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IMPROVING THE METHOD FOR DETERMINING THE PHYSIOLOGICAL AND SANITARY POTENTIAL OF GHERKIN SEEDS

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Abstract

Gherkin seeds usually show irregular physiological quality. Seed production requires fast and reliable tests to evaluate seed quality. Germination test is considered a recognized analysis method; however, seed technology has pursuit the improvement of vigor tests aiming the evaluation of seed's physiological potential. Thus, the objective of this work was to evaluate procedures to perform the test of accelerated aging and determine the physiological and sanitary potential of gherkin seeds. Four seed lots of cultivar Liso Calcuta were used in the study. To evaluate the initial physiological quality the water content was determined and germination and emergence tests, as well as indices of germination speed and emergence speed were used. The accelerated aging test was performed as traditionally and with saturated saline solution, with 48, 72 and 96 hours, at temperatures of 41ºC and 45ºC. After aging, the water content was determined, and seeds' germination and sanity tests were performed. The experiment was set under a completely random design in factorial 4x3x2 (lots x aging periods x temperatures). The standard accelerated aging test and the test with saturated saline solution at 41ºC for 96 hours were efficient to evaluate the vigor of gherkin seeds. Saturated saline solution provides uniform water absorption and deterioration in gherkin seeds, allowing to discriminate seed lots in different vigor levels. The salinity test after accelerated aging with saline solution reduces the incidence of some fungi.

Keywords: *Cucumis anguria*. Seeds Quality. Seeds Vigor. Seed Sanity. Vegetable.

1. Introduction

Gherkin (*Cucumis anguria*) is a widely used vegetable in the folkloric cooking from the Northern, Northeastern and West-Center regions in Brazil (Oliveira et al. 2017). Beyond Brazil, this vegetable is commonly cultivated in India, Cuba, United States and South Africa (Yoon et al. 2015). Seed production is considered scarce when compared with other vegetables and those seeds generally exhibit low quality. The utilization of seeds with high physiological potential is important to set up crops (Kavan et al. 2019) once it enables higher yield (Catão et al. 2019).

Germination test is the official and standard method to determine the physiological quality of seed lots. However, is not always correlated with results in the field and it can be a time-consuming operation, thus generating a demand to develop faster tests which may be consistently correlated with the real emergence of seed lots under field conditions (Rocha et al. 2018; Leite et al. 2019).

In seeds of *C. anguria,* the germination test lasts for a period of 8 days (Brasil 2009), a relative long period when considering a decision-making process. Thus, the use of vigor tests is essential, once these are consistent and produce rapid information concerning processes of physiological deterioration in seeds (Catão et al. 2019).

Among the existing vigor tests, accelerated aging is one of the most used in Brazil and the whole globe. This test has as a basic principle the increase of deterioration rates due to the exposition of the seeds to high temperature and relative humidity levels, considered main factors for the intensity and velocity of deterioration (Bewley et al. 2014; Marcos-Filho 2015). Nevertheless, the size of the seed is a variable which must be considered.

Small seeds such as vegetables seeds absorb water in uneven and fast manner at the end of the accelerated aging period, due to the high humid atmosphere, resulting in an accentuated variation in the degree of seed humidity and promoting inconsistent test results, due to the different degrees of seed deterioration (Radke et al. 2016; Duarte et al. 2017). In addition, the performance of seeds may be influenced by the presence of saprophytic fungi and its high incidence may limit the estimative of seed vigor, once they influence the deterioration interfering in the interpretation of data (Monteiro et al. 2017).

Under such context, the use of saturated saline solutions, substituting distilled water during the performance of accelerated aging tests, constitutes a feasible alternative once it promotes a humid atmosphere and an adequate and controlled water absorption rate (Lima et al. 2015). In addition, it reduces contamination by some seed storage pathogens (Kikuti et al. 2005). This leads to lower deterioration intensity and lower variation of results (Lima et al. 2015).

Therefore, the present study was executed with the objective to evaluate methods to perform the accelerated aging test and to determine the physiological and sanitary potential of gherkin seeds.

2. Material and Methods

The experiment was executed at the Laboratory of Seeds from the Ourinhos University Center (UNIFIO), Ourinhos, São Paulo – SP, Brazil. Samples from four gherkin seed lots, cultivar Liso Calcuta, were purchased at the local market. Water content was determined in these samples, as well as the physiological quality of seeds, using the regular germination and vigor tests.

Water content was determined in an oven at 105 ± 3ºC for 24 hours, using two sub-samples with approximately 2g for each lot (Brasil 2009). Four replicates, with 500 seeds each were used in the germination test, uniformly distributed over two germitest paper foils. The germitest foils were dampened with distilled water at a proportion of 2.5 times the weight of the dry paper foil and set to germinate at 25ºC and 12 hours photoperiod, with an evaluation performed eight days after seeding (Brasil 2009). The results were expressed as germination percentage.

Seedling emergence test was accomplished with four sub-samples (with 50 seeds from each lot), distributed at 0.5 cm depth in expanded polystyrene trays with 128 cells containing commercial substrate (Carolina II®), composed of pine bark, vermiculite and chemical fertilizers (information from manufacturer). Trays were kept in greenhouse, irrigated and evaluated daily until the eleventh day, determining the emergence percentage of seedlings per lot.

Evaluations of germination speed index (IVG) and emergence speed index (IVE) were performed simultaneously with germination and emergence tests, with daily counting the number of seeds germinated and emerged seedlings during the same period of the day. To calculate the indices the formula proposed by Maguire (1962) was used. A completely random design was used to execute the described tests.

The accelerated aging test was studied both under the standard methodology and with saturated saline solution. 'Gerbox' boxes containing aluminum net designed to avoid seed contact with the solution were used. Seeds were set over the aluminum net in a single and uniform layer. Inside each box 40 mL of deionized water were added to test seeds by the standard aging method or 40 mL NaCl saturated solution (40g NaCl/100mL deionized water) to test seeds under the saturated saline solution method. Boxes were closed, identified and kept in B.O.D. chamber for three aging periods (48, 72 and 96 hours), using two temperatures (41ºC and 45ºC). After that, four replicates of 50 seeds per treatment were set to germinate according to the methodology previously described for germination test. The evaluation occurred four days after the test began and the results were expressed as percentage of normal seedlings within each lot. To

keep track of the test, the water content of seeds was determined before and after the aging periods, as previously described. A complete randomized design with factorial scheme 4x3x2 (four lots: L1, L2, L3, L4; three aging periods: 48, 72, 96 hours: two temperatures: 41°C and 45°C) was used.

In order to test seed sanity, the filter paper or "*Blotter test"* was used. Seeds from all four lots were analyzed to verify the incidence of fungi after using the standard and saturated saline solution aging methods. Two hundred seeds were evaluated, distributed in four replicates of 50 seeds in previously disinfected 'gerbox' boxes with a solution of sodium hypochlorite 1% and alcohol 70%. These seeds were previously submitted to an imbibition period of 24 h and then freezing for 24h in order to avoid germination. On each box two sheets of filter paper were placed and wetted with distilled water until saturation. Seeds were distributed over these dampened filter paper sheets, remaining under incubation during seven days at a temperature of 25 ± 2ºC, under alternate photoperiod (12 hours light and 12 hours darkness). After the incubation period seeds were individually evaluated regarding the presence and identification of fungi at a level of genus or species, under stereo microscope (640x magnification) and light microscope. The percentage of contaminated seeds was then determined for each fungus identified.

To statistically analyze the data the *F* test and variance analysis at 5% probability were applied and when significant effects were detected the qualitative means were clustered by the Scott- Knott test at 5% probability, using the software Sisvar 5.0 (Ferreira 2011).

3. Results

The initial evaluation of physiological quality and moisture content of the gherkin seed lots are shown in Table 1. It was not possible to identify significant differences in lots of gherkin seeds using the standard germination test.

Table 1. Mean values of water content, percentage of germination and emergence of seedlings, germination speed index (IVG) and emergence speed index (IVE), form four seed lots of gherkin, cultivar Liso Calcuta.

Lots	Water contente (%)	Germination (%)	IVG	Emergence (%)	IVE
1	6.3	90[a]	30.5[c]	31[c]	6.3[c]
2	7.1	92[a]	32.3[b]	55[b]	7.6[b]
3	8.1	96[a]	31.6[b]	68[a]	8.4[b]
4	7.5	94[a]	34.1[a]	71[a]	9.7[a]
CV (%)		4.12	3.67	8.15	5.84

*Means followed by the same letter within the column are not statistically different by the Scott-Knott test at 5% probability.

The results from the emergence test allowed to observe differences in seed vigor between lots. Lots 3 and 4 were the most vigorous according to the emergence test. Differences were also verified in seed lots for the indices of germination speed and emergence speed.

The initial water content of seeds in the test was of 6.3%, 7.1%, 8.1% and 7.5% in lots 1, 2, 3 and 4, respectively (Table 1). Water content was similar among seed lots. Water content attained after the accelerated aging test is showed in Figure 1.

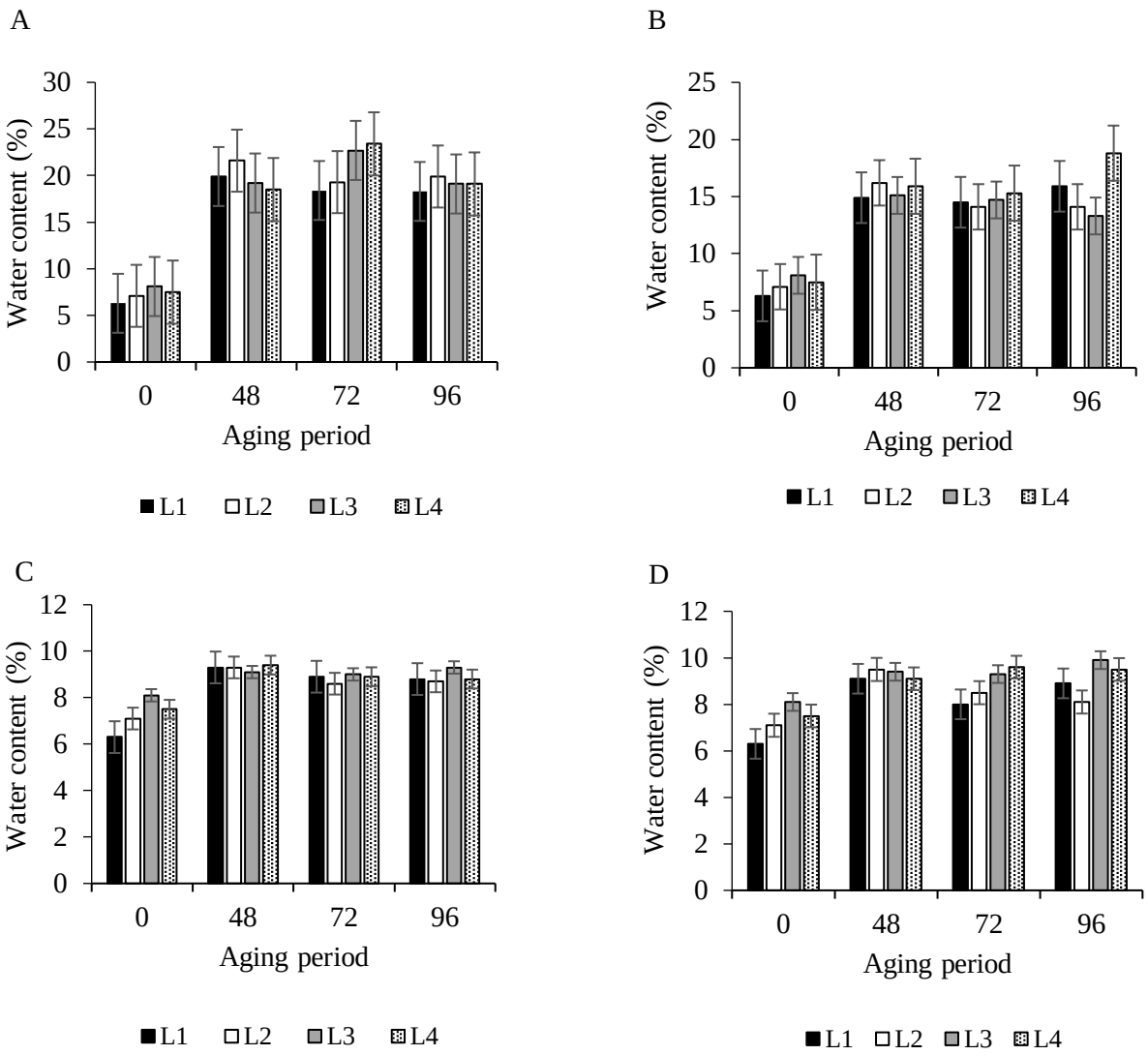

Figure 1. Water content (%) in lots of gherkin seeds cultivar Liso Calcuta. A – traditional accelerated aging at 41ºC; B – traditional accelerated aging at 45ºC; C – accelerated aging with saturated saline solution at 41ºC; D – accelerated aging with saturated saline solution at 45ºC.

Analyzing the results from combinations of temperature and exposition period, an increase in seeds water content is verified as the period of seed exposition to accelerated aging increased. In a general way, temperature of 41°C promoted higher increase in the water content when compared to the temperature of 45°C.

On the other hand, in the accelerated aging test using saturated saline solution a lower water content was absorbed by the seeds (Figure 1C and D), once the relative humidity in the air was of around 76%. In the standard aging test seeds are in contact with the air, which has a relative humidity of 100%.

The saturated saline solution provided uniform water absorption, providing consistence in the degree of seed deterioration. Therefore, a minor deterioration occurs when compared to the standard method and that is fundamental when studying the evaluation of seed vigor in vegetables. This fact may be verified observing that saturated saline solution showed a lower experimental error (Figure 1C and D) than the standard accelerated aging (Figure 1A and B), regardless of temperature.

Another important fact is that even with an increase of water content in the standard method, there were not variations above 5%. The water content at the end of the accelerated aging test is one of the indicators of the homogeneity performance of the test.

It is possible to analyze germination under the unfolding effect of temperature within each lot, as well as the germination of lots within each temperature, during standard accelerated aging and with saturated saline solution at temperatures of 41 and 45ºC (Table 2).

Table 2. Percentages of germination of gherkin seeds cultivar Liso Calcuta, under standard accelerated aging and with saturated saline solution, during 48, 72 and 96 hours, at 41 and 45ºC.

Standard Accelerated Aging (%)				
Temperature (ºC)	Lots	Aging periods (h)		
		48	72	96
41	L1	62^{Ba}	46^{Bb}	42^{Db}
	L2	82^{Aa}	81^{Aa}	62^{Cb}
	L3	87^{Aa}	85^{Aa}	75^{Bb}
	L4	89^{Aa}	90^{Aa}	86^{Aa}
45	L1	62^{Ba}	47^{Bb}	21^{Bc}
	L2	71^{Aa}	68^{Aa}	63^{Aa}
	L3	62^{Ba}	57^{Ba}	58^{Aa}
	L4	74^{Aa}	69^{Aa}	69^{Aa}
Lots	Temperature (ºC)	Aging periods (h)		
		48	72	96
L1	41	62^{Aa}	46^{Ab}	42^{Ab}
	45	62^{Aa}	47^{Ab}	21^{Bc}
L2	41	82^{Aa}	81^{Aa}	62^{Ab}
	45	71^{Aa}	68^{Ba}	63^{Aa}
L3	41	87^{Aa}	85^{Aa}	75^{Ab}
	45	62^{Ba}	57^{Ba}	58^{Ba}
L4	41	89^{Aa}	90^{Aa}	86^{Aa}
	45	74^{Ba}	69^{Ba}	69^{Ba}
CV (%)				11.24
Accelerated Aging with Saturated Saline Solution (%)				
Temperature (ºC)	Lots	Aging periods (h)		
		48	72	96
41	L1	32^{Ba}	29^{Ba}	21^{Da}
	L2	84^{Aa}	77^{Aa}	48^{Cb}
	L3	83^{Aa}	82^{Aa}	73^{Ba}
	L4	91^{Aa}	87^{Aa}	89^{Aa}
45	L1	36^{Ba}	35^{Ba}	15^{Bb}
	L2	73^{Aa}	70^{Aa}	67^{Aa}
	L3	70^{Aa}	60^{Aa}	57^{Aa}
	L4	73^{Aa}	69^{Aa}	68^{Aa}
Lots	Temperature (ºC)	Aging periods (h)		
		48	72	96
L1	41	32^{Aa}	29^{Aa}	21^{Aa}
	45	36^{Aa}	35^{Aa}	15^{Ab}
L2	41	84^{Aa}	77^{Aa}	48^{Bb}
	45	73^{Aa}	70^{Aa}	67^{Aa}
L3	41	83^{Aa}	82^{Aa}	73^{Aa}
	45	70^{Ba}	60^{Ba}	57^{Ba}
L4	41	91^{Aa}	87^{Aa}	89^{Aa}
	45	73^{Ba}	69^{Ba}	68^{Ba}
CV (%)				13.18

*Means followed by the same upper-case letter within the column and lower case letter in the line are not statistically different by the Scott-Knott test at 5% probability.

At a temperature of 41ºC after 96 hours, it was possible to stratify seed lots of gherkin seeds. Lot 4 showed higher viability than the rest. Starting at 48 hours after aging, a reduction in germination from all lots was verified, however in a less accentuated manner in lot 4. The reduction of germination of seed lots

was also verified at a temperature of 45ºC. However, at this temperature it was not possible to stratify seed lots in a consistent way.

When analyzing germination of seed lots at a temperature of 41ºC, a reduction of seed germination was verified starting after 72 hours in lot 1; while in lot 2 and lot 3 the reduction occurred after 96 hours; and no reduction was observed in lot 4. At 45ºC a reduction of seed germination was also verified, however, such reduction after aging was clearly noticed only in lot 1 starting at 72 hours.

While analyzing Figure 2A it is possible to verify the exposition period to the standard accelerated aging at 96 hours was efficient to differentiate seed lots regarding vigor at a temperature of 41ºC. The most vigorous was lot 4, while lot 1 showed lower vigor from 48 hours onward.

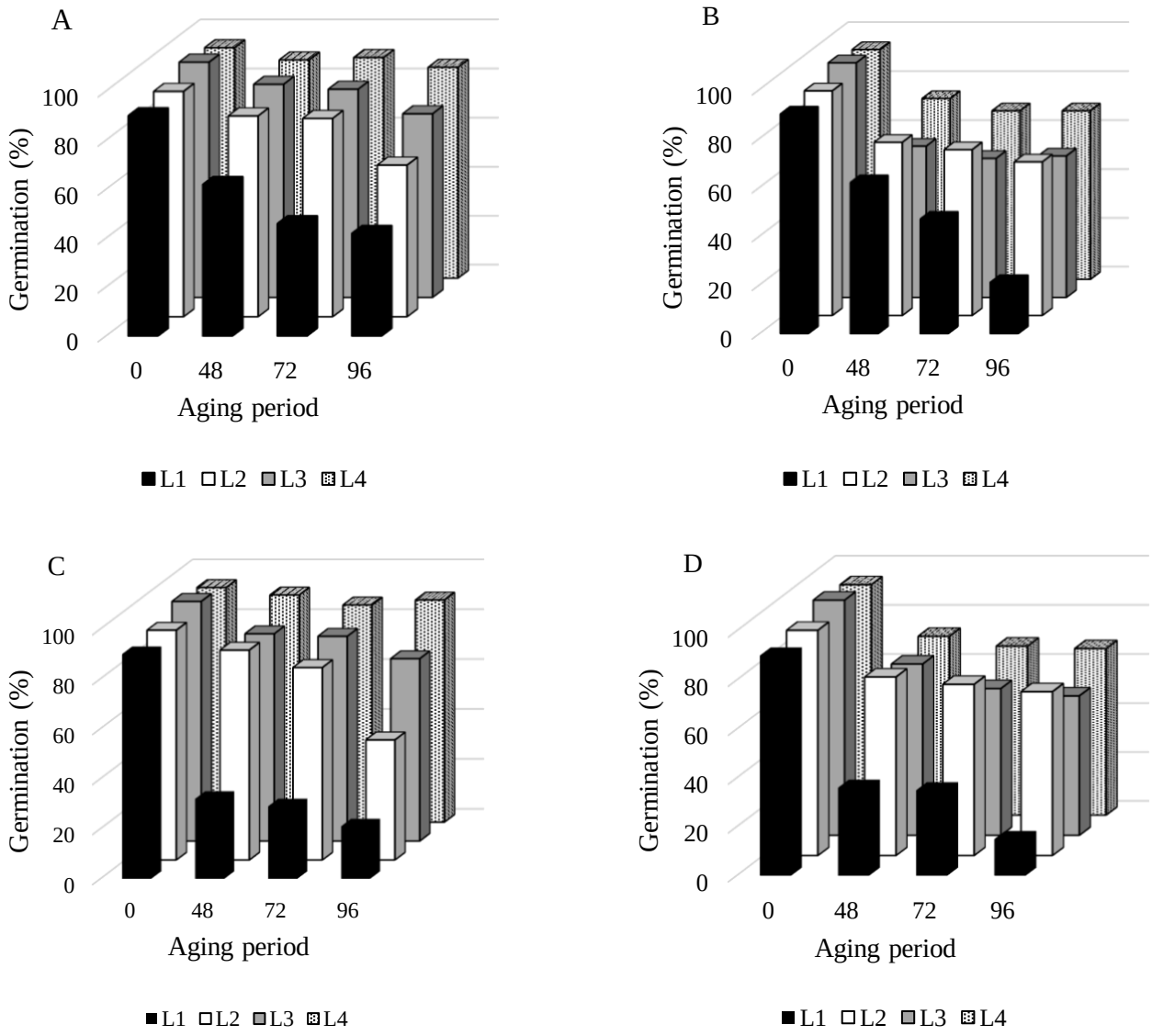

Figure 2. Germination (%) of gherkin seeds, cultivar Liso Calcuta. A – traditional accelerated aging at 41ºC; B – traditional accelerated aging at 45ºC; C – accelerated aging with saturated saline solution at 41ºC; D – accelerated aging with saturated saline solution at 45ºC.

Analyzing Figure 2B is evident that seed lot 4 is the most vigorous after standard accelerated aging at 45ºC. However, the consistent stratification of lot 2 and lot 3 was not possible. Lot 1 also shows the lowest vigor when evaluated at 45ºC.

Using the accelerated aging method with saturated saline solution allowed stratifying seed lots of gherkin in vigor classes (Table 2). By the unfolding of temperature of 41ºC within each seed lot, the period of 96 hours was the most consistent to classify seed lots. Lot 4 had the highest vigor, followed by lots 3, 2 and 1. Other periods of accelerated aging were not efficient to stratify seed lots. At a temperature of 45ºC it

was possible to verify that lot 1 had the lowest vigor. It was not possible to observe statistical differences regarding seed vigor for all other seed lots.

Analyzing the unfolding of germination within each temperature it was possible to verify that lot 1 (45ºC) and lot 2 (41ºC) showed germination reduction 96 hours after exposition to the accelerated aging with saturated saline solution. Using the saturated saline solution, it was possible to verify the stratification of seed lots at 41ºC (Figure 2C) after 96 hours of accelerated aging. At 45ºC the vigor was drastically reduced, especially in lot 2, lot 3 and lot 4, which were impossible to be separated in classes (Figure 2D).

Based in the physiological results observed, the viability and vigor of gherkin seeds are reduced by the stress conditions imposed by the accelerated aging, independently of the method used.

The incidence of fungi *Alternaria cucumerina, Fusarium oxysporum*, *Cladosporium cucumerinum*, *Penicillium* sp., *Aspergillus* spp., *Rhizopus stolonifer* and *Phoma* sp. was verified in seeds of gherkin in both methods of accelerated aging (Table 3). The incidence of fungus was observed in all the combinations of lots and conditioning periods, to a greater or lesser extent, depending on the treatments applied to the seeds.

Table 3. Incidence of fungi (%) in lots of gherkin seeds submitted to periods (48, 72 and 96h) of standard and saturated saline solution accelerated aging at temperatures of 41 and 45ºC.

Fungi	Lot 1			Lot 2			Lot 3			Lot 4		
	48	72	96	48	72	96	48	72	96	48	72	96
Incidence of fungi (%) with standard accelerated aging at 41ºC												
ALT	1	-	-	-	-	-	2	6	-	-	-	-
FUS	28	-	-	14	5	-	8	13	1	3	-	-
CLAD	17	-	-	22	15	3	-	-	-	2	-	-
PEN	13.5	4	-	10	9	2	3	5	-	7	-	-
ASP	69	74	82	44	71	32	68	45	79	16	22.5	37.5
RHI	23	14	2.5	14	2.5	-	2.5	0.5	-	-	-	-
PHO	4	8	6	0.5	-	-	-	1	-	-	-	-
Incidence of fungi (%) with standard accelerated aging at 45ºC												
ALT	3	0.5	-	-	-	-	-	-	-	-	-	-
FUS	33	24	-	22.5	7.5	-	19.5	26.5	3.5	6.5	8	2
CLAD	13	-	-	27.5	8.5	13.5	5	9	2	5.5	3.5	-
PEN	20.5	9.5	7	4.5	4.5	0.5	10.5	-	-	1.5	-	-
ASP	56.5	90.5	91	85.5	88	94.5	93	99	97.5	25.5	17	34.5
RHI	32.5	4	6	14	6.5	1	15.5	5.5	-	0.5	0.5	1
PHO	5.5	3	-	2.5	1	1	-	-	-	0.5	-	-
Incidence of fungi (%) with saturated saline solution accelerated aging at 41ºC												
ALT	-	-	-	-	-	-	-	-	-	-	-	-
FUS	12	1	-	3.5	0.5	0.5	-	-	-	-	-	-
CLAD	0.5	0.5	-	1	1	-	0.5	-	-	-	-	-
PEN	2.5	-	-	-	-	-	0.5	1.5	-	0.5	0.5	-
ASP	45.5	61.5	79.5	56	35.5	26.5	17.5	39	48.5	14.5	8.5	24.5
RHI	3.5	-	-	6.5	0.5	-	-	-	-	0.5	-	-
PHO	1.5	-	-	-	-	-	0.5	-	-	-	-	-
Incidence of fungi (%) with saturated saline solution accelerated aging at 45ºC												
ALT	0.5	-	-	-	-	0.5	-	-	-	-	-	-
FUS	27.5	13.5	2.5	10	1.5	0.5	18	15.5	-	-	-	-
CLAD	0.5	2	-	7.5	3.5	1.5	-	-	0.5	1.5	0.5	0.5
PEN	-	-	-	-	-	-	-	-	-	1	-	-
ASP	21.5	68	84.5	49.5	51.5	55.5	70.5	92.5	86.5	18	20.5	28.5
RHI	14.5	-	0.5	2.5	1.5	1	9.5	1	0.5	-	-	-
PHO	-	-	-	-	-	-	-	-	-	-	-	-

**Alternaria cucumerina* (ALT), *Fusarium oxysporum* (FUS), *Cladosporium cucumerinum* (CLAD), *Penicillium* sp. (PEN), *Aspergillus* spp. (ASP), *Rhizopus stolonifer* (RHI) and *Phoma* sp. (PHO).

In all gherkin seed lots analyzed, it was possible to observe that the highest incidence of fungi occurred within 48 hours under traditional aging. The main fungi developed were *Fusarium oxysporum*, *Penicillium* sp. and *Aspergillus* spp. The use of NaCl decreased fungal growth.

To evaluate seed vigor, the accelerated aging test with saturated saline solution may reduce the incidence of fungi and consequently increase seed vigor, being considered as one advantage of this test. Evaluation of the initial sanitary quality of seeds is important to avoid dissemination of fungi by seeds and consequently its introduction in production areas. Information concerning the main pathogens transmitted by gherkin seeds is scarce. The identification of fungi found in gherkin seeds may help to control and manage crop diseases.

4. Discussion

When evaluating the physiological quality of seeds through the germination test, it is possible to obtain the maximum potential quality of seed lots. However, this test can overestimate quality and does not provide information on seed behavior under adverse field conditions (Ventura et al. 2012). Thus, it is necessary to use the vigor test to obtain the true potential of these lots under field conditions, adding information to the germination test (Gray et al. 2011). The emergence test is considered the best indicative to infer about seed vigor, once during its execution similar conditions from those seeds will endure in the field are provided (Pereira et al. 2015). One of the objectives of vigor tests are to reveal differences in physiological quality, which are not detected in the germination test (Marcos-Filho 2015).

Water content was similar among seed lots. This is important for the test performance once the uniformity of the initial water content of seeds contributes to obtain consistent results. In addition, Marcos-Filho (2015) emphasized that samples with differences between 1 and 2% in the water content do not jeopardize the results and the tests may proceed. Similar results were verified by Rocha et al. (2018) while evaluating physiological quality of popcorn seeds through the accelerated aging test. This happens due to the penetration velocity of water being controlled basically by the initial content of water in the seed, by the absorption rate and principally by the environment temperature (Zucchi et al. 2018; Silva et al. 2019). Radke et al. (2016) and Marcos-Filho (2015) sustained that water content corresponds to the equilibrium point, which rises with the increase of the relative humidity of the air.

The accelerated aging test is one of the vigor tests that simulates conventional storage conditions, through exposure to high temperatures and relative humidity, causing seed deterioration (Moncaleano-Escandon et al. 2013). However, in this method, water absorption may occur unevenly and promote uneven seed deterioration. Therefore, Jianhua and McDonald (1996) recommended the use of saturated salt solutions instead of using water. With the use of this methodology, there is a reduction in the ambient relative humidity, and this provides a uniform deterioration using the same temperature and exposure time (Moncaleano-Escandon et al. 2013). With the use of saturated saline solution are promoted an adequate humid atmosphere, rate of water absorption by the seed, intensity of deterioration and consequently a lower variation of the results which corroborate with data showed by Radke et al. (2016) and Lima et al. (2015). Marcos-Filho (2015) observed variations of 4% and 5% between samples are considered tolerable in tests of accelerated aging.

The hygroscopic balance is achieved by the seeds as the relative humidity increases and their water content increases. This was verified in forage radish seeds (*Raphanus sativus* L. var *oleiferus* Metzg.) by Nery et al. (2009) and on sunflower seeds (*Helianthus annus* L.) by Braz et al. (2008). However, with the use of saturated salt solutions during aging, the water content of the seeds is lower and guarantees greater uniformity of deterioration.

It was also verified that seed deterioration increases with the increment of the exposition period to the accelerated aging test, once the reduction of germination was verified within each temperature tested (Araújo et al. 2017). Frandoloso et al. (2017) sustain that temperature increment promotes more drastic effects in germination than the extension of the exposition period to accelerated aging, as we verified at a temperature of 45^{o}C.

Peng et al. (2011) found in aging that high temperatures and high relative humidity caused lower selectivity of plasma membranes, in addition to reactive oxygen species triggering lipid peroxidation. This allows the entry of water in an uneven way into the cells and results in different degrees of deterioration,

obtaining contradictory and uneven results. Possibly, uneven results in traditional aging may be due to inactivation of antioxidant enzymes (Catalase and Superoxide desmutase), release of free radicals, reduction of respiration, reduction of ATP (adenosine triphosphate) and assimilated during seed germination (Lehner et al. 2008; Demirkaya et al. 2010). Using saturated saline solutions controls the absorption of water and these metabolic aspects in seeds (Jianhua and McDonald 1996).

Freitas et al. (2018), while using standard accelerated aging (water), observed a great discrepancy in germination of arugula seeds, where lots showed different quality levels. According to Rocha et al. (2018) this fact may be caused by the consumption of seed reserves, due to the accelerated metabolism under conditions of high humidity and temperature. However, when using saturated saline solution with NaCl, it is possible to differentiate seed lots, once there is not drastic reduction of germination percentages of seeds.

This is an indication that, when exposed to aging, seeds are not able to repair the membrane and make them more sensitive in germination and promote loss of vigor (Rajjou and Debeaujon 2008; Samarah and Al-Kofahi 2008). In studies carried out by Medeiros et al. (2019) and Marchi and Cicero, a reduction in germination of common bean and carrot seeds was observed after accelerated aging, with germination decay increasing with temperature from 42 to 45°C, in the first case, and from 41 to 45°C, in the second one. Tests performed in seeds of scarlet eggplant provided stratification of seed lots only at the temperature of 41°C for 48 hours (Alves et al. 2012).

Tests performed on *Jatropha curca*s L. seeds by Pereira et al. (2012) provided stratification of seed lots only at the temperature of 41 °C, considering temperatures of 42 and 45°C as limiting, while in wheat (*Triticum aestivum* L.) seeds the temperature of 45°C was lethal (Maia et a. 2007).

Deuner et al. (2018) found that an environment with 75% relative humidity provides less aging sensitivity than an environment with 100% humidity in the evaluation of eggplant seeds (Solanum melongena L.). Due to less deterioration in this type of environment, the seed exposure period has to be increased for a safe evaluation of seed quality. The use of saturated saline solutions, in addition to controlling relative humidity, water absorption and spoilage, also controls fungal infestation.

A reduction of incidence of fungi in the gherkin seeds was verified using the saturated saline solution method. The use of saturated saline solution during the accelerated aging also reduced the incidence of fungi in broccoli seeds (Fessel et al. 2005).

NaCl is recommended to detect storage-environment fungi such as the genus *Aspergillus* and *Penicillium,* due to their capacity of growing in high osmotic concentration media and due to exert an inhibitory effect to other fungi (Bento et al. 2012). According Kikuti et al. (2005) and Lopes et al. (2010) the use of saline solution allows water contents to remain sufficiently low to expressively reduce or avoid the development of microorganisms in bell pepper and okra seeds, respectively.

Higher incidence of fungi from the genus *Aspergillus* was observed in gherkin seeds, considered toxic and causing seed decay. It is a saprophytic fungus of easy dissemination, once the spores are light and dry (Monteiro et al. 2017). This fungus may growth under low water potential, commonly being the first one to develop under low humidity conditions and disturbing the development of other genera which require higher humidity (Monteiro et al. 2017).

Fungal development occurs because during the conditioning period the seeds acquire high moisture and accelerate biochemical processes due to their exposure to high temperatures and relative humidity (Kapoor et al. 2011). The main fungi involved in seed deterioration are *Aspergillus spp*., *Rhizopus spp*. and *Penicillium spp*. (Demirkaya et al. 2010). However, it is possible that chlorine and sodium ions are released into the aging environment with the use of saline solution. It is possible that the reduction of fungi occurs because these ions have an antifungal action (Ávila et al. 2006).

The incidence of *Alternaria cucumerina* (Lopes et al. 2008), *Fusarium oxysporum* and *Cladosporium cucumerinum* (Kurozawa et al. 2005) has been described causing diseases in watermelon and cucumber. These pathogens survive and are disseminated through seeds of the *Cucurbiataceae* family and were identified in seeds of gherkin, being potentially harmful to this crop.

5. Conclusions

The accelerated aging test performed through the standard method or with saturated saline solution at 41°C for 96 hours is efficient to evaluate vigor of gherkin seeds.

The saturated saline solution supplies uniform water absorption and less drastic deterioration in gherkin seeds, allowing to differentiate seed lots by vigor levels.

The sanitary test after accelerated aging with saline solution reduces the incidence of fungi in gherkin seeds.

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