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Physiological deterioration of pigeon pea seeds during storage

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The pigeon pea appears as a good option for farmers to be used directly in food and feed and/or green manure. In spite of this potential, its cultivation area in our conditions is still incipient, mostly due to the lack of seeds of superior quality. It is known that the process of physiological deterioration of seeds varies according to species, constituting a relevant factor in seed technology. Storage problems in Brazil are still an issue and the decrease of physiological quality of seeds at this stage is considerable, especially for seeds containing high concentrations of protein (> 22%) as for the pigeon pea. In this context, this study aimed to evaluate the physiological deterioration of pigeon pea seeds stored in different containers and environments for 10 months. The completely randomized design in a factorial 2 × 4 × 6 with four replications was used. The treatments were constituted of two storage environments (natural condition of laboratory (25 ± 2°C) and refrigerator (4 ± 2°C), combined with four types of packaging (PET bottle, plastic bag, burlap bag and kraft paper) and six storage periods (0, 2, 4, 6, 8, 10 months). The physiological seed quality was determined in a timeframe of two months by the following tests: Standard Test Germination - TPG, TPG first count, accelerated aging and electrical conductivity. It is concluded that the percentage of normal germination seedlings by TPG decreases linearly along the storage period; the PET bottle and Plastic bag preserved the vigor and viability of the seeds more efficiently along the storage, being that the PET bottle for being waterproof and tightly sealed has obtained better performance; and the refrigerator-controlled environment is the most suitable for storage of the pigeon pea among the tested environments.

Key words: *Cajanus cajan* L., seed quality, physiological deterioration, preservation, packaging.

INTRODUCTION

Cajanus cajan is the species in which pigeon pea belongs, still with a controversial origin between the African and Asian continents (Odeny, 2007). This culture can be found all over tropical countries for being easily adaptable to several soil and climatic conditions (Azevedo et al., 2007). In India, the largest producer of this fabacea accounting for 90% of production is an important food in many other tropical regions, including Asia, Africa, Central America and the Caribbean and Latin America (Torres et al., 2007; Mula and Saxena,

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2010). Pigeon pea is grown in 5 million hectares, being the sixth most important legume in the world (Varshney et al., 2012).

The pigeon pea is capable of producing abundant harvests of seeds rich in protein with values around 21 to 25% (Mula et al., 2010), even in low fertility soils, being adapted to high temperatures and drought conditions (Akande et al., 2010). Pigeon pea can survive quite well in degraded soils and tolerate water stress (Odeny, 2007). Thus, this culture has a good potential for farming in the “Cerrado” region, especially for the latter season. Nevertheless, the cultivation area of pigeon pea in Brazil is practically stagnant, especially due to the lack of quality seeds offered to farmers. In this sense, the influence of the tropical climate in the problems of seed storage, with high temperatures and humidity in natural environment has contributed to getting a poor quality product (Alencar et al., 2009).

After the seeds reach physiological maturity, the storage starts immediately, even before the beginning of the harvest known as the storage field (Baudet, 2003). However, being hygroscopic, the seed presents considerable variant in amount of water, depending on the relative humidity of air, and because of this low water amount of the seed associated with low temperature storage and lower relative humidity environment are key factors for the viability maintenance in extended periods (Rubim et al., 2013).

Storage is set as an important stage in the seed production process. The preservation of seed quality during this phase, that is, from harvest to the time of its use is an essential aspect to be regarded in the production process, because the efforts spent in the production phase may not be as effective if the seed quality is not maintained, at least until the time of sowing (Oliveira et al., 1999). It is also observed that every seed must be carefully processed and preserved during the storage period, until the moment of its usage, to ensure the preservation of its physiological quality (Marcos Filho, 2005). However, it is noteworthy that storage in tropical regions is one of the greatest hindrances to the maintenance of physiological seed quality.

Numerous factors influence the maintenance of viability and vigor of seed during storage for instance: initial seed physiological quality, parent plant vigor, climatic conditions during maturation, mechanical damage, drying conditions, adequate water content, relative air humidity, storage temperature, the action of microorganisms and insects, types of packaging and storage duration (Carvalho and Nakagawa, 2000; Toledo et al., 2009).

The utilization of top quality seed lots constitutes one of the major responsible factors for the success of a crop. With the application of the tests, one arrives to more correct decisions about the quality of seeds. Meantime, it is difficult to establish a relationship between the results of germination or seed vigor tests in the laboratory and field emergency, due to the interaction with environmental conditions at sowing time (Krzyzanowski et al., 1999).

The pigeon pea seed contains a considerably amount of starch (<50%) and protein (>22%), being classified as protein-amyl from the medium to long run, due to the concentration of protein as compared to storing starchy seeds - corn, rice, wheat, etc. A clear example of this affirmative was obtained by Martins Filho et al. (2001) by evaluating the physiological quality of soybean seeds whose protein levels are greater than those in the pigeon pea - around 45% when it was observed that there had been a decrease in vigor and viability starting at the 120th day of storage and after 210 days which presented a void effect. This behavior can be attributed as shown by Peske et al. (2006), a deviation in the chemical composition of the seed, hence the metabolism of proteins promotes partial breaking of these same amino acids presenting within this process, changes in chemical composition during the deterioration generating difficulties in obtaining seeds with high capacity growth and vigor.

Various techniques are used for storage of seeds, for example, bulk, porous or permeable containers (fabric bags – cotton or jute, sackcloth, multifold paper, plastic or polypropylene tracing) in packages resistant to moisture intrusion or semi-permeable (thin plastic bag or polyethylene and multifold paper bag laminated with polyethylene - kraft paper), and impermeable packages or moisture proof or completely sealed (aluminum cans).

Peske et al. (2006) could have direct influence over the final physiological quality of the harvested seed. However, there is no consensus in the literature over this hypothesis because there is a case of positive response as in a study by Bilia et al. (1994) about the behavior of hybrid corn seeds stored in three different conditions: cold chamber, dry chamber and atmosphere condition packed into kraft paper bags for six months, and it was found that the dry chamber has favored the quality of corn seeds. Conversely, Azevedo et al. (2003) in an evaluation on the physiological quality of sesame seeds stored in atmosphere conditions in a laboratory environment not controlled in a dry chamber, and in paper bags, plastic bags, and metal containers, have not shown any significant differences as to vigor for different containers tested.

Currently, there is few information available for research about pigeon pea, especially those related to the production and quality of seeds (Pedroso et al., 1988; Giomo, 1999) being narrowed down those for the seed storage in our conditions Nakagawa et al. (2009), and even internationally Godoy and Souza (2004) and Asalmol and Zade (1998). Therefore, investigative work about storage techniques of pigeon pea seeds is nonexistent. That way, it is essential the search for these detailed information, making it possible for technicians and producers a better data bank about the aspects related to the cultivation of this fabacea, especially, those related to the correct procedures for conservation of top seed physiological quality, since this is an extremely important factor for achieving high productivity. Furthermore, it emphasizes that the storage is set as a fundamental practice for the control of seed quality, due to the fact of being a method in which the viability of the seeds can be preserved and the vigor kept at a reasonable level in the period between sowing and harvest. This study aimed to evaluate the physiological quality of pigeon pea seeds stored for ten months in different environments and packaging in the climatic conditions of the Central region of Goiás State, Brazil.

MATERIALS AND METHODS

General information

The pigeon pea seeds produced in the 2010/2011 season were purchased directly from producing firms, so that the performance of the work could take place on the premises of UEG/UnUCET more precisely in the Laboratory of drying and storage plant products of the Agricultural Engineering Course. Midget pigeon pea seeds were used. The seed samples were homogenized to sort the seeds out from undesirable inert material.

Experimental design and treatments

A completely randomized design was used in a factorial 2 × 4 × 6 frame with four replications. The treatments were constituted of two storage environments (natural laboratory conditions - 25 ± 2°C and refrigerator - 4 ± 2°C), four types of packaging (PET bottle, plastic bag, burlap bag and kraft paper) submitted to 10 months of storage every two months ratings (0, 2, 4, 6, 8 and 10 months).

Establishment and management

Before seed packing, water content was determined according to the rule for the analysis of seeds (BRASIL, 2009), the standard greenhouse method where the seeds were submitted to drying at 105°C ± 3° = 221 F for 24 h with results expressed in percentage (BRASIL, 2009). The determination of water content has been repeated at each assessment time of the physiological seed quality in all storage conditions.

Evaluated characteristics

The evaluation of physiological seed quality coming from the different treatments was checked by the following tests: Test pattern of germination – TPG, first count of TPG, accelerated aging and electrical conductivity. Test pattern of germination was conducted with four replications where 50 seeds/replicants were placed over three sheets of germ test paper, moistened with equivalent to three times its original water weight, rolled up and put up in a germination chamber at a temperature of 30°C = 86 F. The evaluation was made on the 10th (tenth) day after the performance of the test. The percentage of normal seedlings was logged in BRASIL (2009). The first germination test - conducted joint with TPG and considering the percentage of normal seedlings present on the 5th (fifth) day after the start. For the aging acceleration **-** 100 seeds / repetitions were distributed on the surface of a wire mesh fixed inside a plastic box - gerbox containing 40 ml of water, maintained at 42°C = 107 F and 100% of relative humidity for 48 h in a germination chamber (Krzyzanowski et al., 1999). After this period, the seeds were subjected to the TPG previously described to determine the percentage of normal seedlings on the 5th (fifth) day after the assembly of the test. The electrical conductivity test was conducted in the cup system recommended by Krzyzanowski et al. (1999) in which 50 pre-weighted seeds / repetitions were placed in plastic cups containing 75 ml of deionized water, placed and kept into a germination chamber at a constant temperature of 25°C = 77 F for 24 h. The reading for the electrical conductivity was performed by a conductivity meter and the results expressed in µS /cm /g of seeds.

Statistical analysis

The data was subjected to analysis of variance and when pertinent were submitted to the Tukey test at 5% probability. Statistical analysis was performed with the computer program Sisvar 4.6 (Ferreira, 2011).

RESULTS AND DISCUSSION

Through, the analysis of variance from the data obtained in the Standard Germination Test, First Count of TPG, Accelerated Aging and Electrical Conductivity were observed that the environment (E) influenced only the variable electrical conductivity ($P>0.05\%$). The packaging (P) influenced Accelerated Aging variable ($P>0.05\%$) and electrical conductivity ($P<0.01\%$). The period (PE) of storing influenced all the variables ($P<0.01\%$). The double interactions E×P and PE×P significantly influenced the variable in Electrical conductivity ($P<0.01\%$). But the double interaction E×P influenced in the Accelerated Aging and Electrical Conductivity variables ($P<0.01\%$). Regarding the triple interaction E×P×PE, we have the influence under the variables in the first count and electrical conductivity ($P<0.01\%$) (Table 1).

There was linear decrease in the percentage of pigeon pea seed germination during the ten month-period storage (Figure 1). This behavior was expected and confirms Villela and Peres (2004) when it demonstrates that the seed quality does not improve during storage except in some cases of seeds endowed with a dormancy phenomenon which was not observed in this study. Moreover, Marcos Filho (2005) explains that from the physiological maturity point, a process of seed deterioration starts in a progressive rate until the unfeasibility of the seed or embryo death.

Figure 2 shows that for the first count of seedling test along the ten month-period store in different packaging and environments, it was found that PET packaging followed by plastic packaging were those which obtained the highest percentage of normal seedlings in both tested environments. This behavior can be explained by the permeability to water vapor that the featured by the packages tested for the PET packaging is classified as an impermeable plus being hermetically sealed not allowing interactions with water vapor from the external

Table 1. Results of analysis of variance (average squares) test applied in pigeon pea seeds for different environments, packaging and storage period.

Cause of variation	G. L.	Average Square			
		Germination	First count	Acceleration of aging	Electrical conductivity
Environment (E)	1	3.685^{ns}	12.505^{ns}	0.117^{ns}	372.681*
Packing (P)	3	3.920^{ns}	14.410^{ns}	154.223*	271.855**
Period (PE)	5	204.220**	321.324**	336.073**	1350.367**
E x P	3	98.782^{ns}	129.329^{ns}	87.543^{ns}	635.375**
E x PE	5	47.703^{ns}	56.255^{ns}	509.321**	742.521**
P x PE	15	67.046^{ns}	69.306^{ns}	47.361^{ns}	327.744**
E x P x PE	15	73.708^{ns}	81.721**	44.642^{ns}	263.777**
Residue	144	41.882	41.177	44.668	77.453
C.V.(%)	-	9.60	9.72	11.07	11,45

G.L. Degrees of liberty; * Significant at 5% probability by F test; ** Significant at 1% by F test; ns Not Significant.

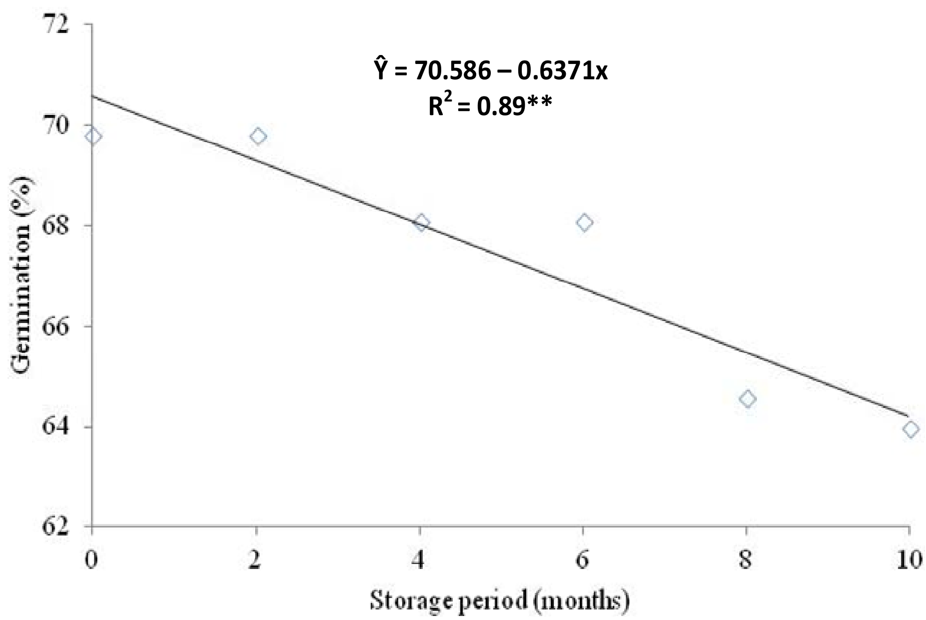

Figure 1. Percentage of normal seedling by TPG test applied in pigeon pea seeds during ten month-period storage.

environment. For Copeland and McDonald (1995), when seeds are stored in low temperature environments, they will probably acquire moisture due to the high relative humidity in these locations. Therefore, the use of impermeable packaging prevents the increase in moisture and deterioration rate.

The plastic container is characterized by being semi-permeable, since it only restricts the exchanges with the environment. To Stubsgaard (1992), plastic packaging is the most sensible for seed storing.

For PET and plastic packaging, the uncontrolled laboratory environment presented slightly higher averages than the controlled refrigerator environment. The lowest averages observed are from the kraft packaging and sackcloth respectively in both environments. It is noteworthy that the refrigerator controlled environment has obtained higher average values related to the ones in environment non-controlled Natural, concerning the packaging kraft and sackcloth. This phenomenon can be explained by the fact that these packs allow water vapor exchange with the environment, when the natural environment is subjected to greater fluctuations in temperature and relative humidity, speeding up the process of deterioration. According to Fowler (2000), packs that allow the exchange of moisture are recommended for seed storage for a short term or for

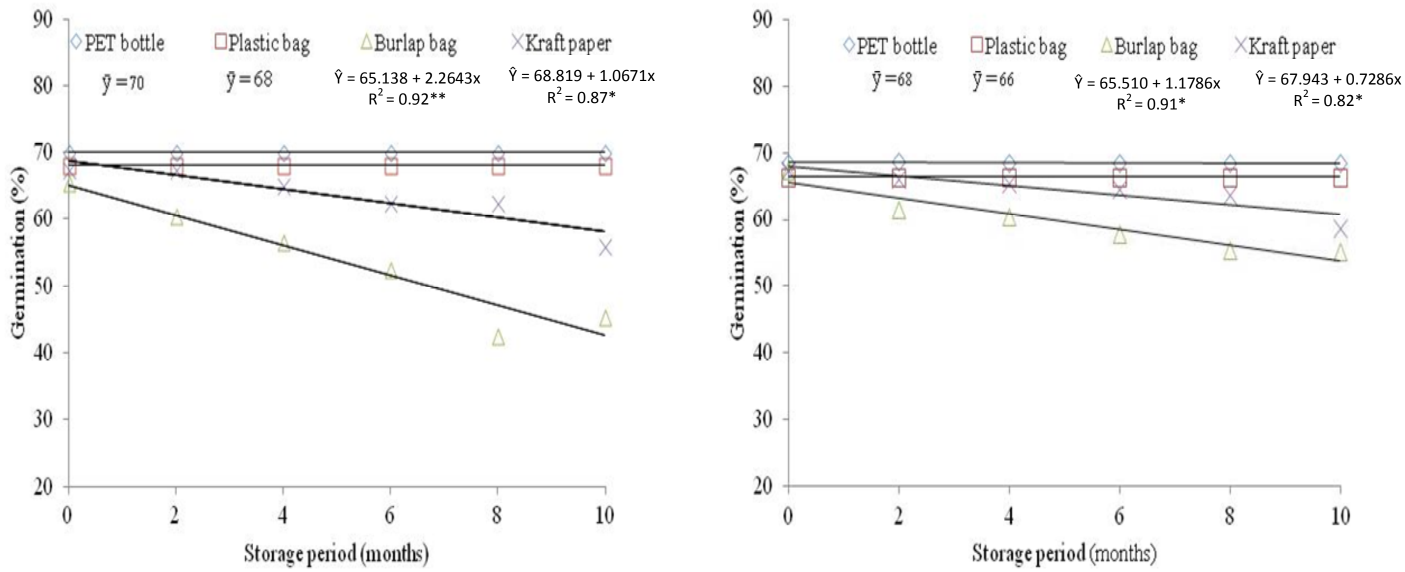

Figure 2. Percentage of germination of normal seedlings at the first count test applied for pigeon pea seeds during 10 months of storage in uncontrolled environment – laboratory (a) and controlled - refrigerator (b).

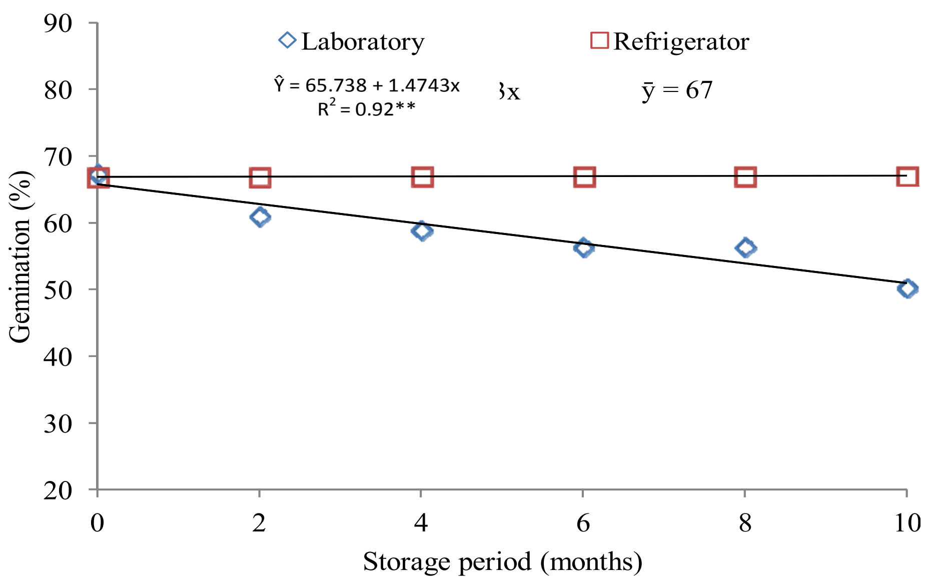

Figure 3. Percentage of germination for normal seedlings by accelerated aging test applied in pigeon pea seeds during 10 months of storage in different storage environments.

orthodox humid seeds. The moisture content of the seeds in this type of packaging varies with change in humidity.

Generally speaking, the percentage of germination of normal seedlings in PET and plastic packs for the uncontrolled environment and controlled-natural-refrigerator, maintained a steady linear behavior along the storage period with higher average values for the uncontrolled natural environment. The remaining packs kraft and sackcloth had a decreasing linear behavior in both environments with smaller percentage values for uncontrolled natural environment.

Besides the intrinsic characteristics of the seeds, the packaging, the environments and the storage period, there was the interference of external factors such as the attack from two pests from the Coleopteran species and two distinct species: The beetle (*Lasioderma cerealella*) and caterpillar (*Euphestia*). These two pests damaged the membrane, embryo and seed coat of the seed stored in kraft packs and sackcloth in the non-controlled natural environmental from the 8th month of storage.

Figure 3 compares the performance of the environments from the results of the first count of

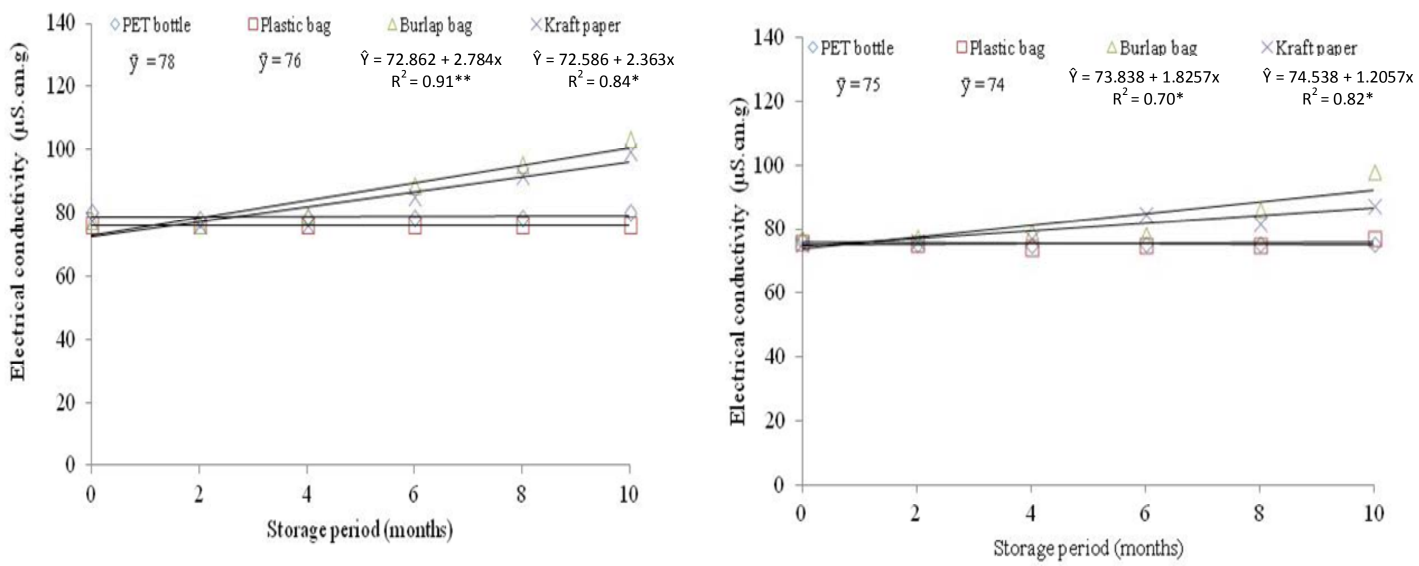

Figure 4. Reading of the electrical conductivity test applied for pigeon pea seeds during 10 months of storage in an uncontrolled laboratory environment - (a) and refrigerator controlled (b).

germination test of accelerated aging. In this test of vigor, it is obvious the superiority of the refrigerator controlled environment related to uncontrolled Natural environment, along the storage period. This can be explained because refrigerator environment maintains a low and constant temperature, contributing to a decrease in cellular metabolism of seeds, whereas the natural uncontrolled environment is subjected to oncoming changes in the external environment. Generally speaking, the lower the temperature and moisture content, the longer is the seed viability with some exceptions (Schmidt, 2007).

The results shown in Figure 4 in relation to the electrical conductivity test applied to pigeon pea seeds for ten months of storage in different packaging and environments, and demonstrate that burlap and kraft packs in both environments show an increase of µS/cm/g higher than other containers over the time, however, it is worth highlighting the results of the non-controlled natural environment because they are superior to the refrigerator controlled. Regarding PET and plastic packing, slightly higher values were observed for PET-packaging in a non-controlled natural environment. As for the refrigerator controlled environment both packing showed similar values during storage.

Moreover, the uncontrolled natural environment obtained higher readings than the refrigerator controlled environment for all packaging studied over the period of storage for the electrical conductivity test. This behavior can be attributed to the fact that the seeds stored under uncontrolled natural environment have suffered greater influence of the external environment, causing acceleration to its deterioration process. The readings of electrical conductivity in both storage environments grow linearly over time for packaging that allow greater exchange with the environment (burlap and kraft), demonstrating that there was a progressive increase in the breakdown and loss of integrity of the cell membrane system seed along storage time.

The packaging impermeable and semi-permeable, PET and plastic respectively, maintained a good overall quality of the seed cell membrane system during the period of storage.

Conclusion

The percentage of normal seedling germination in the TPG decreases linearly along the storage period. The packaging PET and plastic maintained the vigor and viability for the pigeon pea seeds over the ten-month storage period, independent of the tested environments. The vigor and viability of pigeon pea seeds were better maintained over the ten months of storage for impermeable and hermetically sealed packaging PET. The refrigerator controlled environment is the fittest for the storage of pigeon pea among the tested environments.

Conflict of Interest

The authors have not declared any conflict of interest.

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