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Substrates and duration for conducting the safflower seed germination test

Substratos e tempo de duração para condução do teste de germinação de sementes de cártamo

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Abstract

Safflower (*Carthamus tinctorius*) is an annual, rustic plant that has multiple uses. Seed laboratory analysis showed discrepancies in germination depending on the substrate used in a given lot. Therefore, studies are needed to determine the best substrate and the time required to perform the first and final count of the safflower seed germination test. Five lots were used, which were characterized by moisture degree and thousand seed weight. These lots were evaluated on four substrates (between paper, on paper, paper roll, and between sand). The five lots and four substrates were distributed in a 5x4 factorial scheme in a randomized block design with four replicates of 25 seeds. The influence of the factors was evaluated by daily germination count until stabilization, determining the percentage of normal and abnormal seedlings and dead seeds; germination speed index; initial, mean, and final germination time; synchrony and relative frequency; and the date of the first and final germination count. After obtaining the data, analysis of variance was performed. When there was a significant effect, the means were compared using the Tukey test at 5% and 1% probability. Therefore, it is recommended that the safflower seed germination test be conducted on the substrate between paper, with the first and final count being performed at 3rd and 8th days, respectively.

Additional keywords: *Carthamus tinctorius*; germination index; germination method.

Resumo

O cártamo (*Carthamus tinctorius*) é uma planta anual, rústica, que possui múltiplos propósitos de uso. Em análise de laboratório de sementes, verificou-se discrepâncias na germinação, dependendo do substrato adotado em um mesmo lote. Assim, fazem-se necessários estudos para determinar o melhor substrato e o tempo necessário para a realização da primeira contagem e da contagem final do teste de germinação para sementes de cártamo. Foram utilizados cinco lotes, caracterizados quanto ao grau de umidade, e a massa de mil sementes. Estes lotes foram avaliados em quatro substratos (rolo de papel, entre papel, sobre papel e entre areia). Os cinco lotes e os quatro substratos foram distribuídos no esquema fatorial 5x4, em delineamento de blocos casualizados, com quatro repetições de 25 sementes. A influência dos fatores foi avaliada por meio da contagem diária da germinação até a estabilização, determinando-se a porcentagem de plântulas normais e anormais, sementes mortas, índice de velocidade de germinação, tempos de germinação inicial, médio e final, sincronia e frequência relativa, além de ser estabelecida a data da primeira contagem e da contagem final da germinação. Após a obtenção dos dados, foram efetuadas as análises de variância. Quando houve efeito significativo, as médias foram comparadas, utilizando-se do teste de Tukey, a 5% e 1% de probabilidade. Assim, recomenda-se que o teste de germinação para sementes de cártamo deve ser conduzido em substrato entre papel, com primeira contagem e contagem final ao 3º e 8º dias, respectivamente.

Palavras-chave adicionais: *Carthamus tinctorius*; índice de germinação; metodologia de germinação.

Introduction

Safflower (*Carthamus tinctorius* L.) is an annual oleaginous plant belonging to the family Asteraceae, with high economic value due to its use as an ornamental plant. Its oil is used both as edible oil and industrial oil (Anicésio et al., 2015), its seeds are used for bird feeding, and its bran for ruminant feeding.

Moreover, it is a medicinal plant, consisting of an alternative for crop rotation in Brazil for being herbaceous and with good adaptability to hot and dry climatic conditions due to its deep root system (Bonfim-Silva et al., 2015).

Quality seeds are needed for crop establishment. Within the quality control program of a seed company, germination evaluation is fundamental for successful production and lot approval for national and international trade (Brasil, 2009; Carvalho & Nakagawa, 2012; Marcos Filho, 2015).

According to both the Brazilian and the International Rules for Seed Analysis (Brasil, 2009; ISTA, 2009), the recommended period for safflower seed germination testing is 14 days, which may be extended up to 21 days if dormant seeds are found. This period is considered long by laboratories, company technicians, and ranchers who produce and market these seeds. The recommended substrates for safflower are between paper, on paper, and between sand. However, preliminary tests performed at the Seed Laboratory of the Federal University of Goiás showed a difference in the germination percentage of the same seed lot when using the different substrates recommended by RSA (Brasil, 2009).

The standards and procedures of the Rules for Seed Analysis (Brasil, 2009) are periodically reviewed by a committee of researchers and practitioners, but modifications can only be made based on research results (Tomaz et al., 2016). Research on this matter was conducted with forage turnip (Nery et al., 2009), brachiaria grass (Gaspar-Oliveira et al., 2008), and humidicola grass (Tomaz et al., 2016).

This study determines the best substrate and the time required to perform the first and final count of the safflower seed germination test.

Materials and methods

Five lots of seeds from the second crop of 2017, produced under different rates of potassium and phosphate fertilization, were used at the Farm School of the Federal University of Goiás - Regional Jataí. These lots were characterized as to moisture degree in an oven at 105 ± 5 ºC. Furthermore, thousand seed weight was determined with eight replicates of 100 seeds according to Brasil (2009), being corrected to 8% moisture, which is the value recommended for safflower. After these procedures, the seeds were placed in Kraft paper bags, which were stored in plastic bags in a refrigerator, in the absence of light, at a constant temperature of 10 °C, for approximately three months.

The seeds from the five lots were sown on four substrates (paper roll, between paper, on paper, and between sand) for evaluation. The five lots and the four substrates were distributed in a 5x4 factorial scheme in a randomized block design with four replicates of 25 seeds.

The influence of the factors was evaluated by conducting the germination test at a temperature of 25 ± 2 ºC, with a 12-hour photoperiod, in Biochemical Oxygen Demand (BOD) chambers according to the evaluated substrates, as follows:

- Between paper (BP) - 25 seeds were sown on blotter paper moistened with a volume of water (mL) equivalent to three times the dry paper mass (g) (Brasil, 2009). The seeds were covered with a sheet of the same paper inside a clear plastic Gerbox (11.0 x 11.0 x 3.5 cm).
- On paper (OP) - 25 seeds were sown on blotter paper moistened with a volume of water (mL) equivalent to three times the dry paper mass (g) (Brasil, 2009), inside a clear plastic Gerbox (11.0 x 11.0 x 3.5 cm).
- Paper roll (PR) - 25 seeds were sown on a paper towel (Germitest®) moistened with a volume of water (mL) equivalent to three times the dry paper mass (g) (Brasil, 2009). The seeds were covered with a sheet of the same paper and wrapped in a roll after the bottom part was folded to 2 centimeters. The rolls were suspended upright, supported by the germination chamber grid.
- Between sand (BS) - The sand used was previously sieved in a 0.8-mm mesh and sterilized in a forced air oven at 200 ºC for two hours (Brasil, 2009). The substrate was moistened with distilled water in the amount of 60% of sand retention capacity, following the calculation procedure described in Brasil (2009). Thus, 25 seeds were sown on a 3 cm uniform layer of moistened sand, in plastic trays (30 x 30 x 5 cm), being subsequently covered with a 2 cm uniform layer of the same sand.

In the germination chambers, after installing the tests, the substrates and their containers were kept in sealed 45 x 35 cm clear plastic bags to prevent dehydration (Coimbra et al., 2007).

Evaluations were performed daily at the same time, from epicotyl emergence in the sand until the stabilization of the number of seedlings classified as normal seedlings. At the end of the test, seedlings and nongerminated seeds were removed from sand, paper roll, and blotter, being analyzed and counted for the percentage calculation of germination (total normal seedlings), abnormal seedlings, and dead seeds, as described by the RSA (Brasil, 2009).

The Germination Speed Index (GSI) was evaluated using the formula described by Maguire (1962), and the initial (Ti), final (Tf), and mean (Tm) times, the synchrony (Z), and the relative frequency of germination (RF) were calculated using the formulas described in Santana and Ranal (2004).

In addition, the date of the first germination count was established based on 50% + 1 of the total germinated seedlings of the lots, and the final germination count took place on the day from which germination stabilized and/or seedling emergence occurred in the evaluated lots.

The data were submitted to statistical analysis using the statistical program AgroEstat®. Analysis of variance was performed by the F test at 5% and 1% probability and, when there was significant effect, the means were compared by the Tukey test at 5% and 1% probability.

Results and discussion

Table 1 shows the moisture content and thousand seed weight of five safflower lots. The seeds of the five safflower lots had a moisture content between 6.01 and 7.92%, being considered low. Similar moisture levels (6.8%) were reported by Girardi et al. (2013) when studying the quality of stored safflower seed lots.

Table 1 - Moisture degree and seed mass of five lots of *Carthamus tinctorius*.

Lots	Moisture degree (%)	Mass of 1000 seeds (g)*
1	7.92	37.04 a
2	6.40	32.65 b
3	6.19	30.47 b
4	6.01	37.27 a
5	6.82	37.36 a

*Adjusted to 8% of moisture.

Oilseeds such as safflower absorb less water because they are hydrophobic (Brooker et al., 1992), thus requiring low moisture content to maintain their balance compared to starchy seeds under ambient or stored conditions (Carvalho & Nakagawa, 2012). It is important to emphasize that oilseeds have lower storage potential than starchy seeds due to the lower chemical stability of lipids compared to starch. For this reason, oilseeds should be stored with a moisture content lower than recommended for starchy seeds (Marcos Filho, 2015).

Henning et al. (2010) show that more vigorous seeds have higher values of thousand seed weight compared to seeds with lower vigor. Differences in vigor associated with seed characteristics are generally attributed to chemical composition, especially regarding the amount of reserves or deficiency in metabolism (Hampton, 1973). The higher the seed reserve content, the greater the vigor of seedlings; thus, the water supply during seed development can indirectly influence seed vigor due to its effect on chemical composition (Carvalho & Nakagawa, 2012).

Marcos Filho (2015) states that seed water content is a factor that directly interferes with seed weight and may vary according to the conditions of the harvesting site, age, and degree of ripeness.

Table 2 shows a significant interaction between substrates and lots for the variables germination, dead seeds, initial time, mean time, final time, germination speed index, and synchrony. For abnormal seedlings there was an isolated effect of both substrate and lot. For initial time, there was an isolated effect only for lots.

Table 2 - Summary of the variance analysis of the data obtained from the germination test of five seed lots of *Carthamus tinctorius* conducted on four substrates.

Source of variation	Mean squares								
	FD	G (%)	AP (%)	DS (%)	IT (dias)	MT (dias)	FT (dias)	GSI -	Z -
Substrate (S)	3	421.68**	253.57**	84.67**	0.05ns	0.75**	8.71**	9.26**	0.26**
Lots (L)	4	321.58**	55.55*	130.62**	0.45*	0.14*	2.48**	1.82**	0.02ns
S x L	12	159.25**	28.76ns	75.90**	0.05ns	0.18**	2.29**	1.03**	0.04**
Residue	57	23.25	18.60	11.78	0.13	0.04	0.62	0.31	0.01
CV (%)		5.34	78.97	79.60	11.91	6.00	13.82	8.40	21.15

FD: freedom degree; CV: coefficient of variation; G: germination; AP: abnormal seedlings; DS: dead seeds; IT: initial time; MT: mean time; FT: final time; GSI: germination speed index; Z: synchrony; **significant to F test ($p < 0.01$); *significant to F test ($p < 0.05$); nsnon significant.

In general, Table 3 shows that lots 1, 2, 4, and 5 stood out for germination percentage, and lot 3 was the one that had the lowest germination in all substrates, except for the substrate on paper, in which lots 1, 2, 3, and 4 were statistically higher than lot 5. Regarding germination percentage, lots 1 and 4 showed no difference among the evaluated substrates. For lots 2 and 3, the paper roll showed lower values; for lot 5, the substrate on paper had lower values, with the substrates paper roll and between sand showing intermediate values. Hence, the substrate between paper stands out with higher germination percentage in all evaluated lots.

Table 3 - Unfolding Substrate-Lot Interaction for germination (%), dead seeds (%), average time (days), final time (days), germination speed index and synchrony obtained from the germination test of five seed lots of *Carthamus tinctorius* conducted on four substrates.

Characteristics	Substrates	Lots				
		1	2	3	4	5
Germination (%)	Between sand	95 aA	90 abAB	83 aB	90 aAB	98 abA
	On paper	92 aA	97 aA	91 aA	95 aA	76 cB
	Between paper	97 aA	97 aA	87 aB	97 aA	99 aA
	Paper roll	90 aA	84 bA	69 bB	89 aA	89 bA
Dead seeds (%)	Between sand	4.0 aA	6.0 aAB	12.0 abB	7.0 aAB	2.0 aA
	On paper	0.0 aA	1.0 aA	0.0 aA	1.0 aA	13.0 bB
	Between paper	0.0 aA	2.0 aAB	7.0 bB	1.0 aAB	1.0 aAB
	Paper roll	1.0 aA	4.0 aA	17.0 cB	5.0 aA	3.0 aA
Mean time (days)	Between sand	3.09 aA	3.20 aAB	3.16 aAB	3.55 abB	3.26 aAB
	On paper	3.59 bAB	3.82 bB	3.54 aAB	3.68 bAB	3.34 aA
	Between paper	3.60 bA	3.23 aA	3.44 aA	3.19 aA	3.52 aA
	Paper roll	4.10 cC	3.64 bAB	3.38 aA	3.82 bBC	3.47 aAB
Final Time (days)	Between sand	4.25 aA	5.25 aA	4.25 aA	5.25 aA	4.75 aA
	On paper	6.00 bA	7.25 bA	6.25 bA	6.00 aA	5.75 aA
	Between paper	7.50 cB	5.25 aA	6.00 bAB	5.00 aA	6.00 aAB
	Paper roll	7.25 bcB	6.25 abAB	5.00 abA	5.25 aA	5.75 aAB
Germination speed index	Between sand	7.18 aAB	6.64 aB	7.66 aAB	7.50 aA	6.53 bAB
	On paper	6.70 aA	6.43 aA	6.88 abA	5.22 bB	6.48 abA
	Between paper	7.68 aA	6.63 aA	7.74 aA	7.42 aA	7.17 aA
	Paper roll	5.65 bAB	5.04 bAB	6.08 bA	6.65 aAB	5.86 bB
Synchrony	Between sand	0.9 aA	0.8 aA	0.8 aA	0.5 bB	0.6 aAB
	On paper	0.5 bcA	0.5 bA	0.6 aA	0.5 bA	0.6 aA
	Between paper	0.5 bA	0.7 aA	0.6 aA	0.7 aA	0.6 aA
	Paper roll	0.3 cB	0.4 bAB	0.6 aA	0.4 bAB	0.5 aAB

Means followed by the same letter, lowercase in column and uppercase in row, do not differ by Tukey test (p > 0.05).

When germination time was analyzed by the variables initial and final time, synchrony, and germination speed index, substrates between sand and between paper provided faster germination for most safflower seed lots.

For the variable mean time, in days, a better performance was observed for the substrate between paper, except for lot 1. For the variable final time, the substrate between sand performed better for all lots. For the variable germination synchrony, substrates between sand and between paper stand out, except for lot 4 (between sand) and lot 1 (between paper). The substrate between paper provided better germination speed and emergence for all lots except lot 3. Synchrony is a test that evaluates speed and uniformity in the germination process, being thus of paramount importance in the present study, since in the end we were able to infer the reduction in the conduction time of the test.

Regardless of substrate, lot 4 showed a smaller quantity of abnormal seedlings (Table 4) than lot 3, which, in turn, did not differ from lots 1, 2 and 5. Moreover, lot 1 had a lower initial time than the others, taking longer to start germination. For the isolated effect of substrates on the percentage of abnormal seedlings, it is recommended to use substrates between sand or between paper, as they provided lower abnormality values compared to the others.

Table 4 - Abnormal seedlings (%) and initial germination time (days) of five lots of *Carthamus tinctorius* seeds sown on different substrates.

Characteristics	Lots				
	1	2	3	4	5
Abnormal seedlings (%)	5 AB	5 AB	9 B	4 A	5 AB
Initial time (days)	3.37 B	3.00 A	3.00 A	3.00 A	3.00 A
	Substrates				
	Between sand	On paper	Between paper	Paper roll	
Abnormal (%)	3 A	7 B	2 A	10 B	

Means followed by the same letter in row do not differ by Tukey test (p > 0.05).

Figures 1, 2, and 3 show the relative frequency of germination. Studying this frequency helps to understand the distribution of germination over time. Through the frequencies, it is possible to observe whether seeds germinate until they reach a maximum value and then decline, or if germination reaches a maximum point, declines, and then rises again (Santana & Ranal, 2004).

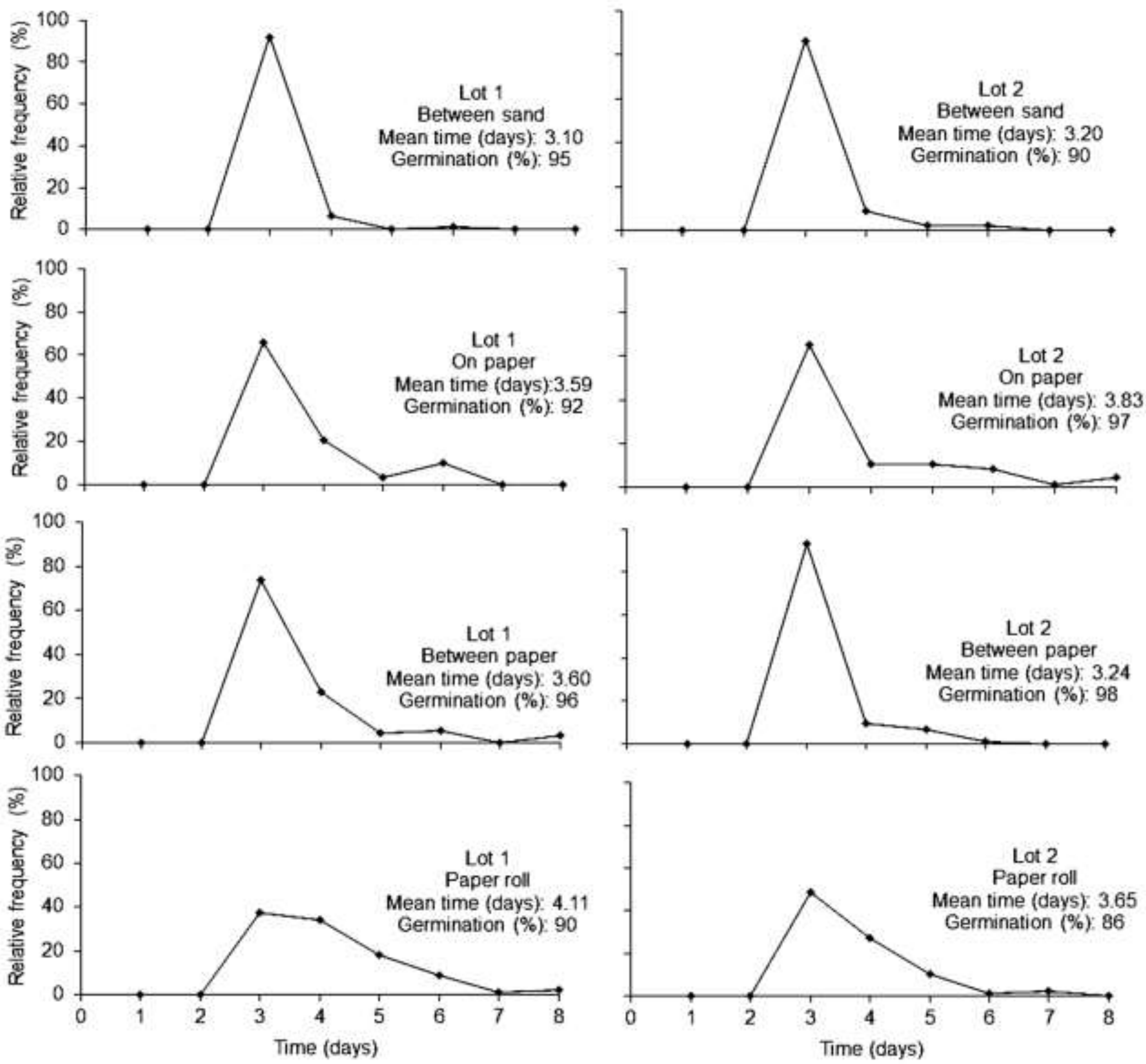

Figure 1 - Distribution of the relative frequency of *Carthamus tinctorius* seed germination from lots 1 and 2 on substrates between sand, on paper, between paper, and paper roll.

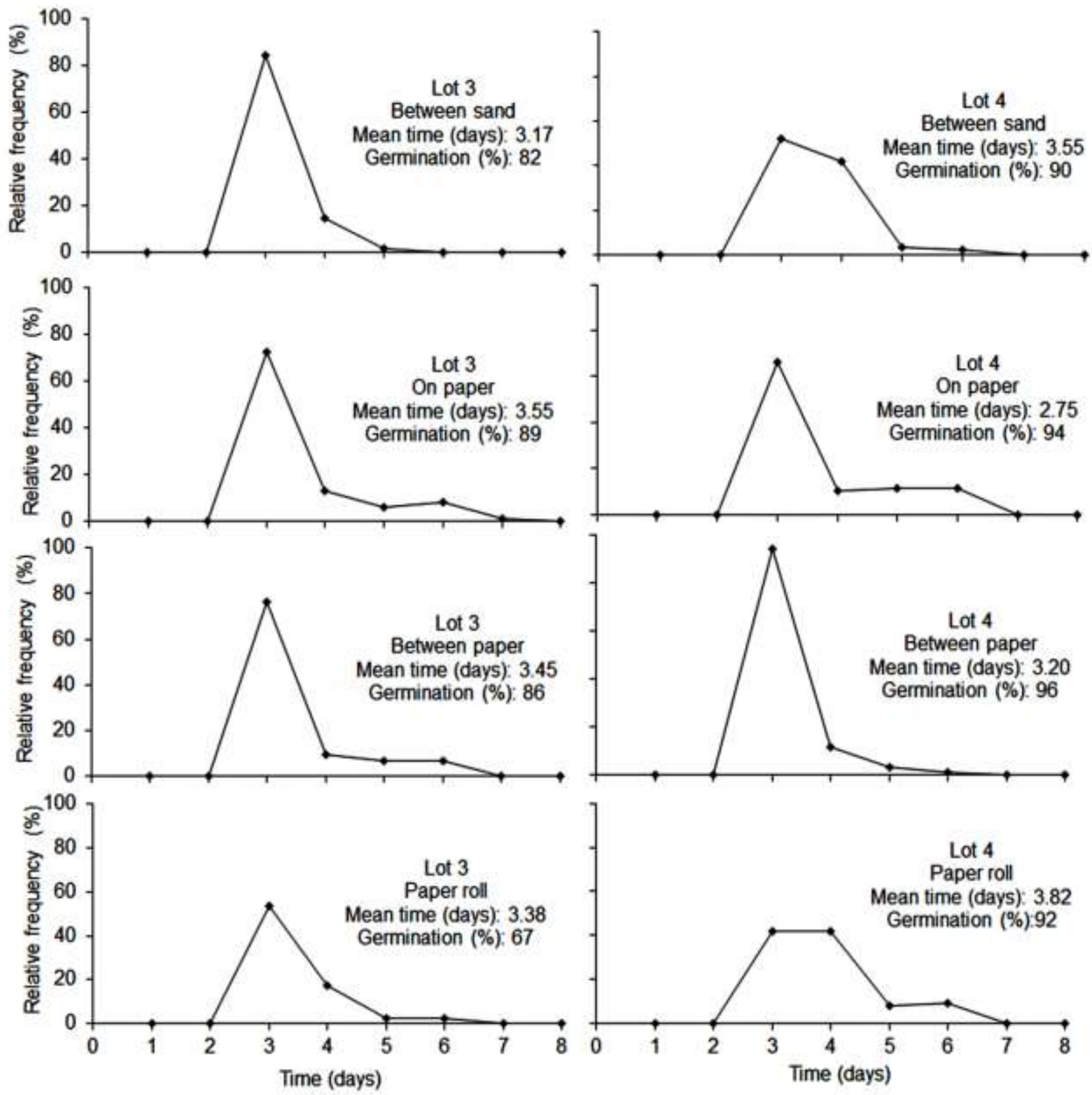

Figure 2 - Distribution of the relative frequency of *Carthamus tinctorius* seed germination from lots 3 and 4 on substrates between sand, on paper, between paper, and paper roll.

The germination peak occurs around the third day, with the germination sequence altered according to the substrate. For the substrate between sand, germination usually occurs until the fifth day. For the substrate between paper, germination extends until the seventh day. Finally, for substrates on paper and paper roll, germination takes place until the sixth day. Thus, germination time varies as a function of the substrate in which the seed will grow.

It should also be noted that the first count of safflower germination can be done on the 3rd day after sowing, since the final germination percentage of all lots evaluated in the substrate between paper, on that day, presented 50% + 1 of the total germinated seedlings. Thus, it can be used as an indicator of vigor, in addition to reducing sources of contamination, if any, and facilitating the final germination reading. This final reading can be done on the 8th day after sowing, since from that day safflower seedling germination stabilizes. Thus, we can reduce the evaluation time prescribed by Brasil (2009), in which the first count is made after four days and the final count after 14 days. The possibility of reducing the germination test duration was also observed for *Brachiaria brizantha* cultivar Marandu (Gaspar-Oliveira et al., 2008) and for Tanzania grass (Tomaz et al., 2010), similarly to that observed in the present study.

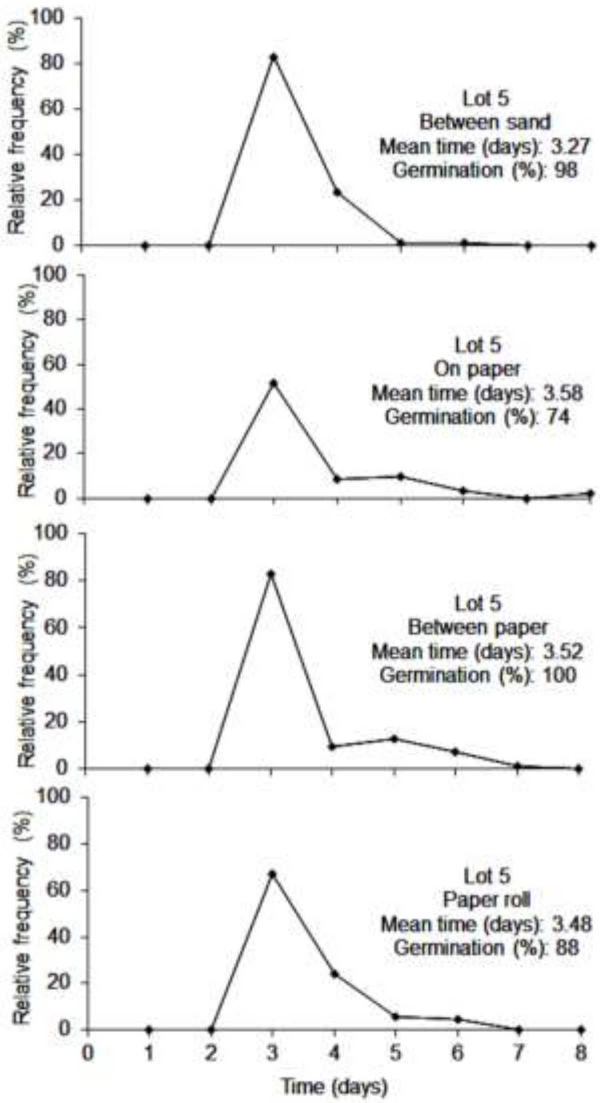

Figure 3 - Distribution of the relative frequency of *Carthamus tinctorius* seed germination from lot 5 on substrates between sand, on paper, between paper, and paper roll.

Conclusions

The safflower seed germination test should be conducted on the substrate between paper or between sand, with the first and final count being performed at 3rd and 8th days, respectively.

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