors have assessed different storage conditions, and found a reduction in the quality of *J. curcas* L. seeds after a period of 5 to 6 months of storage (Höring et al, 2011; Pinto Jr. et al, 2012; Pereira et al., 2013). However, seeds stored at 10 °C and 55% RH maintained their physiological quality for 12 months when stored in paper bags (Chaves et al., 2012).

It is apparent, therefore, that the majority of studies mentioned above are related to setting appropriate conditions for the preservation of *J. curcas* seeds, with little information about the changes in the seeds resulting from the deterioration process. Thus, this research aimed to evaluate the physiological changes in *J. curcas* seeds under different storage conditions.

## Material and Methods

The *J. curcas* seeds used were extracted manually from fruits collected at a brown yellow stage, as recommended by Silva et al. (2012). The seeds were placed to dry in the shade until they reached moisture content of approximately 10%. The experiment was conducted at the Seed Laboratory of the Plant Sciences Department of the Federal University of Viçosa, in Viçosa-MG.

Initially, the initial moisture content of the seeds was determined using the oven method at  $105 \pm 3$  °C for 24 hours (Brasil, 2009). The results were expressed as percentages. The seeds were then placed in three different types of packaging: multiwall (or Kraft) paper bags; cloth bags; and high-density plastic bags, with a thickness of 40 µm. The packages were sealed and maintained under the following conditions: a laboratory environment ( $23 \pm 3$  °C;  $64 \pm 11\%$  RH); a refrigerated room ( $20 \pm 2$  °C,  $55 \pm 5\%$  RH) and a cold chamber ( $10 \pm 2$  °C,  $55 \pm 5\%$  RH). Initially and thereafter every three months for a period of 12 months, the seeds were submitted to physiological quality assessments, as follows:

*Moisture content (MC)*: the oven method at  $105 \pm 3$  °C was used for 24 hours with four samples of approximately 10 g for each treatment (Brasil, 2009), with the results expressed as percentage.

*Germination (G)*: eight subsamples of 25 seeds were sown on paper towels, moistened with water equivalent to 2.7 times the weight of dry paper. The rolls were kept in a germination chamber at 25 °C. The evaluations were performed at seven and 12 days after sowing, and the results were expressed as a percentage of normal seedlings (Oliveira et al., 2014a).

*First count of germination test (FC)*: is the record of the number of normal seedlings obtained on the date set for the first count of the germination test, i.e. after seven days. The results were expressed as a percentage of normal seedlings (Oliveira et al., 2014a).

*Seedling emergence (SE)*: conducted in a greenhouse, using plastic trays containing soil mix and sand in the ratio 2:1, respectively, moistened up to 60% of its holding capacity. Four replicates of 50 seeds were distributed in longitudinal grooves of 2 cm in depth and spaced 5 cm apart. Irrigation was performed when necessary. Daily counts were carried out by registering the number of seedlings which had cotyledons exposed above the ground level. This was done until the twelfth day after seeding, to obtain the percentage of seedlings. The data obtained from the daily counts were used to calculate the emergence speed (ES - days), according to Nakagawa (1999).

*Accelerated aging (AA)*: the methodology described by Oliveira et al. (2014b) was adopted. A single layer of seeds was arranged on a plastic wire screen attached to a

plastic gerbox, containing, in the bottom, 40 mL of distilled water. The boxes were covered so as to obtain about 100% RH inside, and maintained in a BOD chamber at 42 °C for 48 hours. After this period, eight subsamples of 25 seeds were evaluated using the germination test, according to the methodology described above, by calculating the percentage of normal seedlings obtained seven days after sowing.

**Electrical conductivity (EC):** eight replicates of 25 seeds each were weighed and immersed in 200 mL of distilled water and then kept in a chamber at 25 °C for 24 hours. After this period, the electrical conductivity of the solution was determined in a conductivity meter. The results were expressed in  $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$  of seeds.

**Statistical procedure:** the experiment was conducted in a completely randomized design in a split plot scheme, with four replications. In the plots, storage conditions were allocated in a factorial design (3 packages x 3 environments) and, in the sub-plots, the storage times. Data were subjected to analysis of variance and the means for packaging and environments were compared by Tukey's test at 5% probability. Data of the

storage periods were subjected to regression analysis.

## Results and Discussion

The moisture content of the seeds during storage was 9.4%. There were no changes in the moisture content during the storage, and the values were maintained between 8 and 10% (data not shown). These values are considered suitable for the storage of orthodox seeds (Marcos-Filho, 2015) because they prevent the reactivation of cellular metabolism, slowing down the deteriorating process.

The germination of the seeds was substantially maintained during 12 months of storage (refrigerated room and freezer), giving values close to 80%, except for the seeds stored in the laboratory environment (Figure 1), which showed reduced germination. *J. curcas* seeds stored under low temperature conditions also had a substantially maintained germination over one year (Guzman and Aquino, 2009). A reduction in metabolic activity of the seeds under cooled ambient conditions contributes to the maintenance of viability in the storage (Marcos-Filho, 2015).

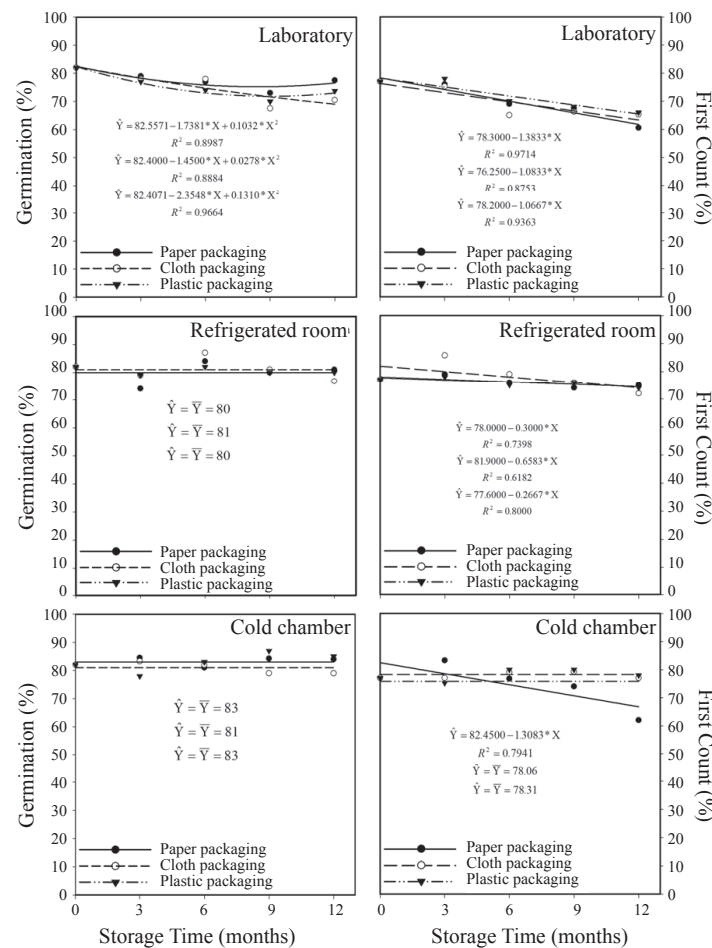


Figure 1. Germination and first count of *J. curcas* seeds stored in different environments and packaging, in relation to the storage time.

When comparing seed germination in different storage environments and packaging (Table 1), it was verified that, from the ninth month of storage, there were no differences between environments when using the cloth and plastic packaging, with lower germination for seeds kept under laboratory conditions. These results are aligned with the results from Worang et al. (2008), who observed reduction

in viability and vigor using plastic containers during storage in an environment without temperature control and relative humidity. It is observed even after 12 months, that the average value of germination for seeds kept in the laboratory environment was 74% (Table 1), which allows the affirmation that the reduction in germination during storage was not severe.

Table 1. Germination and first count of germination of *J. curcas* seeds stored in different environments (LAB-Lab, chilled RFR-Refrigerated Room and CC-Cold Chamber) and packaging (Kraft paper bag, cloth bag and plastic bag) for 12 months.

Germination (%)									
Storage time (months)									
Packaging	Initial	3				6			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper		79 Aa	74 Aa	85 Aa	79	77 Aa	84 Aa	81 Aa	81
Cloth	82	78 Aa	79 Aa	83 Aa	80	78 Aa	87 Aa	82 Aa	82
Plastic		77 Aa	79 Aa	78 Aa	78	74 Aa	82 Aa	83 Aa	80
Means		78	77	82		76	84	82	
Packaging	Initial	9				12			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper		73 Ab	80 Aa	84 Aa	79	78 Aa	81 Aa	84 Aa	81
Cloth	82	68 Ab	81 Aa	79 Aa	76	71 Aa	77 Aa	79 Aa	76
Plastic		70 Ab	80 Aab	87 Aa	79	74 Ab	80 Aab	85 Aa	80
Means		70	80	83		74	79	83	
CV (%)		6.14							
First count (%)									
Storage time (months)									
Packaging	Initial	3				6			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper		76 Aa	79 Aa	83 Aa	79	69 Aa	76 Aa	77 Aa	74
Cloth	77	75 Aa	86 Aa	77 Aa	79	65 Ab	79 Aa	79 Aa	74
Plastic		78 Aa	78 Aa	75 Aa	77	70 Aa	75 Aa	80 Aa	75
Means		76	81	78		68	77	79	
Packaging	Initial	9				12			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper		68 Ab	74 Aa	74 Aa	72	61 Ab	75 Aa	62 Bb	66
Cloth	77	66 Ab	76 Aab	79 Aa	74	65 Ab	72 Aab	77 Aa	71
Plastic		68 Ab	76 Aab	80 Aa	75	66 Ab	74 Aa	78 Aa	73
Means		67	75	78		64	74	72	
CV (%)		5.71							

Means followed by the same lowercase and uppercase on the same line in the column, in each storage time, do not differ by Tukey's test at 5% probability.

Some studies about the storage of *J. curcas* seeds have shown a reduction in germination during storage, especially under ambient conditions (Santoso et al., 2012; Chaves et al., 2012; Pereira et al., 2013). Moreover, Pinto Junior et al. (2012) didn't observe a sharp drop in germination for *J. curcas* seeds stored for 180 days in permeable packaging (multiwall paper bags) and waterproof packaging (high density plastic bags and glass containers) in different storage environments.

Similar results, as to those for germination, were observed for the first count of the germination test (Figure 1, Table 1). A reduction in the germination rate was found only for seeds stored in laboratory conditions (Figure 1) regardless of the packaging used. It can be noted from Table 1 that, after three months of storage, there was no difference between the different environmental conditions. From the sixth month onwards, there is a lower germination at first

count for seeds kept in a laboratory environment. Similar results were obtained by Pinto Junior et al. (2012) for seeds in plastic bags at ambient conditions without controlled temperature and relative humidity.

In general, a reduction of seedling emergence can be observed in soil over the course of storage time, especially after six months of storage, being more severe at ambient conditions (paper and cloth packaging) and in a refrigerated room (paper and plastic packaging) (Figure 2). Similar

results were also obtained by Worang et al. (2008), who observed reduction in seedling emergence over the course of storage time for paper bags under ambient conditions. For seeds kept in a cold chamber (Figure 2) there was a linear reduction of the emergence during storage, although less severe than in other conditions (Table 2). Values above 70% for the seedling emergence were obtained for seeds stored in the laboratory and in the cold room while using the plastic packaging (Table 2).

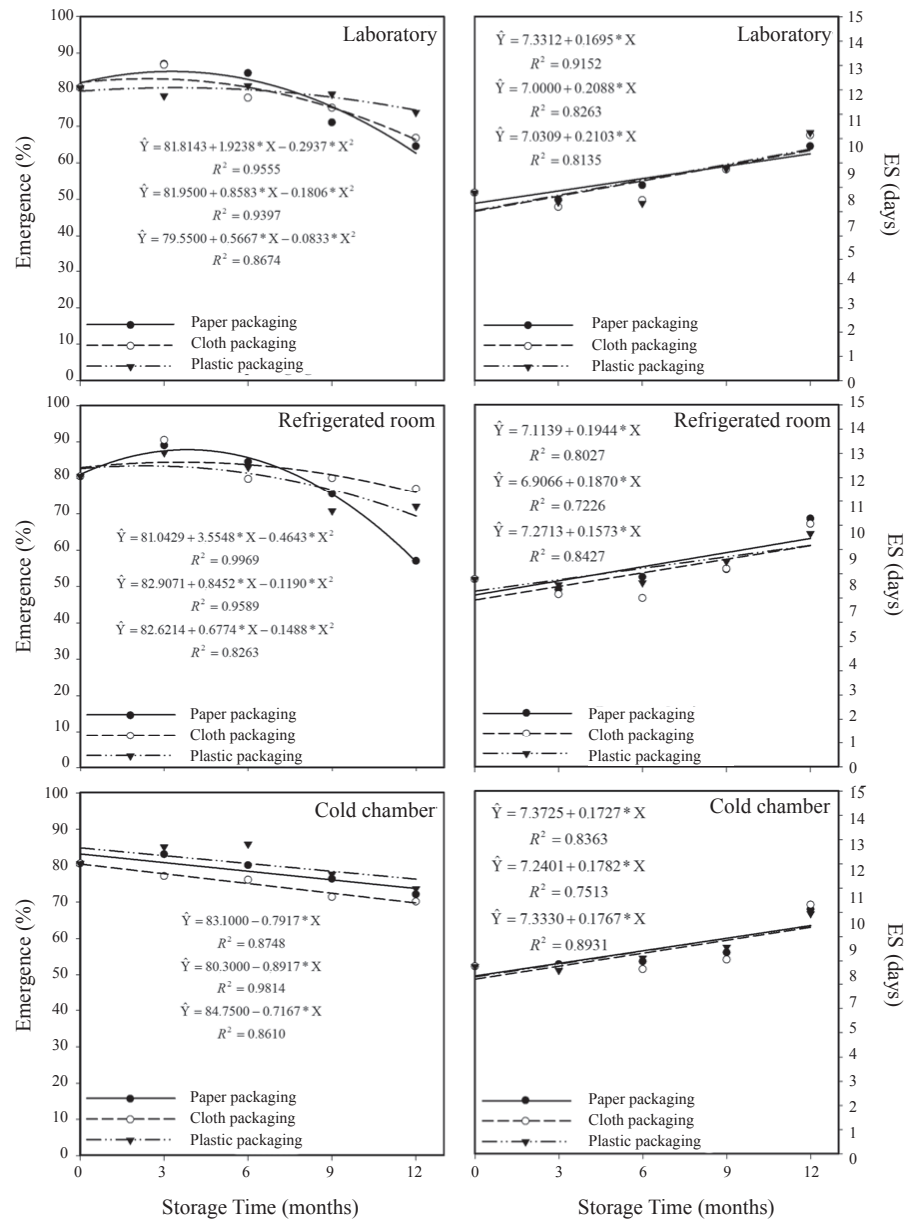


Figure 2. Emergence and emergence speed (ES) of *J. curcas* seedlings obtained from seeds stored in different environments and packaging, in relation to the storage time.

Table 2. Emergence and Emergence Speed of *J. curcas* seedlings obtained during storage of the seeds in different environments (LAB-Lab, RFR-Refrigerated Room and CC-Cold Chamber) and packaging (Kraft paper bag, cloth bag and plastic bag) for 12 months.

Emergence (%)									
Storage time (months)									
Packaging	Initial	3				6			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper	81	87 Aa	89 Aa	83 Aa	86	85 Aa	85 Aa	80 Aa	83
Cloth		87 Aa	91 Aa	77 Aa	85	78 Aa	80 Aa	76 Aa	78
Plastic		78 Aa	87 Aa	85 Aa	83	81 Aa	83 Aa	86 Aa	83
Means		84	89	82		81	83	81	
Packaging	Initial	9				12			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper	81	71 Aa	76 Aa	76 Aa	74	65 Aab	57 Bb	72 Aa	65
Cloth		75 Aa	80 Aa	71 Aa	75	67 Aa	77 Aa	70 Aa	71
Plastic		79 Aa	71 Aa	78 Aa	76	74 Aa	72 Aa	74 Aa	73
Means		75	76	75		69	69	72	
CV (%)		6.42							
Emergence speed (days)									
Storage time (months)									
Packaging	Initial	3				6			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper	7.77	7.48	7.34	7.86	7.56 A	8.07	7.86	7.97	7.97 A
Cloth		7.18	7.15	7.78	7.37 A	7.46	6.99	7.65	7.37 A
Plastic		7.38	7.54	7.6	7.51 A	7.33	7.62	8.11	7.69 A
Means		7.35 a	7.34 a	7.75 a		7.62 a	7.49 a	7.91 a	
Packaging	Initial	9				12			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper	7.77	8.75	8.17	8.33	8.42 A	9.67	10.26	10.11	10.01 A
Cloth		8.73	8.2	8.05	8.33 A	10.13	10.04	10.31	10.16 A
Plastic		8.75	8.49	8.55	8.6 A	10.24	9.64	9.94	9.94 A
Means		8.74 a	8.29 a	8.31 a		10.01 a	9.98 a	10.12 a	
CV (%)		8.79							

The means followed by the same lowercase and uppercase on the same line in the column, in each storage time, do not differ by Tukey's test at 5% probability.

For each storage period, there was no significant difference between packaging and storage environments on the seedling emergence speed (Figure 2). With the increase of the storage time, an increasing trend was observed for the number of days (ES) required to obtain the stabilization of seedling emergence in all combinations of packaging and environments; a result which characterizes the loss of seed vigor. A similar result was observed by Santoso et al. (2012), when storing *J. curcas* seeds from different maturation stages in plastic bags at ambient conditions; these authors found that the average time spent to stabilize seed germination from yellow-brown fruits increased from six to 10 days.

The results obtained in the accelerated aging test (Table 3 and Figure 3) also show a reduction in the vigor

of *J. curcas* seeds during storage in all environments, with a significant reduction in the laboratory environment (Figure 3). Similar results were also obtained by Freitas et al. (2000), who stored cotton seeds in paper bags under laboratory conditions (without temperature control and relative humidity) for 12 months.

Seeds packed in cloth and plastic bags in a cold chamber (Figure 3) showed a less severe reduction in vigor after the third month of storage when compared with those packed in paper bags. However, it can be stated generally that the vigor of the seeds stored in the cold chamber was almost maintained during storage, which can be explained by low temperature conditions (10 °C) of this environment. Furthermore, the relative humidity in both the refrigerated room and the cold chamber was similar throughout the storage; about 50 to

60%. Therefore, in this case, the factor that most influenced the storage of seeds was the temperature, which in the cold chamber was approximately 10 °C, while in the refrigerated room was approximately 20 °C.

Table 3. Germination obtained in accelerated aging test and electrical conductivity values of *J. curcas* seeds stored in different environments (LAB-Lab. chilled RFR-Refrigerated Room and CC-Cold Chamber) and packaging (Kraft paper bag, cloth bag and plastic bag) for 12 months.

Accelerated aging (%)									
Storage time (months)									
Packaging	Initial	3				6			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper		81 Aab	71 Ab	87 Aa	80	73 Aab	66 Ab	82 Aa	74
Cloth	80	72 Aa	76 Aa	79 Aa	76	67 Aa	74 Aa	77 Aa	73
Plastic		69 Ab	81 Aa	78 Aa	76	66 Ab	75 Aa	69 Aa	70
Means		74	76	81		69	72	76	
Storage time (months)									
Packaging	Initial	9				12			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper		71 Aab	69 Ab	80 Aa	73	55 Aa	60 Aa	53 Ba	56
Cloth	80	57 Bb	67 Aab	73 Aa	66	49 Ab	65 Aab	75 Aa	63
Plastic		67 ABb	76 Aa	70 Aa	71	59 Aa	69 Aa	66 ABa	65
Means		65	71	74		54	65	65	
CV (%)		6.65							
Electrical conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ )									
Storage time (months)									
Packaging	Initial	3				6			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper		37.2 Aa	37.3 Aa	36.1 Aa	36.87	43.5 Aa	41.7 Aa	41.7 Aa	42.3
Cloth	33.5	35.4 Aa	37.6 Aa	36.3 Aa	36.43	39.7 Aa	42.6 Aa	41.8 Aa	41.36
Plastic		36.1 Aa	38.7 Aa	35.9 Aa	36.9	42.9 Aa	43.5 Aa	40 Aa	42.13
Means		36.23	37.87	36.1		42.03	42.6	41.17	
Storage time (months)									
Packaging	Initial	9				12			
		LAB	RFR	CC	Means	LAB	RFR	CC	Means
Paper		44.4 Aa	47.1 Aa	49.4 Aa	46.97	53.6 Aa	54.7 Aa	57.4 Aa	55.23
Cloth	33.5	46.2 Aa	46.7 Aa	45.6 Aa	46.17	50.1 Aa	53.7 Aa	48.6 Ba	50.8
Plastic		48.7 Aa	47.1 Aa	49 Aa	48.27	54.4 Aa	55.3 Aa	51.8 ABa	53.83
Means		46.43	46.97	48		52.7	54.57	52.6	
CV (%)		5.53							

The reduction in temperature contributes to reduce the respiratory activity of seeds, slowing down the deterioration process (Marcos-Filho, 2015; Bewley et al, 2013). Note that there was practically no change in seed germination (Figure 1) for seeds stored in a refrigerated room as well as in a cold room. However, when evaluating the effect of temperature, it appears that the lower temperature (10 °C) was more adequate for the maintenance of seed quality (Figures 2 and 3).

Figure 3 and Table 3 show the electrical conductivity of *J. curcas* seeds during the storage period. It was observed that there was a linear increase in conductivity with increasing storage time of the seeds in all environments, regardless of

the packaging used. Freitas et al. (2000) also observed a linear increase in electrical conductivity of cotton seeds stored in multiwall paper bags under ambient condition without temperature control and relative humidity.

The electrical conductivity test allowed the identification of physiological and biochemical changes related to the beginning of the deterioration process. As stated by Delouche and Baskin (1973), the disruption of the cell membrane system is one of the first signs of deterioration of seeds. Thus, the results obtained after three months of storage for all conditions studied, indicate an increase in electrical conductivity and hence reduction of seed vigor.

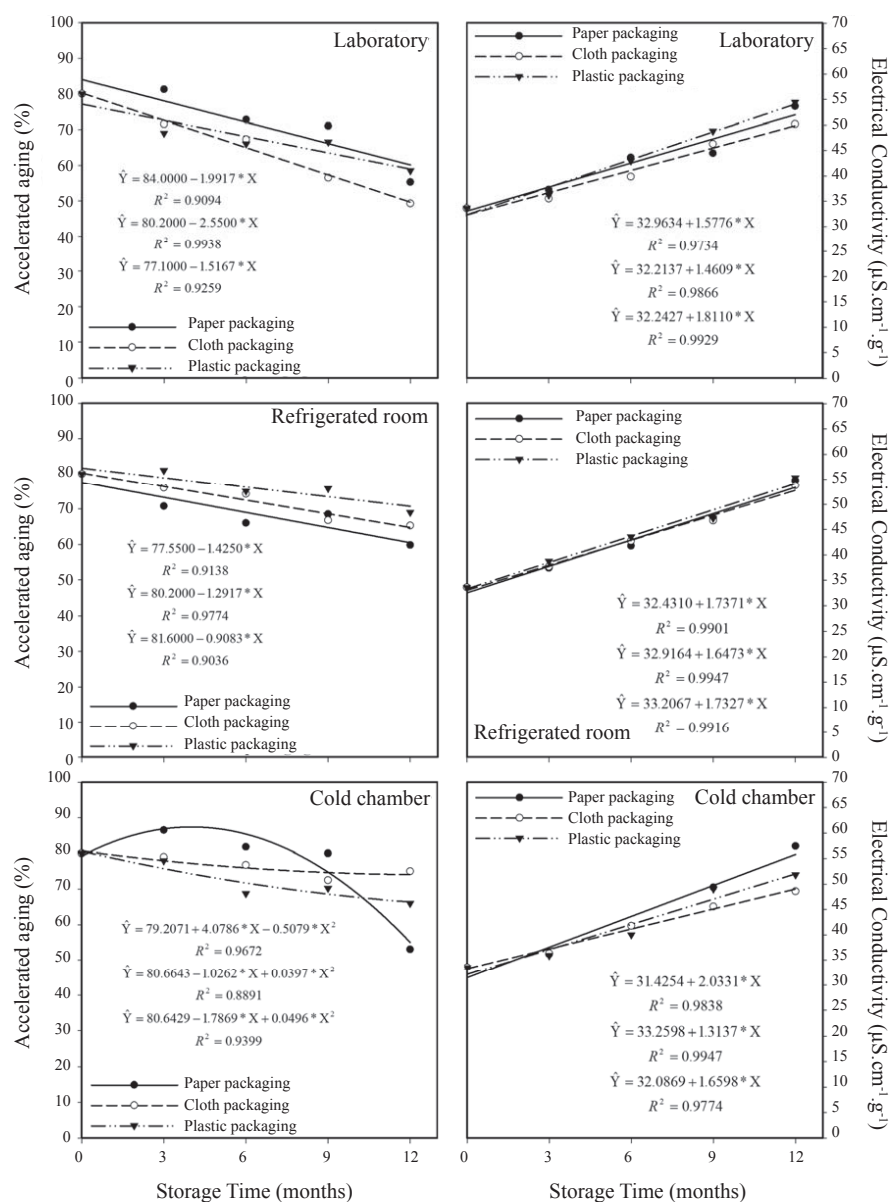


Figure 3. Accelerated aging and electrical conductivity of *J. curcas* seeds stored in different environments and packaging, in relation to the storage time.

It is also observed that after only twelve months of storage, the type of packaging influenced the seed vigor, when a greater electrical conductivity was observed (lower vigor) for the seeds kept in paper packaging, especially when compared with those kept in a cloth bag (Table 3).

In general, the germination of *J. curcas* seeds was substantially maintained during 12 months of storage under refrigerated room conditions (20 °C) and cold chamber conditions (10 °C), independent of the packaging (Figure 1). The seed vigor however suffered severe reduction during storage under laboratory conditions, which can be determined, mainly by the results of the

stress test, i.e., the accelerated aging test, and also the electrical conductivity test (Figure 3), which is a biochemical test. For the accelerated aging test, it was found that the reduction in seed vigor was less drastic for seeds stored in a refrigerated environment. Accordingly, the worst performance was obtained for seeds in paper bags, as indicated by the results of the seedling emergence test (Figure 2) and the accelerated aging test (Figure 3). The less severe reduction in vigor under low temperatures was not observed by the results of the electrical conductivity test, under the three storage conditions; there was linear reduction of seed vigor over 12 months (Figure 3).



## Conclusions

The storage of *J. curcas* seeds in a cold chamber ( $10 \pm 2$  °C,  $55 \pm 5\%$  RH), combined with the packaging of the seeds in plastic bags, is the most suitable condition for the maintenance of seed quality for up to 12 months.

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