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# Freezing, cooling and storage of pumpkin (*Cucurbita moschata* Dusch) seeds produced in biodynamic system

**Carolina Olga da Fonseca Ribeiro*, Higino Marcos Lopes and Felipe Kuhn Leão de Salles**

Instituto de Agronomia, Departamento de Fitotecnia, Universidade Federal Rural do Rio de Janeiro, Seropédica-RJ, Brasil.

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**Farmer's dominance depends on seed processing practices that allow seed longevity and sanity throughout storage. In organic systems, seeds cannot be exposed to chemical pesticides. Therefore, organic seed treatment must be conducted by applying techniques which do not contaminate seeds and that can provide physiological and sanitary quality, and also a safe storage. This work aimed to evaluate the physiological and sanitary quality of pumpkin seeds frozen at -18°C and cooled at 15°C, for 36 h and 7 days in polyethylene terephthalate (PET) bottles and in plastic bags with vacuum. Results showed that freezing seeds at -18°C for 36 h in PET bottle provided an increase in the first count of germination and seedling length reduction. The physiological and sanitary qualities of pumpkin seeds frozen at -18°C for 36 h in PET bottle and stored for up to six months under ambient conditions and in a cold chamber were also evaluated. Refrigerated storage provided increased seed viability as compared to ambient storage. In the sixth month of storage, a reduction of the genera *Aspergillus* and *Penicillium* occurred in both environments.**

**Key words:** Freezing, package, storage, vigor, viability, organic seed treatment, *Cucurbita moschata* Dusch.

## INTRODUCTION

In organic systems, seeds play an important role in ensuring farmers' sovereignty and independence of external inputs. In Brazil, organic standards allow organic farmers to use conventional seeds in their systems when organic seeds are not found in the market, as long as they are not treated with pesticides. There are a few companies producing organic seeds in Brazil and a few conventional companies have a collection of non-treated seeds for sale. Pumpkin is a plant of American origin, from the *Cucurbitaceae* family. It stands out as a culture that is part of the traditions of the ancient civilizations that inhabited the Americas, and is widely cultivated in the Brazilian regions by farmers (Jovchelevich, 2011). Hybrid and genetically modified seeds, commonly found in

*Corresponding author. E-mail: carol.olga@gmail.com. Tel. +55 22 999003211.

commercial establishments are improved to generate higher yields in conventional systems and to create varieties resistant to chemical agents. In contrast, conventional systems pose a threat to the environment, leading to contamination of soil, water, air, plants, animals and humans (Yadav, 2010). Climate change can also affect crop production and seed recruitment, increasing the need for adaptive and resilient cultivars (Mondoni et al., 2012).

The correct cleaning and treatment of post-harvested seeds and a safe storage can contribute to the minimization of the deterioration process (Tripathi and Lawande, 2014). Seed sanitary quality also determines the success of vegetable production, since the presence of pathogens has direct effects on vigor, seedling establishment and yields in the field, and can cause considerable damage to production systems (Nascimento et al., 2011).

Orthodox seeds are long-lived seeds. They can be successfully dried to moisture contents as low as 5% without injury and are able to tolerate freezing temperatures. Most storage seed fungi belong to one of two principal genera, *Aspergillus and Penicillum* and generally grow at seed moisture contents in equilibrium with relative humidity from 65 to 90% and the optimum temperature for growth of storage fungi is about 30 to 33°C, with maximum at 55°C and minimum of 0°C. They cause seed deterioration not only through invasion, but also by producing toxic metabolites that destroy cells that provide the dead tissues on which they subsist (Schmidt, 2000; Copeland and McDonald, 2001). According to Berjak and Pammenter (2008), the best way to maintain the viability of the seed is to keep it at a low temperature and reduce contamination with fungi.

According to Brito et al. (2013), freezing green bean seeds make it possible to eliminate insects and microorganisms capable of damaging and compromising the viability of stored seeds. This study aims to evaluate the effects of seed freezing and cooling on its vigor, viability, sanity and storage up to six months in different ambients. Simple techniques like this might help farmers to preserve their seeds in an efficient way, on-farm, without using pesticides or chemicals.

## MATERIALS AND METHODS

The experiments were conducted at the Seed Quality Control Laboratory of the Agronomy Institute of the Federal Rural University of Rio de Janeiro, and in the Laboratory of Seed Analysis of the State Center for Research in Organic Agriculture (CEPAO)/Agricultural Research Company of the State of Rio de Janeiro (PESAGRO-RIO RJ). Samples of pumpkin seeds of the variety *Biocosta* were submitted for initial evaluations of:

### Mass of one thousand seeds

Eight replicates of 100 seeds were weighed and the average obtained was multiplied by 10, according to MAPA (2009).

### Standard germination test

This was performed with four replicates of 50 seeds on moistured germitest paper rolled and covered in plastic bags. The rolls were kept in a chamber at a temperature of 25°C, with light regime periods recommended for the species as prescribed in the Rules for Seed Analysis (MAPA, 2009).

### First germination count

This was performed in conjunction with the germination test. Normal seedlings were removed from the paper and counted.

### Moisture content

It was determined by a forced-air seed drier at 105±3°C for 24 h, with four replications (MAPA, 2009).

### Moisture adjustment

Seeds were then submitted to a forced-air drier at 38°C until it reached a moisture content of 6%, recommended for freezing (Justice and Bass, 1978) and storage (James, 1967).

### Seedling length

Four replicates of 20 seeds sown in the upper third of the germitest paper were carried out and conducted according to the germination test methodology. On the 8$^{th}$ day of the germination progress, the normal seedlings were measured, obtaining the value of the average length of the seedling (Vieira and Kryzanowski, 1999).

### Blotter test

100 seeds were distributed in plastic gerbox with three sheets of filter paper moistened with water, in laminar flow chamber, with four replicates. The seeds were placed under illumination, photophase 12 h, at room temperature (20 ± 2°C) for a period of 7 days. Then, the morphological identification and quantification of the genus of fungi present in the seeds were determined (Cirio and Lima, 2003). The experiment was divided into two stages. In the first stage, eight treatments were carried out with one control (seeds that were not subjected to any treatment) and subsequent evaluation of the effects of the treatments on their physiological quality. In the second stage, the seeds were subjected to a single treatment and stored for up to six months.

### First stage

#### *Effects on the physiological quality of seeds subjected to freezing and cooling in two types of packages and two periods of time*

After the initial testing, samples of pumpkin seeds were packed in polyethylene terephthalate (PET) bottles with 17 cm height and 5 cm of base diameter and another sample in bags composed of PET and PE (polyethylene) with 23 x 20 cm from the brand Oster® and vacuum was applied using a Food Saver Oster® Model V2240 vacuum sealer. These packages with the seeds were then placed in two environments, separately. A sample was placed in a freezer set at an average temperature of -18°C using a plastic plate to insulate the samples from the base of the freezer. Another sample was

placed in a cold room at a mean temperature of 15°C. The seeds were exposed to the temperatures for periods of 36 h and 7 days in each environment. After each treatment period, the packages were removed from the freezer and placed in small closed styrofoam boxes and transferred to a cold room at a temperature set at 15°C for 24 h to a complete defrosting of the seeds. Then, the samples from both environments were submitted to the standard germination test, seedling length and first count of germination, as described previously.

After the treatments were carried out, one in which the mean values of the physiological and vigor evaluations more closely approximated to the averages obtained in the physiological evaluations of the untreated seeds (control), was selected. The treatment was then repeated and the blotter test was performed.

The experimental design was completely randomized in a 2x2x2 factorial considering the treatments: two packages, two timing periods and two temperatures applied to the seeds, with four replications. A variance analysis was performed using the F test at 5% probability and the means were compared using the Scott Knott test at 5% probability. For the blotter test, the non-parametric Mann-Whitney test, at 5% probability was applied.

**Second stage**

***Storage of seeds subjected to freezing***

The treatment in which the mean values of the physiological and vigor evaluations were more closely approximated to the averages obtained in the physiological evaluations of the untreated seeds (control), was then repeated and the pumpkin seeds were subjected to freezing treatment at -18°C in PET bottles for a period of 36 h. After thawing for 24 h, the samples were stored in two environments: in the laboratory environment with a temperature of 26±2°C and 55% relative humidity (RH) and in a cold room with a 15±2°C temperature and 77% of RH, for six months. The daily temperature and RH oscillations of the environment and cold chamber were measured using a datalogger (Extech® model RHT 10). Physiological quality evaluations were performed every 45 days, totaling four evaluations throughout the period of storage. In these evaluations, the standard germination test, first count of germination and seedling length test were performed. In the fourth evaluation, at 180 days, the seed moisture content was determined and the blotter test was performed following the methodologies already mentioned. The experimental design was completely randomized in a 2x4 factorial considering the treatments: two environments and four storage periods, with four replications. The means were compared using Scott Knott's test at 5% probability. For the blotter test, Kruskal-Wallis non-parametric test was used at 5% significance level.

## RESULTS AND DISCUSSION

In the initial evaluations of the seeds (Table 1), pumpkin seeds were within the commercial standards determined by the Brazilian law (MAPA, 2013), which recommends a minimum of 60% germination for pumpkin seeds of "S2" type. According to Harrington (1973), the ideal moisture content for seeds under storage in impermeable packages is 6 to 12% for starchy seeds and 4 to 9% for oilseeds. Moisture content greater than 12% for starches and 9% for oilseeds promotes a faster deterioration than those in permeable packages. In the results, the initial data obtained from seed quality assessments (Table 1) is referred to as "control".

When comparing the results of germination between the treatments for pumpkin seeds, as shown in Table 2, it can be noticed that the treatment using PET packaging, the time of 36 h and the temperature of -18°C provided the highest percentage of germination. However, when in the same package and subjected to 36 h of exposure at 15°C, they presented a significant reduction in the percentage of germination. The vacuum packaging caused significant changes, especially when under a temperature of -18°C and exposure time of 7 days, generating the lowest percentage of germination among the treatments. The same results can be observed when comparing the treatments with the control (Figure 1). Croft et al. (2013), when storing pumpkin seeds in a refrigerated environment using vacuum packaging, observed that only the vacuum itself was able to preserve the germination of the seeds.

Comparison between treatments on seedling length results of treated pumpkin seeds (Table 2) shows a great variability. However, the treatment combining PET packaging for 36 h at -18°C presented the highest mean among the results. Treatment using vacuum packaging for 7 days at -18°C reduced the seedling length of pumpkin seeds. When comparing treatments with the control (Figure 2), all treatments resulted in a significant reduction in seedling length. Yeh et al. (2005), when storing *Cucurbita* seeds in vacuum packaging and under refrigeration, noticed a small decrease in seed vigor, probably caused by the vacuum effect of minimizing oxygen pressure and exposure to free radicals.

In the results of the first count of the germination test of pumpkin seeds, comparing the treatments with each other (Table 2), the same behavior that was obtained for the germination test was observed. The PET packaging, the time of 36 h and the temperature at -18°C resulted in the highest average. On the other hand, the vacuum packaging, the 7 day period and the temperature of -18ºC caused significant reductions in the first count of the germination test. When compared with the control (Figure 3), most of the treatments provided a reduction in the percentages of the first count of germination, except for the treatment combining PET packaging, 36 h period and the temperature of -18°C that showed a significant increase in the percentages of first counts. Treatments using PET packaging for 7 days at 15°C and vacuum packaging for 36 h at -18°C did not change the means of the results in relation to the control (Figure 3). The treatment that promoted the greatest reduction in the first count of germination was again the combination of vacuum packaging for 7 days at -18°C. Bee and Barros (1999) stored pumpkin seeds with 13% moisture in vacuum packaging and also observed a decrease in the physiological quality of the seeds.

Decrease in vigor and germination is notable in the treatments that mainly used the vacuum packaging, regardless of the exposure time and temperature applied.

**Table 1.** Initial characterization of the seed lots before being subjected to the treatments (control).

| | |
|---|---|
| Mass of 1,000 seeds (g) | 76 |
| Germination (%) | 94 |
| First count of germination (%) | 70 |
| Seedling lenght (cm) | 18.5 |
| Initial moisture content (%) | 9 |
| Final moisture content (%)* | 6 |

*% of moisture content adjusted using a forced-air seed drier at 38 ± 2°C.

**Table 2.** Germination, seedling length and first count of germination of pumpkin seeds subjected to freezing (-18°C) and cooling (15°C) in PET bottles and in plastic bags with vacuum for 36 h and 7 days.

| Temperature | Germination (%) | | | |
|---|---|---|---|---|
| | 36 h | | 7 days | |
| | PET | Vacuum | PET | Vacuum |
| - 18°C | $95^{aA\alpha}$ | $81^{aB\alpha}$ | $71^{bA\beta}$ | $25^{bB\beta}$ |
| 15°C | $63^{bA\beta}$ | $53^{bB\alpha}$ | $85^{aA\alpha}$ | $57^{aB\alpha}$ |
| Seedling lengh (cm) | | | | |
| -18°C | $15.3^{aA\alpha}$ | $11.2^{bB\alpha}$ | $14.3^{aA\alpha}$ | $11.1^{aB\alpha}$ |
| 15°C | $14.3^{aA\alpha}$ | $13.3^{aA\alpha}$ | $12.6^{bA\beta}$ | $12.5^{aA\alpha}$ |
| First count of germination (%) | | | | |
| - 18°C | $91^{aA\alpha}$ | $68^{aB\alpha}$ | $55^{bA\beta}$ | $7^{bB\beta}$ |
| 15°C | $23^{bA\beta}$ | $27^{bA\beta}$ | $71^{aA\alpha}$ | $41^{aB\alpha}$ |

Means followed by distinct letters, lowercases between temperatures (for the same combination package x time), uppercases between packages (for the same combination temperature x time) and Greek between time (for the same combination package x temperature), differ significantly using the F test of the analysis of variance.

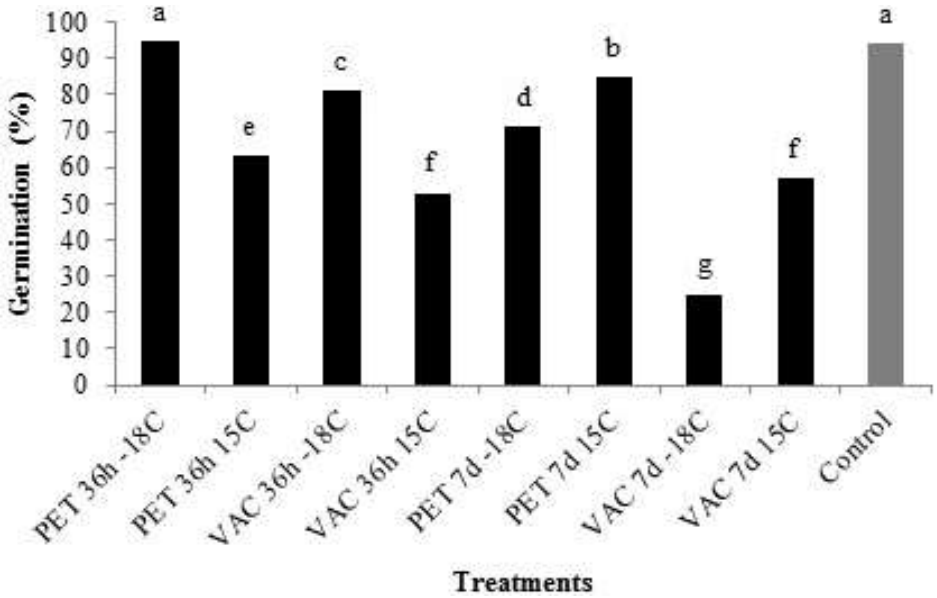


**Figure 1.** Germination of pumpkin seeds in control and subjected to freezing (-18°C) and cooling (15°C) packed in PET bottles and in plastic bags with vacuum for 36 h and seven days, using the Scott Knott test 5% significance.

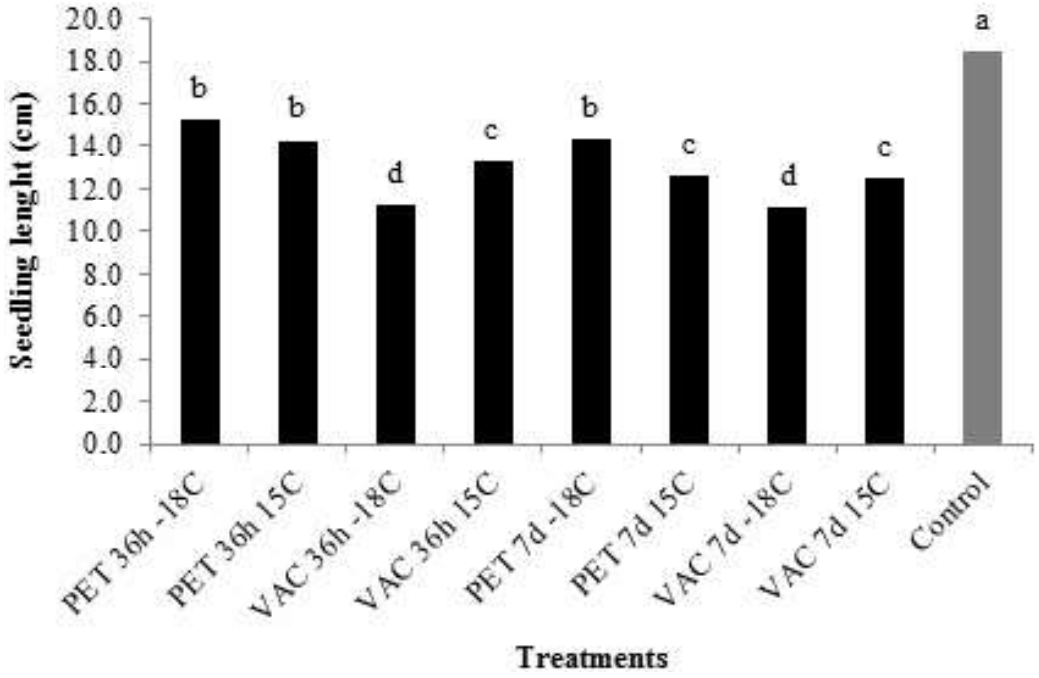


**Figure 2.** Seedling length of pumpkin seeds in the control and subjected to freezing (-18°C) and cooling (15°C) packed in PET bottles and PET and PE bags with vacuum for 36 h and seven days, using the Scott Knott test 5% significance.

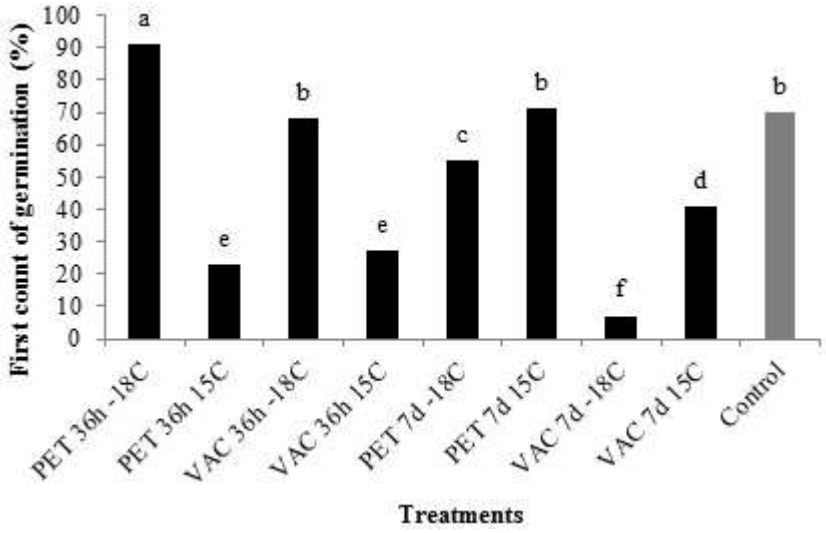


**Figure 3.** First count of germination of pumpkin seeds in control and subjected to freezing (-18°C) and cooling (15°C) packed in PET bottles and in plastic bags with vacuum for 36 h and seven days, using the Scott Knott test 5% significance.

However, the treatment using the PET bottle with 36 h of exposure at a temperature of -1°C increased the vigor of the seeds and did not alter the percentage of germination. There was no change in the moisture content of the seeds after the treatments as shown in the tables.

The sanitary test of pumpkin seeds (Figure 4) was applied for seeds without treatment (control) and for the seeds of the treatment carried out in PET packaging for 36 h at -18°C. The treatment did not alter statistically, the fungus contamination in the seeds. The genus of fungi, *Rhizopus*, *Aspergillus* and *Penicillium* were predominant

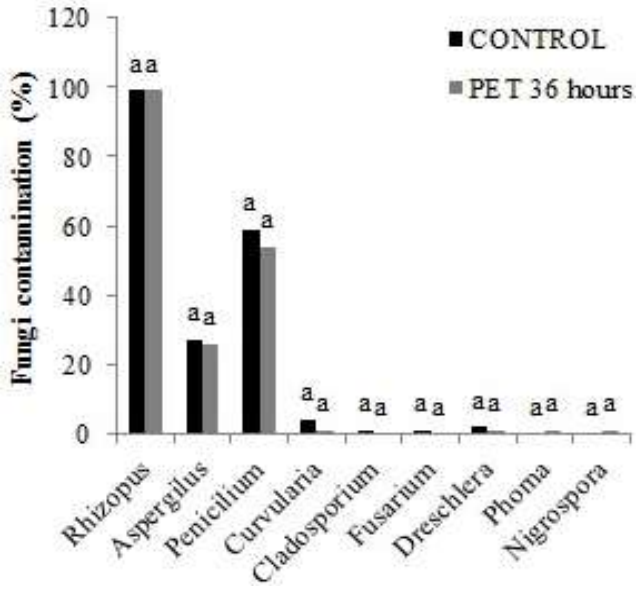


**Figure 4.** Fungi contamination in pumpkin seeds, comparing the treatment (PET packaging for 36 h at -18°C) and the control, using the Mann-Whitney non-parametric test, at 5% probability.

in the treated and untreated seeds, while the genus *Curvularia*, *Cladosporium*, *Fusarium*, *Dreschlera*, *Phoma* and *Nigrospora* were detected in less quantity. Bee and Barros (1999) also found higher quantities of *Aspergillus* and *Penicillium* in pumpkin seeds; however, by storing them in vacuum, it was possible to reduce the incidence of these genus.

The results corroborate with those of Casaroli et al. (2006), who, when working with lots of pumpkin seeds produced in agroecological systems and in conventional systems, treated and untreated, observed that the lots of agroecological systems presented greater contamination by fungi, mainly of the genus *Rhizopus* and *Penicillium*. Paiva et al. (2016), when evaluating the physiological and sanitary quality of lettuce seeds, also found high amounts of the genus, *Rhizopus* in the seeds, although it did not interfere with the germination performance at the laboratory. According to the same author, the genus *Fusarium* and *Cladosporium* are transmitted via plant and seedlings through the seeds. The genus *Rhizopus* is treated as a contaminant and the genus *Aspergillus*, *Phoma* and *Penicillium* are considered as storage fungi.

In the second stage of the experiment, the pumpkin seeds were treated with freezing at -18°C for 36 h in PET packaging and stored for 180 days in the laboratory environment with a temperature of 26 ± 2°C and 55% relative humidity (RH) and in a cold room with a 15 ± 2°C temperature and 77% of RH. The results of the germination test (Table 3) indicate that the time caused a reduction in germination for both storage environments and the seeds in refrigeration had a higher average of germination than those stored in the laboratory environment. At 180 days of storage under environmental conditions, a reduction in the germination of pumpkin seeds occurred, making commercial use unfeasible. These data corroborate with those obtained by Neto et al. (2012), who also observed a reduction in the germination of pumpkin seeds in the sixth month of storage, regardless of the type of packaging used and the environment. However, for the authors, at 360 days of storage, there was an increase in germination percentages, probably due to an overcoming of the physiological dormancy of 'Jacarezinho' pumpkin seeds throughout the storage.

In the evaluations of the seedling length of treated and stored pumpkin seeds (Table 3), the time factor was more significant for the decrease of vigor of the seedlings as compared to the storage site. Similar data was obtained by Croft et al. (2013), who observed that the type of packaging was a more efficient factor in the preservation of the seeds than the refrigeration or not. Yokoyama and Silva Júnior (1988) and Torres et al. (2002) pointed out that pumpkin seeds present physiological shoot and thermoblastic dormancy, which is overcome by the absence of light and high temperatures.

The first count of the germination test results of the treated and stored pumpkin seeds (Table 3) indicate that the environment and time caused a reduction in seed vigor. At 180 days of storage in environment, a drastic descent occurred in the first count of germination. The seeds stored in refrigeration presented higher averages as compared to those stored in the environment. Croft et al. (2013) indicated that storing the seeds in a refrigerated environment can contribute to the preservation of the quality of the seeds. According to Rahim et al. (2013), the *Rhizopus* fungus genus, predominant in almost 100% of the samples of the present work (Figure 5), is capable of causing complete deterioration of seeds and seedlings. This factor may have contributed to the reduction of the physiological quality of pumpkin seeds when evaluating the effects of storage in a laboratory environment. The scarcity of research on pumpkin seed storage techniques makes it difficult to recommend correct procedures for its conservation.

When evaluating the sanity test on pumpkin seeds treated with freezing at -18°C for 36 h and stored for 180 days in laboratory environment (Da 180), under refrigeration (Dg 180) and seeds in control (Figure 5), there was a considerable decrease in the presence of the *Aspergillus* and *Penicillium* fungi genus at both storage sites in comparison with the control. It is possible that the treatment with freezing for 36 h favored the reduction of these fungi and preserved them during the storage period. However, there was no reduction in the incidence of the *Rhyzopus* fungus, which is considered a contaminating fungus. This data corroborates that of Weidenböner (2001) which evaluated the quality of

**Table 3.** Germination, seedling length and first count of germination of pumpkin seeds, treated with PET bottle for 36h at -18°C, during storage up to six months in environment and refrigeration.

| Storage (day) | Laboratory environment | Germination (%) | |
|---|---|---|---|
| | | Refrigeration | General means |
| 45 | 93$^{aA}$ | 89$^{aA}$ | 91$^{A}$ |
| 90 | 85$^{aB}$ | 78$^{bB}$ | 82$^{B}$ |
| 135 | 76$^{aC}$ | 77$^{aB}$ | 77$^{C}$ |
| 180 | 33$^{bD}$ | 75$^{aB}$ | 54$^{D}$ |
| General means | 72$^{b}$ | 80$^{a}$ | |
| **Seedling lenght (cm)** | | | |
| 45 | 16.78$^{aA}$ | 18.68$^{aA}$ | 17.73$^{A}$ |
| 90 | 15.33$^{aA}$ | 15.63$^{aB}$ | 15.48$^{B}$ |
| 135 | 13.85$^{aA}$ | 13.60$^{aB}$ | 13.73$^{B}$ |
| 180 | 15.20$^{aA}$ | 15.03$^{aB}$ | 15.11$^{B}$ |
| General means | 15.29$^{a}$ | 15.73$^{a}$ | |
| **First count of germination (%)** | | | |
| 45 | 80$^{bA}$ | 95$^{aA}$ | 88$^{A}$ |
| 90 | 63$^{aB}$ | 51$^{bB}$ | 57$^{B}$ |
| 135 | 55$^{aC}$ | 50$^{bB}$ | 53$^{C}$ |
| 180 | 4b$^{D}$ | 34$^{aC}$ | 19$^{D}$ |
| General means | 51$^{b}$ | 58$^{a}$ | |

Means followed by the same letters, lowercase on the lines and uppercase on the columns, do not differ using Scott Knott test at 5% of significance.

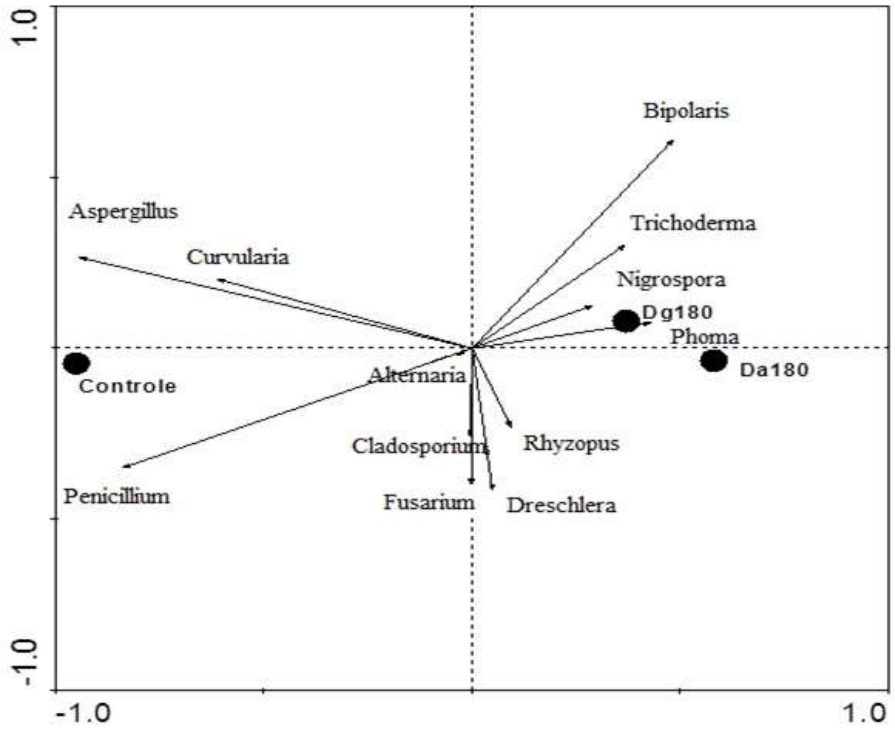


**Figure 5.** Multivariate analysis of the sanity test performed on pumpkin seeds treated and stored for 180 days in laboratory ambient (Da180) and under refrigeration (Dg180), and control (%), using Kruskal-Wallis nonparametric test at 5% of significance.

pumpkin seeds lots and found a predominance of the genus, *Rhyzopus* in all of them. The genera *Curvularia*, *Cladosporium*, *Fusarium*, *Dreschlera*, *Phoma*, *Nigrospora*, *Trichoderma*, *Bipolaris* and *Alternaria* were found in small percentages.

## Conclusion

Biodynamic pumpkin seeds, variety *Biocosta*, with 6% of moisture content can be frozen at -18°C for 36 h without affecting their physiological and sanity quality. Freezing and storage of the seeds in PET packaging proved to be viable and this treatment can reduce the fungus contamination of the genus *Aspergillus* and *Penicillium* after six months of storage. The viability of the seeds treated with freezing reduced after six months of storage. It is not recommended to use the vacuum packaging for seeds with the Oster® machine. Pumpkin seeds should be stored in a refrigerated environment at 15°C and 77% RH for up to six months.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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