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Physiological and sanitary quality of safflower seeds with different storage periods

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Abstract

The safflower crop (*Carthamus tinctorius* L.) is of agro-economic potential due to its wide range of uses, from ornamentation to oil extraction, with good adaptability to cultivation throughout Brazil. However, this crop still faces challenges regarding the quality of its seeds, as there is a high susceptibility to the attack by phytogenes. Thus, the objective of this study was to evaluate the physiological and sanitary quality of safflower seeds submitted to different storage periods. The experiment was conducted in a completely randomized design, 4x7 (seeds grown in the 2017/2018 harvest: sown in winter and spring 2017, and summer and autumn 2018, and seed storage periods: 0; 6; 12; 18; 24; 30 and 36 sequential months, counted from the harvest dates), with four replications. The quality of seeds was evaluated through the following tests: germination, electrical conductivity, dry mass and seedling length, field emergence and sanity. It was observed that the different safflower seed lots were sensitive to a prolonged storage period, with reduction in their physiological potential, especially in the percentage of germination and seedling emergence, and an increased incidence of harmful phytopathogens on these seeds. Thus, it is recommended a period of up to 12 months to obtain the conservation of safflower seeds.

Keywords: *Carthamus tinctorius* L., germination potential, incidence of phytopathogens

Introduction

The *safflower crop (Carthamus tinctorius* L.), belonging to the Asteraceae family and originating in Asia, is characterized by its cultivation rusticity and by presenting a wide aptitude for uses, in particular with its floral stems for ornamentation and its seeds for oil extraction, both for medicinal purposes or for biodiesel. However, the cultivation of this plant in Brazil has still been a challenge, due to its high susceptibility to phytopathogen attacks, which has depreciated the ornamental quality of floral stems and the quality of seeds (Emongor & Oagile, 2017; Menegaes & Nunes, 2020).

For an effective production of floral stems, the quality of the seeds is fundamental. Among the attributes that determine this quality, the physiological and the sanitary aspects act, above all, in the vigor and establishment of the plants in the field. It is known that these qualitative attributes change over time, especially due to seed storage conditions and periods (Marcos-Filho, 2015).

The conservation of these qualitative attributes of seeds during the storage period may be compromised by several factors. The main attribute is controled by the conditions of the seeds, such as seed moisture content and the presence of phytopathogens and insects. Another attribute is storage conditions, such as relative air humidity and temperature, packaging, oxygen availability, among others (Abreu et al., 2013; Menegaes et al., 2021).

Even with all necessary management during seed storage period, some authors have verified the occurrence of deterioration in sunflower (*Helianthus annuus* L.), pepper (*Capsicum chinense* L.) and safflower seeds during their storage under fully controlled conditions (Abreu et al., 2013; Silva et al., 2018; Menegaes et al., 2021). All authors showed that the symptoms of

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seed deterioration are related to their physiological and sanitary attributes. These results were obtained through laboratory and field tests, whereas in both conditions the seeds showed a low potential for germination and initial seedling development.

During storage period, there is a water movement (vapor) at the seed-environment-packaging interface, which seeks to maintain the hygroscopic balance among them. This movement is sensitive to variation in temperature and air humidity. This balance affects positively or negatively the longevity of seeds of several species, such as forestry, horticulture and floriculture. On average, it is recommended a humidity level between 8% and 14% to maintain the quality of seeds and reduce or suppress pathogen activities (Silva et al., 2015; Marcos-Filho, 2015).

In this context, the objective of this study was to evaluate the physiological and sanitary quality of safflower seeds submitted to different storage periods.

Material and methods

This experiment was carried out in the Seed Research and Didactics Laboratory and in the Floriculture Sector, both from the Plant Science Department of the Federal University of Santa Maria (UFSM), located in Santa Maria, RS (29°43' S; 53°43' W and altitude of 95m), between 2017 and 2021. The climate in the region is humid subtropical (Cfa), according to the Köppen-Geiger classification, with average annual rainfall of 1,769 mm, average annual temperature close to 19.2° C and air humidity around 78.4% (Alvares et al., 2013).

A completely randomized experimental design was used, in a 4x7 factorial scheme (seed lots and seed storage periods), with four replications (each experimental unit consisting of 50 seeds).

The lots of safflower seeds from the Lasting Orange cultivar were grown in the experimental area of the Floriculture Sector at the UFSM Plant Science Department, in Santa Maria, in the 2017/2018 harvest. The sowing of each seed lot took place at the beginning of each year season, characterizing the lots as: **Lot 1**: sown in winter (07/05/2017) and harvested 127 days after sowing (DAS); **Lot 2:** sown in spring (10/06/2017) and harvested 98 DAS; **Lot 3:** sown in the summer (01/04/2018) and harvested 95 DAS; and **Lot 4:** sown in autumn (04/03/2018) and harvested 147 DAS.

The seeds were harvested at the physiological maturation stage of the capitula (**Figures 1**a and 1b), according to the description of the phenological stages of safflower plants made by Flemmer, Franchini and Lindström (2015).

After harvesting, the seed lots were stored in a cold chamber (15° C and 40% RH) in kraft paper bags (brown type of 1.0 kg), with an average humidity degree of 9.0%, until the execution of this experiment process. Seed storage periods occurred at 0; 6; 12; 18; 24; 30 and 36 sequential months, counted from the aforementioned harvest dates.

At each storage period, the following parameters were evaluated:

Seed moisture content (SMC): determined by the oven method at 105±3° C for 24 h, using four replications of 5 g (adapted from BRASIL, 2009a).

Thousand seed mass (TSM): determined by the method of Brasil (2009a), with four replications.

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 B.O.D. type germinator, with a photoperiod of 24 h and temperature of 25±2° C (Brasil, 2009a). Germination assessments were performed at four and 14 DAS, and the results expressed as percentage of normal seedlings. For the germination speed index (GSI) (Furbeck et al., 1993) evaluations were performed daily up to four DAS. The germination of normal seedlings was used as a criterion, those that showed elongation of the primary root and emergence of cotyledons (Brasil, 2009a; Abud et al., 2010).

Mass electrical conductivity (EC): the mass of 50 seeds was measured, and they were placed in disposable plastic cups, 200 mL capacity, containing 50 mL of distilled water. Then, the cups were kept in a B.O.D. type germinator, set at 25° C, and readings were taken 22 h after imbibition (before the period of root protrusion 24 h after imbibition), in a table conductivity meter (Kryzanowski et al., 1999).

Seedling length and dry mass: the seeds were kept in the same condition as the SGT, at four DAS it was measured the total seedling length including the aerial part and the radicle of ten normal seedlings of each repetition, 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, then the mass was measured on a digital scale (Kryzanowski et al., 1999).

Emergence in the field: the seeds were distributed in lines of 1 m, spaced at 0.2 m and with a depth of 0.03 m. Final evaluations were performed at 14 DAS, with results expressed as percentage of seedling emergence (Brasil, 2009b). For the emergence speed index (ESI) and for the mean emergence time (MET; days) daily

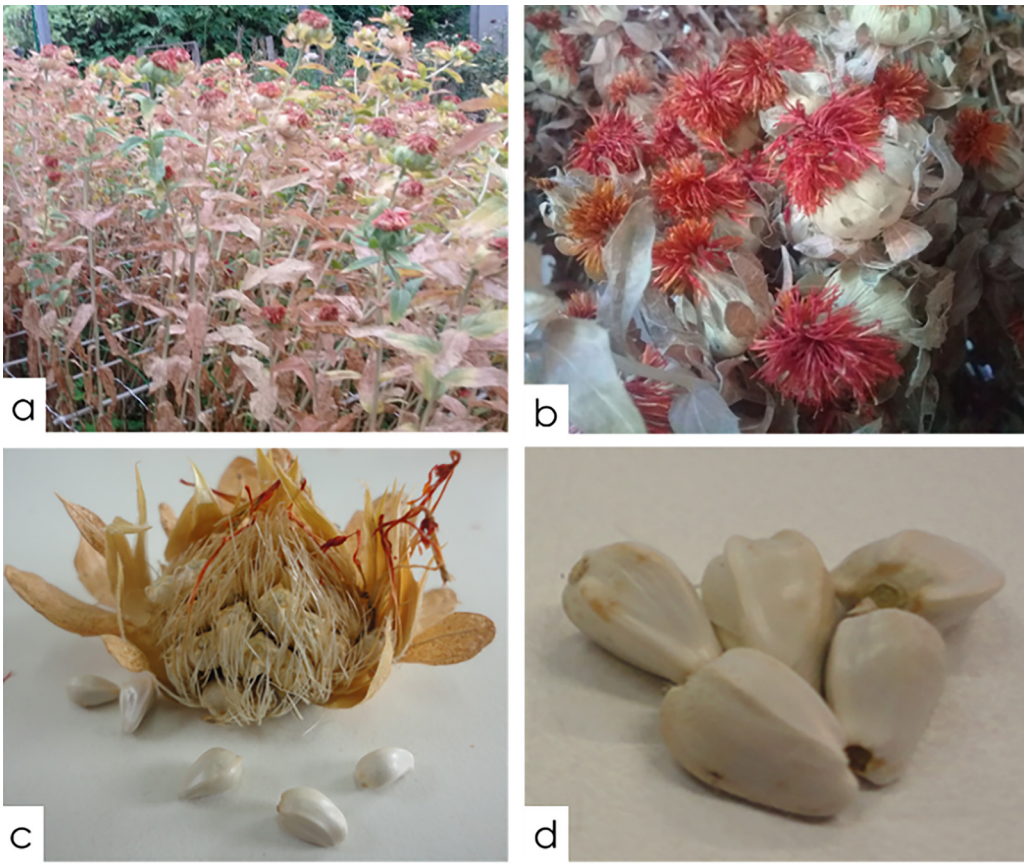

Figure 1. Physiological maturity of floral stems (a) and closed capitula (b), open capitula with exposure of seeds (c) and safflower seeds (*Carthamus tinctorius* L.). Photos: Authors (2018).

assessments were performed up to 14 DAS, according to the methodology of Furbeck et al. (1993), the complete development of the cotyledons and epicotyl was used as a criterion (Abud et al., 2010).

Sanity test: the seeds were distributed in transparent plastic boxes for germination in paper substrate (Blotter Test) moistened with distilled water, corresponding to 2.5 times the dry paper mass. Seed germination was inhibited by freezing for 24 h at a temperature of 06±1° C, then the boxes were kept in B.O.D. for five days with a photoperiod of 12 h of light and 12 h in the dark at a temperature of 20 ± 2° C (Brazil, 2009b). They were evaluated under a magnifying glass (stereoscopic microscope) with the identification of phytopathogens at the genus level, and the results were expressed in percentage of total infested seeds (TIS).

The relative frequency of germination (Fr) was determined by the adapted methodology of Menegaes et al. (2018), expressed in the Equation [$Fr=ni/\sum_{i=1}^{k} ni$], where: Fr = relative frequency of germination; ni = number of seeds germinated per day; Σni = total number of germinated seeds.

Data expressed as percentages were transformed into arcsine (√x/100). Analysis of variance (ANOVA) of the data and comparisons of means by regression or Scott-Knott tests ($p<0.05$) were performed with the aid of the SISVAR software (Ferreira, 2014).

Results and discussion

It was observed that all safflower seeds showed a tendency to reduce moisture content (SMC) over the different storage periods (**Figure 2**a). The four lots of seeds before storage contained an average of 9.0% SMC. It was observed that after 36 months of conservation the average moisture content was 7.5%. In the first months, a greater variation in SMC was observed, which may be attributed to the hygroscopic balance at the seed-environment-packaging interface. Menegaes et al. (2021) found that safflower seeds stored for 12 months in different packages also tended to decrease moisture percentage due to hygroscopicity at the seed-environment-package interface.

Consequently, this balance also negatively affected the mass of one thousand seeds (TSM), with a variation of 35.1; 35.3; 36.2 and 37.6 g for lots 1; two; 3 and 4, respectively, before storage, and after 36 months of storage the lots presented TSM of 33.1; 33.2; 33.4 and 31.2 g, respectively (Figure 2b). Scariot et al. (2017) attributed the variation of the SMC of wheat seeds (*Triticum aestivum* L.) during storage to the interaction of the permeability of

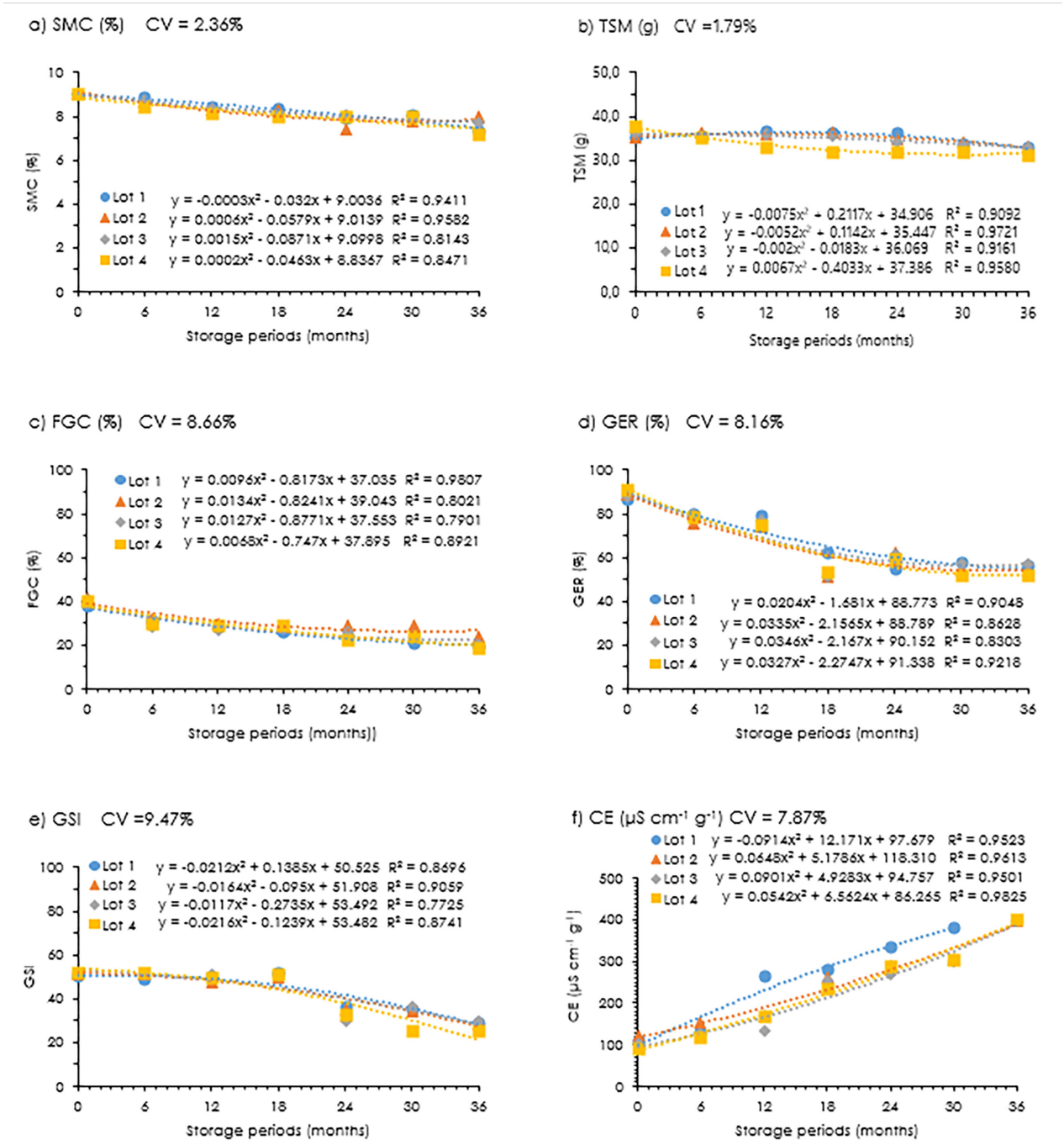

Figure 2. Safflower seed lots (*Carthamus tinctorius* L.) stored for different periods. CV: Coefficient of variation. Moisture degree (SMC, a), thousand seed mass (TSM, b), first germination count (FGC, c), germination (GER, d), germination speed index (GSI, e) and electrical conductivity (EC, f).

the packages with the environment, for the maintenance of the hygroscopic balance.

It was observed in all safflower seed lots that the first germination count (FGC) at 4 DAS and the germination (GER) at 14 DAS followed a similarity in the different storage periods. The reduction in the percentage of vigor in FGC and germination from 12 months onwards was more prominent than in the other periods (Figures 2c and 2d).

Regarding the germination speed index (GSI) it was observed that from 18 months of storage there was a considerable reduction in this parameter for all safflower seed lots, being more pronounced at 36 months (Figure 2e), showing signs of deterioration in these seeds. The physiological quality of seeds is essential for their productive expression in the field, when deteriorated, this expression is reduced and it does not allow a homogeneous plant stand. Electrical conductivity (EC) is a parameter that helps to measure this quality. In Figure 2f, a considerable increase in EC is observed for

all safflower seed lots as the storage period increases. Thus, the deterioration of these seeds is evidenced by the decrease in the REE percentage and the increase in EC, which are inversely proportional.

According to Marcos-Filho (2015), the metabolic process of the initial development of seedlings, expressed by their physiological potential, may be related to the adaptive interaction of seeds and the storage conditions to which they were exposed. Almeida et al. (2010), studying the physiological quality of several oil seeds, such as herbaceous cotton (*Gossypium hirsutum* L.), peanuts (*Arachis hipogaea* L.), sunflower and soybean (*Glycine max* (L.) Merr.), found that after 135 days of storage, there was an accelerated deterioration in this quality due to the interactions of the genetic characteristics of each species with the storage environment.

The physiological potential with field emergence test (FET) and emergence speed index (ESI), added to the total infested seeds (TIS) (**Figures 3**a, 3b and 3c), indicate the deterioration of the four safflower seed lots as the storage period increases, as it was verified that there was a reduction in the expression of emergence in the field, similar to the expression of this potential in the laboratory by the parameters of FGC, REE and GSI (Figure 2).

According to Marcos-Filho (2015), the acceleration of seed deterioration occurs by the sum of several factors resulting in physiological changes under environmental storage conditions, which will be expressed at the time of germination and emergence of seedlings in the field.

Figure 4 shows the relative frequency of emergence of safflower seedlings in the field, where there is a variation in the mean emergence time (MET) and in the total number of germinated seeds (TNG) that originated typical seedlings. There was a disparity in emergence peaks in all safflower seed lots, indicating heterogeneity in the physiological quality of these seeds.

That is, as the storage period increased, the emergence peaks were being shifted, evidencing the deterioration of seeds, since they needed more time to carry out their metabolic reactions. Alves et al. (2011) observed that the displacement of the relative frequency line in the germination process of canafistula seeds (*Peltophorum dubium* (Spreng.) Taubert) occurred in function of mean germination time (MGT), associating the physiological quality of seeds as a limiting factor in this process.

The opposite was verified by Menegaes et

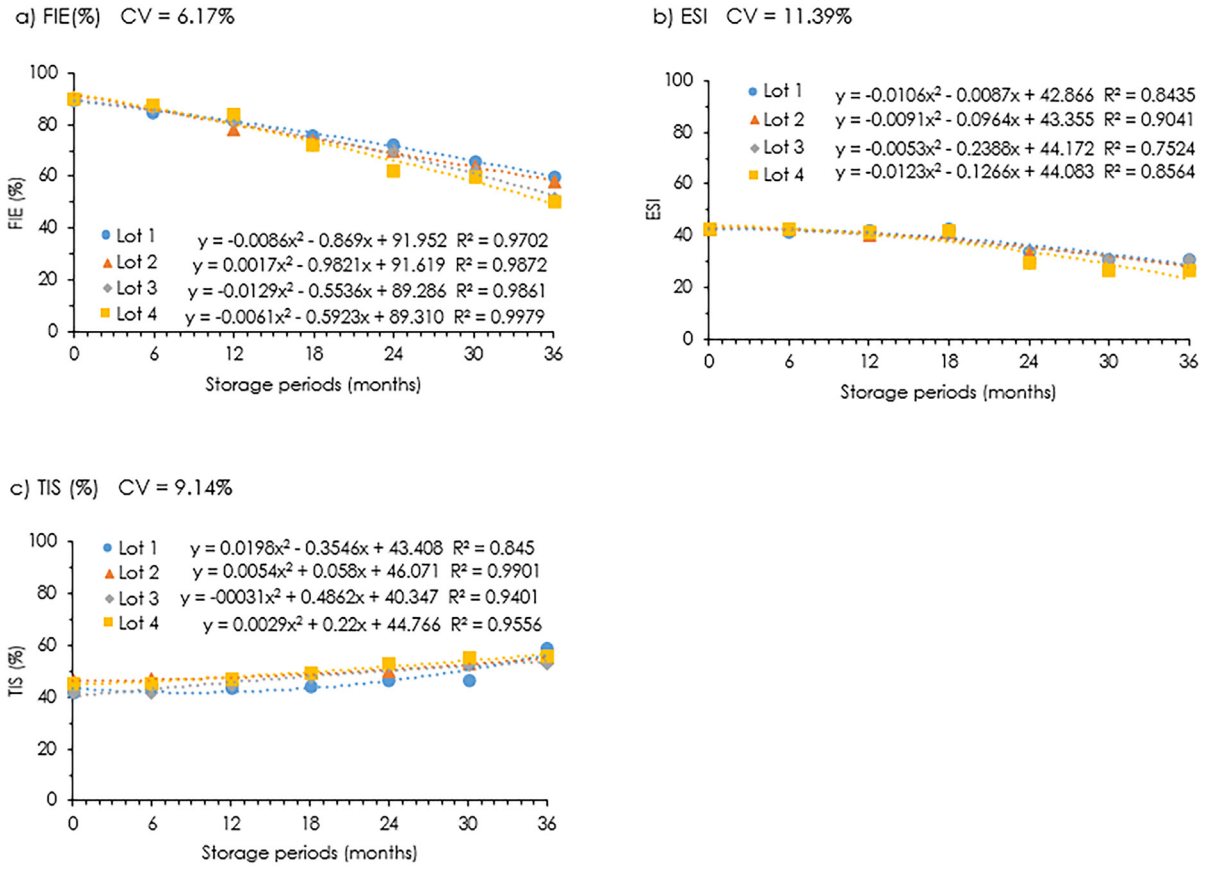

Figure 3. Lots of safflower seeds (*Carthamus tinctorius* L.) stored for different periods. CV: Coefficient of variation. Field emergence (FIE, a), emergence speed index (ESI, b) and total infested seeds (TIS, c).

al. (2019) for the germination of two species of celosia (*Celosia argentea* and *C. cristata*) stored for 16 years, in which the coincidences of the germination peaks were close, thus attributing this homogeneity to the genetic quality of the seeds that were preserved by the storage environment.

In Table 1, in relation to the dry mass and the length of the seedlings, it was verified that the differentiation occurred between the lots, being similar between the storage periods. The average dry mass of seedlings among the lots was 8.8; 9.4; 10.4; 9.9; 9.1; 10.3 and 9.9 mg pl^{-1}, and the mean seedling length was 7.7;

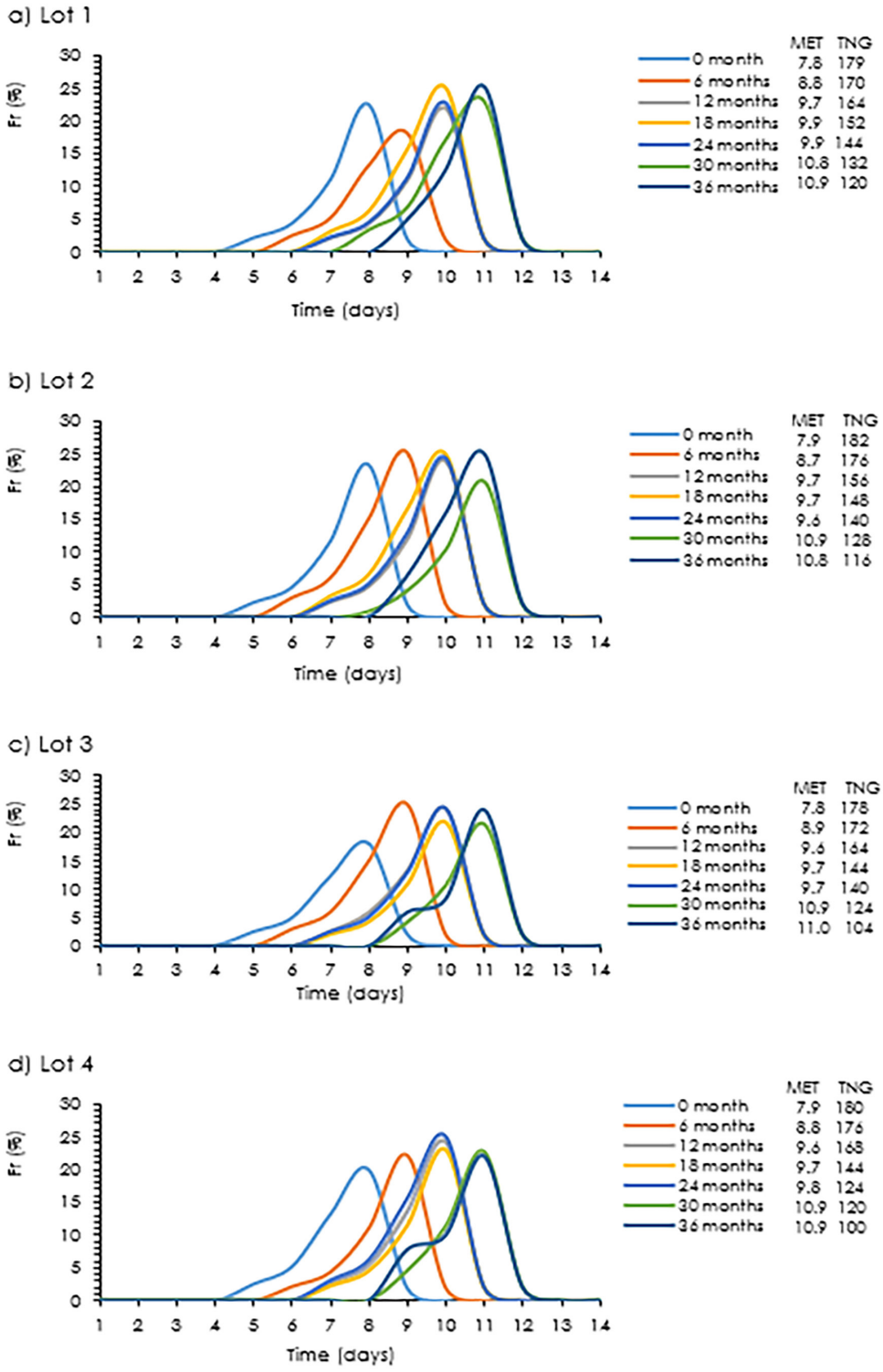

Figure 4. Emergence frequency of different safflower seed lots (*Carthamus tinctorius* L.) submitted to storage for periods of 0; 6; 12; 18; 24; 30 and 36 months. MET: mean emergence time and TNG: total number of germinated seeds.

Table 1. Lots of safflower seeds (*Carthamus tinctorius* L.) stored for different periods.

	Months							Months						
LOTS	0	6	12	18	24	30	36	0	6	12	18	24	30	36
	Dry seedling mass (mg pl^{-1})							Seedling length (cm)						
1	8,4 c*	8,8 c*	10,4 b*	9,8 b*	9,0 b*	10,1 a*	10,0 a*	7,1 b*	8,2 b*	8,4b*	8,7 a*	7,2 c*	8,1 b*	8,9 b*
2	8,9 b	9,2 b	10,3 b	9,4 c	8,8 a	10,5 a	9,9 b	8,2 a	8,3 b	8,6 a	8,3 b	9,2 a	8,2 b	7,4 c
3	9,7 a	9,4 b	10,6 a	9,7 b	9,2 b	10,5 a	9,4 b	7,4 b	8,6 a	8,7 a	8,5 b	8,39 b	9,1 a	9,5 a
4	8,0 d	10,1 a	10,1 a	10,6 a	9,2 b	10,4 b	10,1 a	7,9 a	8,0 b	8,2 b	8,6 a	7,6 c	8,1 b	8,7 b
CV (%)	4,16							2,79						
	Aspergillius spp. (%)							*Botrytis* spp. (%)						
1	24,5 b*	13,3 b*	10,6 b*	76,1 a*	82,7 a*	35,6 d*	63,6 c*	9,6 b*	13,3 b*	10,6 a*	7,2 d*	15,3 a*	16,8 c*	11,2 c*
2	27,9 a	9,0 c	9,2 c	61,7 b	73,5 b	55,4 c	59,8 d	11,1 b	9,0 c	9,2 b	9,1 c	12,4 b	21,0 b	14,9 b
3	24,0 b	19,1 a	10,3 b	59,4 c	65,1 c	59,8 b	70,4 b	13,5 a	19,1 a	10,3 b	11,5 b	10,3 c	15,8 d	22,1 a
4	23,0 b	13,3 b	11,4 a	34,7 d	59,9 d	71,7 a	82,3 a	10,5 b	13,3 b	11,4 a	17,6 a	10,9 c	22,9 a	15,3 b
CV (%)	11,16							7,78						
	Fusarium spp. (%)							*Penicillium* spp. (%)						
1	24,7 c*	25,8 a*	13,7 c*	0,0 d*	0,0 d*	27,2 a*	19,4 a*	34,0 a*	6,8 c*	17,0 d*	10,3 b*	0,0 b*	14,0 a*	3,3 b*
2	24,3 c	14,3 c	21,7 b	10,8 b	9,3 b	17,1 b	0,0 c	29,2 b	17,9 b	22,6 c	7,7 c	0,0 b	4,7 b	21,6 a
3	29,0 b	18,9 b	23,2 a	7,2 c	6,3 a	17,6 b	4,7 b	23,9 d	23,8 a	25,3 b	9,3 b	7,7 a	5,1 b	0,0 c
4	30,3 a	9,1 d	24,0 a	30,2 a	11,7 a	2,1 c	0,0 c	27,1 c	18,0 b	27,2 a	15,9 a	6,5 a	0,0 c	0,0 c
CV (%)	16,72							19,32						
	Sclerotinia spp. (%)													
1	7,3 b*	31,3 a*	20,9 a*	6,4 c*	2,0 c*	6,3 a*	2,6 b*							
2	7,6 b	27,1 b	17,9 b	10,7 b	4,8 b	1,9 c	3,7 a							
3	9,7 a	11,0 d	20,1 a	12,6 a	10,6 a	1,7 c	2,9 b							
4	9,2 a	16,0 c	21,8 a	1,6 d	11,0 a	3,3 d	2,3 b							
CV (%)	20,12													

*Means in a row with the same letter differ by the Scott-Knott test p<0.05). CV: Coefficient of variation.

8.3; 8.5; 8.5; 8.1; 8.4 and 8.7 cm for 0; 6; 12; 18; 24; 30 and 36 months of storage, respectively.

According to Taiz and Zeiger (2009), the growth and development of seedlings are compromised when the seeds are subjected to stress conditions, negatively affecting their enzymatic activities and, consequently, presenting a high variation of these parameters. Corroborating the variation in FGC (**Figure 2c**), GER (**Figure 2d**), EC (**Figure 2e**), FIE (3a) and relative frequency (**Figure 4**).

In the sanity test, there was a high incidence of phytopathogens, with an average of 44% for the four lots of safflower seeds before storage (month zero), and presenting an increase in this incidence with an average of 56% after 36 months of storage (**Figure 3c**). Coronado (2010) attributes the sanitary quality of safflower seeds to low productivity and plant establishment in the field. Öğüt and Oğuz (2006) verified in their studies that the high incidence of phytopathogens during the safflower cultivation cycle reduced the oil yield by up to 75%.

In Table 1, the identification of phytopathogens on safflower seeds occurred at the genus level, with the highest incidences being of *Aspergillius* spp., *Botrytis* spp., *Fusarium* spp., *Penicillium* spp. and *Sclerotinia* spp. In the study by Girardi et al. (2013), the authors found a high incidence of *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. in the percentages of 62%; 42% and 56%, respectively, with a low percentage of germination in safflower seeds harvested at different times.

Coranado (2010) reports that for the safflower crop, phytopathogens of the genus Botrytis spp. are the main cause of diseases, because they stay inside the floral capitulum, attacking the ligules and causing a squashing in the seeds. According to Sharma et al. (2015), phytopathogens of the genus Fusarium spp. are responsible for substantial losses in safflower productivity in the field, as the seeds show early deterioration due to stains, rotting and mold on their surface.

The least expressive phytopathogens were from the genus *Sclerotinia* spp., nevertheless, Venturoso et al. (2015) observed that pathogens of this genus, when incident on oilseeds such as safflower and sunflower, negatively affect the percentage and speed of seedling emergence.

It is well known that the high incidence of phytopathogens before and after the different storage periods negatively affected both germination and emergence of safflower seeds. Thus, confirming the studies of Sharma et al. (2015) and Menegaes et al. (2021), who emphasize the importance of the seed sanitary quality for the establishment of plants in the field, positively impacting productivity, either of flower stems or seeds.

Conclusion

The storage of safflower seeds (*Carthamus tinctorius* L.) negatively affects their physiological and sanitary quality. It reduces their germination potential and increases the incidence of phytopathogens as the storage period was extended for all tested seed lots. This way, a period of up to 12 months is recommended to obtain the conservation of safflower seeds.

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