

# Physiological and sanitary quality of black bean seeds dependent on storage time and initial seed moisture content

Calidad fisiológica y sanitaria de semillas de frijol negro dependiente del tiempo de almacenamiento y de la humedad inicial de la semilla



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**Black bean seeds - cultivar IPR88 Uirapuru.**

Photo: J.F. Menegaes

## ABSTRACT

Black beans (*Phaseolus vulgaris* L.) are a species of great importance for family farming, both for food security and socioeconomic activity. The objective of this work was to assess the physiological and sanitary viability of black bean seeds stored with different initial moisture contents and cold storage periods. It was used a completely randomized design, 3×5 (seed moisture levels: 10, 13 and 16%, and storage periods: 0, 3, 6, 9 and 12 months in cold rooms, with four repetitions). Black bean seeds belonging to variety IPR88 Uirapuru were grown in the municipality of Santa Maria, Brazil, harvested in March 2020, and stored in a cold room (15°C and 40% RH) in Kraft paper bags (brown type, 1.0 kg). Physiological variables and sanitary quality were assessed through laboratory tests. According to the results, it was possible to verify that the physiological and sanitary quality of black beans were negatively affected with the increase of storage period, in all grades. However, it is possible to store these seeds with moisture contents of 10 or 13% for a period of up to nine months in a cold room, with a 70% germination quality.

**Additional key words:** *Phaseolus vulgaris*; conservation environment; seed moisture content; seed storage.

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## RESUMEN

El frijol negro (*Phaseolus vulgaris* L.) es una especie de gran importancia para la agricultura familiar, tanto para la seguridad alimentaria como para la actividad socioeconómica. El objetivo de este trabajo fue evaluar la viabilidad fisiológica y sanitaria de semillas de frijol negro almacenadas con diferentes humedades iniciales y periodos de almacenamiento en frío. El diseño utilizado fue completamente al azar, 3×5 (niveles de humedad de la semilla: 10, 13 y 16, y periodos de almacenamiento: 0, 3, 6, 9 y 12 meses en cámaras frigoríficas). Se utilizaron cuatro repeticiones en cada unidad experimental. Semillas de frijol variedad IPR88 Uirapuru fueron cultivadas en el municipio de Santa Maria, Brazil, cosechadas en marzo de 2020, y almacenadas en cámara frigorífica (15°C y 40% HR) en bolsas de papel Kraft (tipo marrón de 1,0 kg). Se evaluaron las variables fisiológicas y calidad sanitaria mediante pruebas de laboratorio. De acuerdo con los resultados, es posible verificar que la calidad fisiológica y sanitaria del frijol negro se vieron afectadas negativamente, con el aumento del periodo de almacenamiento, en todos los grados, sin embargo, es posible almacenar estas semillas en condiciones humedad de 10 a 13% por un periodo de hasta nueve meses en cámara frigorífica, con calidad de germinación del 70%.

**Palabras clave adicionales:** *Phaseolus vulgaris*; ambiente de conservación; contenido de humedad de la semilla; almacenamiento de semillas.

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The production of black beans (*Phaseolus vulgaris* L.) in Brazil is intended for domestic consumption, with family farming being responsible for a large part of this production. One of the fundamental agricultural inputs for productivity is good quality seeds, which guarantee sustainability and food security, both for this type of agriculture and for domestic consumption (Sousa and Wander, 2014; Fernandes *et al.*, 2019).

Seed longevity and viability are classified into categories, such as orthodox, recalcitrant and intermediate, depending on their germination performance against desiccation tolerance and storage under different environmental conditions (Walters *et al.*, 2013; Marcos-Filho, 2015). Conservation aims to prolong durability, maintain quality and reduce losses after harvest, providing a longer shelf life and marketing of products of agroeconomic interest, such as seeds. The storage period is one of the most important stages in the agricultural sector, ensuring the maintenance of balance from the distributor market to the final consumer.

Storage, as an agricultural management practice, aims to ensure the integrity and viability of seeds for their qualitative attributes, genetic, physical, physiological and sanitary, between storage periods. However, there is an interaction among seeds and moisture content, due to hygroscopic processes, the type of

packaging, and the conservation environment, all of which can have both positive and negative effects, particularly on the physiological quality of seeds.

In general, desiccation and lowering the temperature are techniques used only for orthodox seeds. Other factors for the conservation of seed quality are the presence and action of phytopathogens and insects, relative humidity and air temperature, oxygen availability, and storage period (Carvalho and Nakagawa, 2012).

The inadequacy of storage conditions favors deterioration and, consequently, a reduction in seed quality, evidenced during the germination and initial development of the seedlings. The speed at which this deterioration occurs can be mitigated by adjusting the initial quality of the lot, the maturity stage, the longevity, the moisture content, the physical conditions of the seeds, and the phytosanitary treatment (Gomes *et al.*, 2016; Menegaes *et al.*, 2021a; 2021b).

Several authors, Santos *et al.* (2005), Maia *et al.* (2011), Silva *et al.* (2014) and Zucareli *et al.* (2015), emphasize the importance of the correct storage conditions for black bean seeds, since their qualities, especially physiological ones, are negatively affected by uncontrolled temperatures and humidity conditions, by accelerating deterioration, impacting the establishment

of plants in the field. The objective of this study was to assess the physiological and sanitary viability of black bean seeds after cold storage at different initial seed moisture contents and for different storage time periods.

## MATERIALS AND METHODS

The experiment was carried out from March 2020 to March 2021, at the Seed Research and Teaching Laboratory of the Department of Plant Science of the Federal University of Santa Maria (UFSM), Brazil, located in Santa Maria, RS (29°43' S; 53°43' W and altitude of 95 m). The climate in the region is humid subtropical (Cfa), according to the Köppen-Geiger classification, with average annual precipitation of 1,769 mm, average annual temperature close to 19.2°C and air humidity around 78.4% (Alvares *et al.*, 2013).

Black bean plants belonging to the IPR88 Uirapuru cultivar were grown in the municipality of Santa Maria, Brazil, and harvested in March 2020. The seeds were stored in a cold chamber (15°C and 40% RH) in Kraft paper bags (brown type of 1.0 kg). This cultivar was chosen due to its excellent adaptation in the region, the possibility of harvesting in a mechanized cultivation medium and the potential for harvesting in a culture medium.

The design used was completely randomized, in a 3×5 factorial scheme (seed moisture levels and storage periods), with seed moisture levels of 10, 13 and 16%, following the methodology by the Rules for Seed Analysis manual (Brazil, 2009a), and storage periods of 0, 3, 6, 9 and 12 months in cold chambers, with four replications, each experimental unit composed of 50 seeds. The first assessment of physiological and sanitary qualities was 48 h after seed harvest, considering this as zero storage period (0).

Subsequently, the qualities were assessed by the following tests:

**Standard germination test (SGT):** seeds were distributed on a roll of germination paper, moistened with distilled water at a rate of 2.5 times the dry paper mass. The rolls were kept in a BOD (Box for Organism Development), with a photoperiod of 24 h and a temperature of 25±2°C (Brazil, 2009a). Germination evaluations were performed at 5 and 9 days after sowing (DAS), and the results are expressed as a percentage of seedlings.

**Length and dry mass of seedlings:** the seeds were kept in the same conditions as the SGT, and at 5 DAS the total length of the seedling, including the stem and the root of ten normal seedlings of each repetition, was measured using a millimeter ruler. Subsequently, the total dry mass was determined by drying the material in a forced ventilation oven at 65±5°C for 48 h and then recording the mass on a digital scale (Kryzanowski *et al.*, 2020).

**Mass electrical conductivity:** performed with four repetitions of 50 seeds, weighed and placed in disposable plastic cups, with a capacity of 200 mL, containing 75 mL of distilled water. The cups were placed in a BOD type germinator set at 25°C, and readings were taken 24 h after imbibition using a table conductivity meter (Kryzanowski *et al.*, 2020).

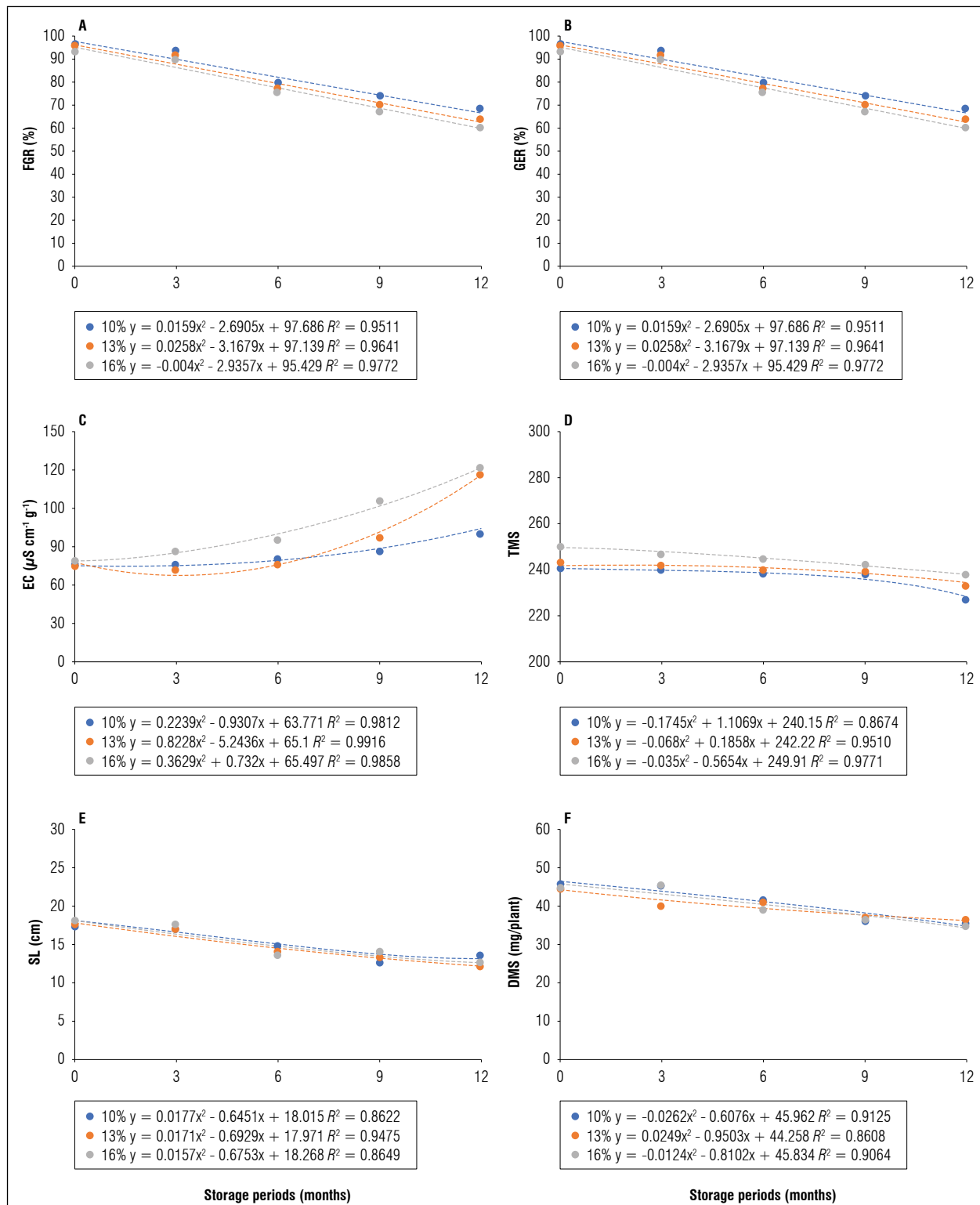
**Sanitary test:** seeds were distributed in transparent plastic boxes for germination in a paper substrate (Blotter Test), moistened with distilled water corresponding to 2.5 times the mass of the dry paper. Seed germination was inhibited by freezing for 24 h at 6±1°C, then the boxes were kept in BOD for 5 d with a photoperiod of 12 h light and 12 h dark at 20±2°C (Brazil, 2009b). They were evaluated under a magnifying glass (stereoscopic microscope) for the identification of phytopathogens at the genus level, and the results expressed as percentage of total infested seeds (SIT).

Data expressed as percentages were transformed into arcsine. Analysis of variance (ANOVA) of the data and comparison of means by the regression test ( $P<0.05$ ) were performed with the aid of the SISVAR program (Ferreira, 2014).

## RESULTS AND DISCUSSION

Tests to verify the physiological quality of black bean seeds showed significant interaction (Fig. 1). The variables of first germination count (FGR) and germination (GER), at 5 and 9 DAS, respectively, gave similar results, with general averages of 95, 92, 77, 71 and 64% of FGR and GER for storage periods of 0, 3, 6, 9 and 12 months in cold chambers, in that order. Fernandes *et al.* (2019) found that black bean seeds showed similar FGR and GER, even when subjected to saline conditions.

According to Normative Directive no. 45/2013 of the Ministry of Agriculture, Livestock and Supply



**Figure 1. (A) First germination count (FGR), (B) germination (GER), (C) mass electrical conductivity (EC), (D) thousand seed mass (TSM), (E) seedling length (SL) and (F) seedling dry mass (SDM) of black bean seeds (*Phaseolus vulgaris* L.) cultivar IPR88 Uirapuru subjected to different storage periods and seed moisture levels.**

(MAPA) (Brazil, 2013), black bean seeds must have at least 70% germination for the basic commercial category and for other categories at least 80% germination. Thus, data from this study indicate that the commercialization of black bean seeds is possible for up to nine months of storage at seed moisture levels of 10 and 13%, and for up to 6 months at 16% seed moisture content.

Seed deterioration was confirmed both by the reduction of germination percentage and by the mass electrical conductivity (EC), which worsened during storage, mainly in seeds at a higher degree of humidity. Boiogo *et al.* (2013) verified by the EC test that storage of cowpea seeds (*Vigna unguiculata* (L.) Walp.) for more than 90 d resulted in increased deterioration with negative implications for germination percentage.

The reduction in germination percentage of these seeds may be attributed to the interaction of the genetic characteristics of the species with the storage environment. These results are similar to those verified by Menegaes *et al.* (2021a; 2021b) for safflower seeds (*Carthamus tinctorius* L.) stored for 0, 4, 8 and 12 months in cold chambers. For the aforementioned authors, the EC test confirms that as the storage period of the seeds increases, their germination potential is reduced, inducing an increase in their deterioration, even under controlled conditions of relative humidity and temperature. According to Marcos-Filho (2015), greater electrolyte leaching in the EC test reflects greater deterioration of seed quality, negatively affecting the emergence of seedlings in the field.

It was observed that the mass of a thousand seeds (TSM) showed an interaction between the storage period and seed moisture content (Fig. 1D), with a reduction of the masses during storage. Scariot *et al.* (2017) attributed the reduction of TSM of wheat seeds (*Triticum aestivum* L.) during the storage period to the maintenance of the hygroscopic balance and the permeability of the storage form, considering that there is an energy expenditure for this maintenance.

It was found that there was a similarity in mean seedling length (SL) and seedling dry mass (SDM) of black beans at all moisture content levels, with averages of 18, 17, 14, 13 and 13 cm, and 45, 44, 41, 37 and 36 mg/plant for storage periods of 0, 3, 6, 9 and 12 months in cold chambers, respectively. Zucareli *et al.* (2015) found that during storage periods of up to 18 months, seeds of the Carioca beans variety had

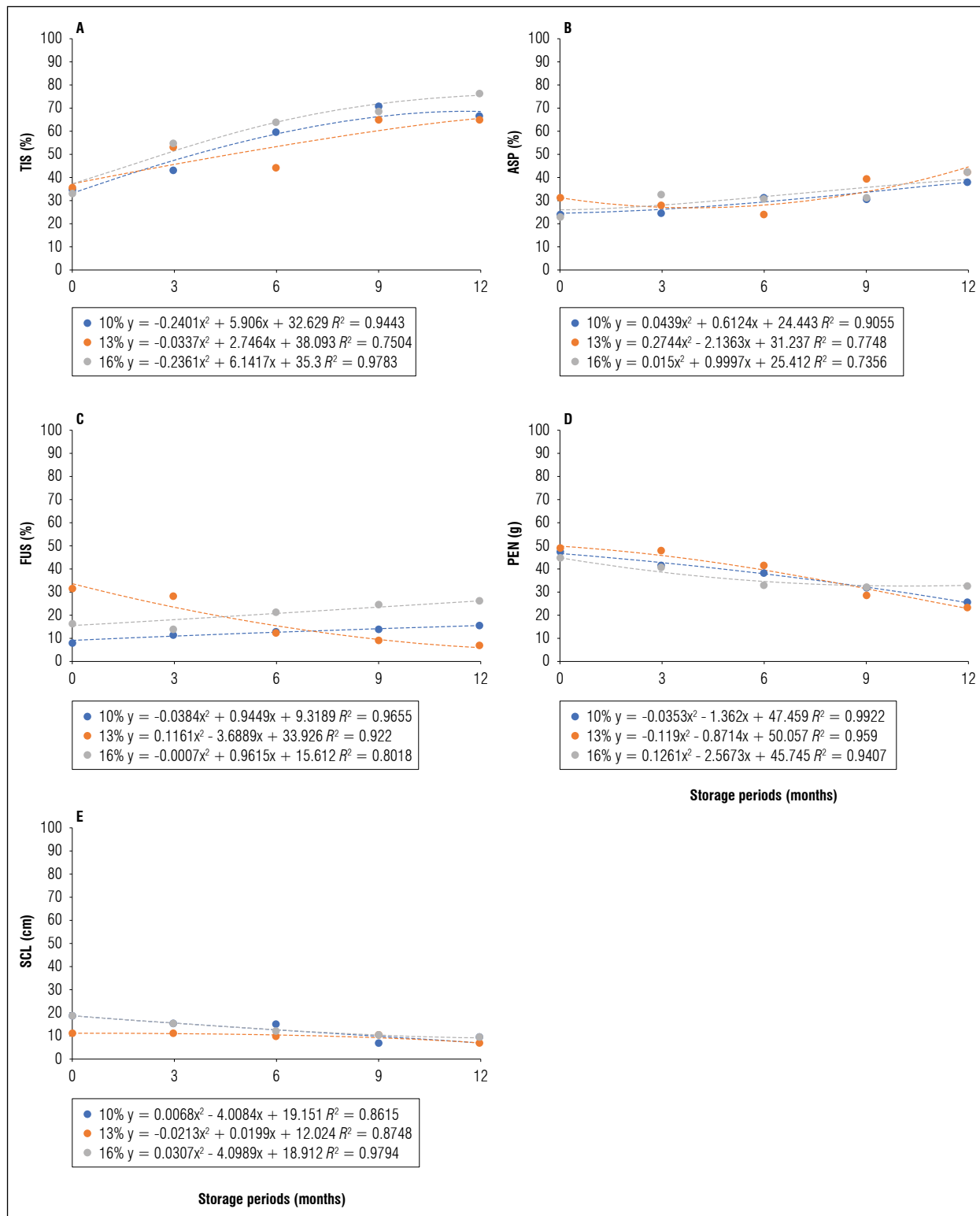
a reduction in physical quality during the general form.

The storage period directly influenced the total number of infested seeds, regardless of the seed moisture levels (Fig. 2). From this evaluation, the presence of phytopathogens of the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Sclerotinia* (Fig. 2B, 2C, 2D and 2E) was verified in the stored black bean seeds. Gomes *et al.* (2016) emphasize the importance of seed health quality for productivity, especially for fava bean (*Phaseolus lunatus* L.) seeds, which, when infested by phytopathogens, show reduced emergence, negatively affecting the establishment of plants in the field.

It was observed that the regression trends on the incidence of the *Aspergillus*, *Fusarium*, *Penicillium* and *Sclerotinia* phytopathogens, were similar at all humidity levels of black bean seeds over different storage periods. On the other hand, it was noted that *Aspergillus* fungi (Fig. 2B) showed an increasing regression trend as the storage period increased for all moisture levels. Menegaes *et al.* (2021b) found similar fungal behavior for safflower seeds stored for 0, 4, 8 and 12 months.

The opposite was verified for *Penicillium* and *Sclerotinia* (Fig. 2D and 2E), which showed a decreasing trend according to the storage period, with the lowest percentage of fungal incidence at 12 months of storage for all moisture levels. Marino *et al.* (2008) attribute the difference in incidence of pathogens on black bean seeds to the storage environment. Care must be taken from this harvest to avoid the association of pathogens with the seeds as much as possible, since the pathogen survives longer, maintaining its viability and characteristics, and can be easily disseminated in different environmental conditions, at levels considered to be contamination, as seen in Fig. 2C for *Fusarium* pathogens.

The results of the present study corroborate the findings of Santos *et al.* (2005), Maia *et al.* (2011), Silva *et al.* (2014) and Zucareli *et al.* (2015), in which all authors verified the importance of storing bean seeds under controlled conditions in order to avoid accelerating their deterioration. It was observed that with the extension of storage periods in all seed moisture levels, there was a reduction in the physiological quality of black bean seeds and a considerable increase in the incidence of pathogens on the seeds. Since the storage conditions were not enough to help in the phytosanitary control of these seeds, further studies are required on this subject.



**Figure 2.** (A) Total infested seeds (TIS), (B) *Aspergillus* spp. (ASP), (C) *Fusarium* spp. (FUS), (D) *Penicillium* spp. (PEN) and (E) *Sclerotinia* spp. (SCL) of black bean (*Phaseolus vulgaris* L.) seeds, cultivar IPR88 Uirapuru, subjected to different storage periods.



## CONCLUSION

It is possible to store black bean seeds at different initial seed moisture contents and periods in a cold chamber. The physiological and sanitary qualities of black bean seeds are maintained for up to nine months at 10 or 13% seed moisture contents and up to 6 months at 16% seed moisture content.

**Conflict of interests:** The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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