

Thermotherapy via Humid Heat for the Treatment of Safflower Seeds

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Abstract

The objectives of this work were to evaluate and adequate the method of thermotherapy via humid heat for the treatment of safflower seeds (*Carthamus tinctorius* L.) and, to verify its effect on the physiological and sanitary quality of seeds. The experiment was performed in the period from September to November, 2016 and from May to July, 2017, in entirely randomized design, arranged in $5 \times 6 + 1$ factorial scheme, with five temperatures: 25, 35, 45, 55 and 65 °C and with six time periods: 5, 10, 15, 30, 45 and 60 min, plus the additional treatment (control), with eight repetitions. The seeds were packaged in glass of 500 mL and disposed in thermodigital water bath device with heated water according to the abovementioned factorial. We evaluated the degree of humidity of the seeds after thermotherapeutic treatments, the germination of normal seedlings, the emergence at field, the speed indexes of germination and emergence, the length and dry mass of seedlings and the sanity test. We observed that the treatment of seeds via humid heat thermotherapy was efficient in the control of phytopathogens, without damage to the physiological quality until 45 °C. The treatment of 45 °C for 15 min provided the greater reduction of the pathogens on the safflower seeds, incrementing its germinative potential and emergence at field.

Keywords: *Carthamus tinctorius* L., physiological quality, sanity, ornamental seeds

1. Introduction

The safflower (*Carthamus tinctorius* L.), Asteraceae, is a species of economic concern with broad fitness for use, from food to ornamental, offering high variability of raw materials. As ornamental plant, we highlight the inflorescences by the beauty, rusticity and versatility of the use form, such as, fresh and/or dry cut flower (Abud et al., 2010; Emongor & Oagile, 2017).

The safflower seeds have high oil content, on average of 35%, which is extracted and used, as raw material in the food, pharmaceutical and oil industry, with cultivation for this purpose in more than 60 countries, highlighting China, United States, Mexico and Argentina. In Brazil, the cultivation of safflower is still incipient both to production of seeds and cut flowers (Faostat, 2017).

The phytosanitary quality of ornamental seeds, mainly, the safflower ones contributes positively for the formation of the plants stand and consequently, for a production of quality of their floral stems, as well as the production of seeds. This way, the treatment of seeds is indispensable to avoid the dissemination of field diseases. Among the treatment techniques of seeds, the thermotherapy via humid heat or hot water immersion becomes a viable alternative and with low environmental impact (Silva et al., 2002; Lazarotto et al., 2013).

The thermotherapy, which uses the temperature-time binomial, as seeds means of treatment, must be adjusted to each species, in a way that it does not affect negatively the physiological quality, for example, the germination. Once that, the thermotherapeutic method via humid heat presents greater thermal capacity, and this requires a shorter time of exposure of seeds, to avoid the early rupture of the tegument and, consequently, the soaking in heated water, in order to mitigate the damage and the exacerbated leakage of the substances via membrane (Machado, 2000; Coutinho et al., 2007).

The thermotherapy for the control of phytopathogens has been showed itself efficient for several species, such as: squash (*Cucurbita pepo* L.) (Cunha et al., 2017), alfalfa (*Medicago sativa* L.) (Mendes et al., 2001), brachiaria (*Brachiaria brizantha* Hochst.) (Marchi et al., 2008), Spondias mombin (*Spondias mombin* L.) (Brito et al., 2008), canafistula (*Peltophorum dubium* Spreng.) (Lazarotto et al., 2013), cumaru (*Amburana cearensis* A.C. Smith) (Oliveira et al., 2011), beans (*Phaseolus vulgaris* L.) (Estefani, Miranda Filho, & Uesugi, 2007), castor oil plant (*Ricinus communis* L.) (Marroni et al., 2009), passion fruit (*Passiflora suberosa* L.) (Marostega et al., 2015), maize (*Zea mays* L.) (Coutinho et al., 2007), tomato (*Solanum lycopersicum* L.) Carmo et al. (2004) Braga et al. (2010), among others, without damage to the physiological quality, offering an alternative to the use of chemical products, commonly used in the seeds treatment. Oliveira et al. (2011) highlight that this form of seeds treatment is viable for the pathogens control, without environmental damage, because it does not use pollutants and it does not emit residues.

Thus, the objectives of this work were to evaluate and adequate the method of thermotherapy via humid heat for the treatment of safflower seeds and, to verify its effect on the physiological and sanitary quality of seeds.

2. Material and Methods

The experiment was carried out in the period from September to November, 2016, and from May to July, 2017, in the Seed Research and Teaching Laboratory (LDPS) of the Department of Plant Science in the Federal University of Santa Maria (UFSM), RS (29°43' S; 53°43' W and altitude of 95 m). The safflower seeds used are from Yellow Saffron cultivar, collected in the crop of 2015/2016, the seeds were stored in cold chamber (15 °C and 40% UR) in kraft paper bags (brown paper type of 1 kg), with average degree of humidity of 9%.

The experiment of thermotherapy via humid heat was in entirely randomized treatment, arranged in 5 × 6 + 1 factorial scheme, with five temperatures: 25, 35, 45, 55 and 65 °C and with six time periods: 5, 10, 15, 30, 45 and 60 min, plus the additional treatment (control), with eight repetitions. The seeds were packed in glass (Becker) of 500 mL having distilled water and disposed in thermodigital water bath device with heated water in the temperatures and time according to the abovementioned factorial. Previously, the seeds were soaked in distilled water that was not heated, during one hour, for elimination of air pockets between the superficial dead tissues, facilitating the conduction of heat in the seeds tissues (Coutinho et al., 2007). After the treatment by thermotherapy, the seeds were put to dry on toilet paper at room temperature by the period of 24 h.

In the sequence, we evaluated the physics, physiological and sanitary qualities of these seeds by the following tests:

Humidity degree of seeds after thermotherapeutic treatment: determined by the greenhouse method 105±3 °C for 24 h, using four repetitions of 5 g (adapted Brasil, 2009a).

Standard test of germination, first count of germination (vigor) and speed index of germination (IVG): sown eight repetitions of 50 seeds, in paper roll of germination, moistened with distilled water in proportion of 2.5 times the mass of the dry paper. The rolls were kept in BOD (Box Organism Development) type germinators, with photoperiod of 24 h and temperature of 25±2 °C. The evaluations of vigor and germination were at four and 14 DAS (days after sowing), respectively (Brasil, 2009a). The IVG with daily evaluations according to methodology described by Maguire (1962), adapted for the culture of safflower used as criteria the elongation of the primary root and emergence of the cotyledons (Abud et al., 2010).

Length and dry mass of seedling: performed with eight repetitions of 20 seeds, sowed in two non-matching lines in the upper third of the germination paper and, maintained in the same condition of the standard test of germination. At the four DAS the length of the aerial and root parts of ten normal seedlings of each repetition were measured. In the sequence, we determined the total dry mass by drying of the material in forced ventilation oven at 65±5 °C by 48 h (Nakagawa, 1999).

Emergence at field and emergence speed index (IVE): eight repetitions of 50 seeds were sown in lines of 1 m, with spacing between them of 0.2 m and in grooves of 0.03 m of depth. The IVE was determined with daily evaluations according to the methodology described by Maguire (1962), adapted for the safflower culture using as criteria the development of the cotyledons and epicotyls, and the evaluation of emergence was performed at 14 DAS (Abud et al., 2010).

For the germination variables of normal seedlings and emergence at field, we used as pattern the Normative Instruction n.45/2013 of MAPA (Ministry of Agriculture, Livestock and Supply) (Brasil, 2013). This normative regulates the standards of production and commercialization of seeds in Brazil; however, the same does not contemplate the safflower seeds, thus, we chose the standard of sunflower seeds (*Helianthus annuus* L.) because

it belongs to the same botanical family of safflower, Asteraceae, and it is demanded between 65-70% of germination (Brasil, 2013).

Sanity test in filter-paper: performed through the incubation in paper substrate (Blotter Test), with eight repetitions of 50 seeds. The sowing occurred in plastic transparent boxes for germination (gerbox), prepared with three sheets of paper of germination moistened with distilled water corresponding to 2.5 times the mass of dry paper. The germination was inhibited by the method of freezing for 24 h. Subsequently, the seeds remained in BOD for five days with photoperiod of 12 h of light and temperature of 20 ± 2 °C. They were evaluated in magnifying glass (stereo microscope) the percentage of seeds infested and the identification of the phytopathogens at level of genus (Brasil, 2009b).

The data expressed in percentage was transformed in $\text{arc-sine} \sqrt{\%/100}$. The analysis of variance of data was performed with the help of SISVAR program (Ferreira, 2014), at level of 5% of error. We used the contrast test of the additional treatment (control) versus the factorial (levels of temperature and periods of time) and, also, the comparison of averages of the factorial by the Scott-Knott test. The Pearson correlation, at level of significance of 5%, was performed among the total infested seeds and the first count, germination, emergence of dead seedlings and seeds in the germination test.

3. Results and Discussion

The physical, physiological and sanitary quality of safflower seeds was measured by the analysis of the variables by the contrast test of the additional treatment (control) with the thermoherapeutic factorial (levels of temperature and periods of time), which were significant at 5% of error, as well as the interaction between the levels of temperature and the periods of time. In general, the control treatment presented superior average to the combinations of temperature and time, except for the variable degree of humidity, which presented negative estimate and non-significance in the factorial (Table 1).

For the seed preservation, Coutinho et al. (2007) indicated the necessity of soaking the seeds in non-heated distilled water with the intention of eliminating the air pockets between the superficial dead tissues, facilitating the heat conduction in the seed tissues and avoiding greater damage to the membranes. Marcos-Filho (2015) reports that the maintenance of the membrane system integrity is fundamental for the vitality preservation and viability of seeds.

In relation to the seed vigor obtained by the germination first count, at four DAS (days after sowing), we observed that in temperatures of 25; 35 and 45 °C when the same were exposed to 5; 10 and 15 min of humid heat, in a general way, the vigor was similar to the additional treatment (without thermotherapy). We verified that the most accentuated reduction of the safflower seed vigor occurred in the exposure to temperatures superior to 45 °C and for more than 30 min of soaking in heated water. Marcos-Filho (2015) reports that the initial seed hydration in form of water vapor, when in high temperatures, can cause damage to the membranes by leaching of cellular content, resulting in the increase of the speed deterioration and in reduction of germinative potential.

The initial physiological quality of the safflower seed lot used, in this work, was of 66% of germination of normal seedlings and 76% of emergence at field, characterizing itself as a commercial lot according to the standards of MAPA (Brasil, 2013). The evaluation of germination was performed at 14 DAS, in which, we verified that the standard demanded by MAPA maintains itself in the temperature of 25 °C in all the tested periods of time, obtaining the same performance for the emergence at field. Yet, in the temperatures of 35 and 45 °C the germination standard remains until the period of 15 min of exposure in heated water.

Among the thermotherapeutic treatments, we verified that the treatment of 45 °C for 15 min stood out from the others, resulting in 75% of germination of normal seedlings in laboratory and 83% of emergence at field. Oliveira et al. (2011) verified that according to the increase of the exposure period of cumaru seeds to humid heat (60 °C for 5, 10 and 20 min) there was a greater expression of both the vigor and the germination potential, as it was verified in the treatment of 45 °C 15 min⁻¹ for the safflower seeds.

The germination of the seeds and their emergence in the field depend directly on the favorable environmental conditions to which they were submitted. Acting directly in metabolic processes related to them, since the hydrolysis of their reserves to biosynthesis of new tissues (Marcos-Filho, 2015). For the safflower seeds, we observed that the thermal capacity to maintain the metabolic processes is at 45 °C, favoring the expression of germinability.

However, Coutinho et al. (2007) verified that the vigor loss and the germinative potential loss of the maize seeds treated by thermotherapy via humid heat was increasing according to the exposure period of seeds to this treatment, being the vigor and germination close to zero in the most stringent treatment (60 °C, 20 min⁻¹). These results corroborate with the ones obtained in the present work, because in the most stringent treatment of 65 °C 60 min⁻¹ affected the physiological quality of the seeds, inhibiting totally the germination (Table 1).

The physiological quality of the safflower seeds measured by the emergence test at field verified that in these conditions the emergence supports greater thermal amplitude, obtaining emergence values until 55 °C 30 min⁻¹ (Table 1). Thus, the emergence test at field enables to the safflower seeds treated by thermotherapy an improvement in the production potential expression of seedlings in relation to the germination standard test. This improvement can be explained by the interaction of the environment (conditions not controlled), substrate (soil) and to the phytopathogens incident in the seeds, because in the germination standard test there is a constancy in the environmental conditions, which provides the pressure of the inoculum on the seeds and substrate (germination paper).

We verified that the safflower seeds presented high incidence of phytopathogens with 42% of total infested seeds in the sanity test (Table 1). Nevertheless, we observed a significant reduction in the infestation of phytopathogens in the seeds according to the thermotherapeutic treatments, in which the increase of the period of exposure of the same in heated water soaking was beneficial to this reduction, being similar among the tested temperatures. Coutinho et al. (2007) using thermotherapeutic treatment via humid heat for phytopathogenic control in maize seeds, concluded that the increase of the exposure period of the seeds in heated water reduces the infestation, corroborating with the results of this work. Braga et al. (2010) highlighted that the success of the thermotherapy depends directly of the satisfactory combination between the control of the phytopathogens incident in the seeds and the minimum maintenance of its physiological quality.

The phytopathogens of greater incidence identified in the safflower seeds were the ones of the genus: *Aspergillus* spp., *Botrytis* spp., *Fusarium* spp., *Penicillium* spp., and *Sclerotinia* spp. In the additional treatment (without thermotherapy) the incidence of phytopathogens was of 42% of total seeds, from this percentage 81% was of *Aspergillus* spp. in the same treatment (Table 2).

(2000) mentions that *Aspergillus* spp. and *Penicillium* spp. are the phytopathogens that contribute the most for the deterioration of seeds depreciating their physiological quality.

However, Schneider et al. (2015) concluded that the thermal treatments 25; 45; 50 and 55 °C for 15 min were not efficient to the control of the *Fusarium* spp. pathogens incidents in the oilseeds of jatropha (*Jatropha curcas* L.), confirming the results of the present work. Lazarotto et al. (2013) verified that the thermotherapeutic treatments via humid heat (80 °C for 0; 5; 10; 15 and 20 min) were not efficient to prevent the infestation of phytopathogens on the seeds of cassia fistula without affecting negatively its physiological quality. Nevertheless, Cunha et al. (2017) concluded that the thermotherapy via humid heat at 60 °C for until 18 min reduces the incidence of phytopathogens in squash seeds without damage in their physiological quality.

The indexes IVG and IVE (index of speed of germination and emergence, respectively) were decreasing according to the increase of temperature and the period of exposure of seeds to humid heat, with values that confirm the vigor, demonstrating viability of germination and emergence until 45 °C 30 min⁻¹ (Table 3). This indicates that according to the exposure of the safflower seeds to the humid heat, the trigger of the metabolic processes of the nutritional reservation of these seeds was slower. Marcos-Filho (2015) reports that the deterioration process of seeds depends on the genetic heritage since the rearrange of the membranes for the beginning of the germinative three-phase process, added to the degree of humidity and heat exposure.

Among the safflower seedlings, in general, we observed that the reservations of the seeds germinated were mobilized for the seedling length at 76.7 and 23.3% for the root and aerial parts, respectively. Yet, the partition of dry mass of safflower seedlings was of 16.4 and 83.6% for the root and aerial parts, respectively.

We noted that the safflower seedlings have initially greater expansion in the root part, however, with the lower dry mass. Abud et al. (2010) report that the safflower seeds presented germination of the epigeal type and the phanerocotylar seedling, firstly, occurs the protrusion of the root, with greater expansion in volume, and in the sequence the emergence of the cotyledons originating the aerial part.

In Table 3, we presented the coefficients of linear correlation among the total infested seeds in the sanity test and the standard tests of germination and emergence at field. We observed that there was positive correlation among the total infested seeds and the vigor of safflower seeds, highlighting the thermotherapeutic treatments of 35 °C 10 mim⁻¹ and 45 °C 10 mim⁻¹, with 13% of infested seeds (Table 1).

The positive correlation that had the greatest highlight for the germination of normal seedlings (0.96) was in the period of zero exposure in water in all the tested temperatures, in these treatments there were 42% of infested seeds. Yet, the positive correlations for the emergence at field were in the treatments of 45 °C 10 mim⁻¹ and 55 °C 10 mim⁻¹ (Table 4), these treatments presented total infested seeds of 13 and 12% respectively.

Table 4. Pearson correlation coefficient between the total infested seeds in sanity test and the first count, germination of normal seedlings, emergence at field and dead seeds in germination test of *C. tinctorius* submitted to thermotherapy via humid heat

Additional Treatment		Temperature (°C)	Factorial					
			Periods of time (minutes)					
			5	10	15	30	45	60
<i>First count of germination (vigor)</i>								
Control	0.43**	25	-0.76	-0.39	-0.66	-0.3	0.42**	-0.43
		35	0.65**	0.93**	-0.72	-0.52	0.86**	-0.5
		45	0.30**	0.76**	-0.16	-0.33	-0.99	-0.48
		55	0	-0.96	-0.65	0	0	0
		65	0	0	0	0	0	0
<i>Germination of normal seedlings</i>								
Control	0.96**	25	-0.63	-0.8	-0.28	0.90**	0.17**	-0.85
		35	-0.41	0.50**	-0.76	-0.47	-0.07	-0.85
		45	-0.63	-0.17	0.66**	0.58**	-0.14	-0.88
		55	0.04	0.06	-0.31	-0.49	0	0
		65	0	0	0	0	0	0
<i>Emergence at field</i>								
Control	-0.02	25	0.22**	0.08	-0.76	0.48**	0.69**	0.58**
		35	0.22**	-0.05	-0.76	0.49**	0.69**	0.58**
		45	-0.52	0.84**	-0.13	-0.82	0.14**	0.29**
		55	-0.52	0.84**	-0.13	-0.82	0	0
		65	0	0	0	0	0	0
<i>Dead seeds in germination test</i>								
Control	-0.23	25	0.22**	0.35**	0.33**	0.67**	0.87**	0
		35	0.36**	-0.54	0.85**	0.40**	0.24**	0.24**
		45	0.60**	-0.68	-0.31	-0.44	0.11*	0.73**
		55	-0.43	0.27**	0.32**	0.82**	0.99**	-0.48
		65	0.73**	-0.58	0.18**	-0.13	0.37**	0.54**

Note. ** and * are significant by *t* test in 0.01 and 0.05 of error probability, respectively.

The positive correlations of the dead safflower seeds in the germination test were distinct in virtue of the high variation of infested seeds. In general, we determined that the greater the incidence of phytopathogens, the more the vigor, the germination of normal seedlings and the emergence at field were negatively affected. Härter et al.

(2014) report the importance of exemption of phytopathogens in the seeds, because the contamination of the same can cause damage in the future development of the seedlings at the field.

In general, the relation to sanity of safflower seeds after the submission to thermotherapy via humid heat, we observed efficiency in the percentage control of phytopathogens in all the tested temperatures and periods of time. However, this thermotherapeutic technique preserves the physiological quality of the safflower seeds until the temperature of 45 °C.

Among the variation of the thermotherapeutic treatments tested on the safflower seeds, we highlight the treatment at 45 °C, 15 min⁻¹, because it promotes the improvement in the physiological expression of the same in vigor (40%), germination (75%) and emergence at field (83%), besides the control on the total infested seeds (5%).

Despite the emphasis and the international investment, the safflower cultivation in Brazil is incipient. Initial research on crop improvement and adaptability indicate the species as an alternative to off-season cultivation, especially for seed production. Results of the study suggest good prospects for the use of thermotherapy via humid heat for the treatment of safflower seeds, offering an option to the use of chemical products. Due to the fact of the low environmental impact, without residual effect and efficiency to phytosanitary control, beyond the easy handling of the method, appropriate for its use in familiar or agroecological agriculture.

4. Conclusion

The treatment of seeds by means of thermotherapy via humid heat was efficient for the control of incident phytopathogens in safflower seeds, without damage to the physiological quality. Among the treatments of thermotherapy of seeds, the combination of 45 °C, 15 min⁻¹ provided better phytosanitary quality for safflower seeds, incrementing also its potential of germination and emergence at field.

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