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ARTICLE

Physiological potential of soybean seeds treated and stored under uncontrolled conditions

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Abstract: The aim of this study was to evaluate the isolated effect and the combination of products in the chemical treatment on the physiological potential of soybean seeds with different vigor levels, after different storage periods. The study was divided into four experiments, with different storage periods: 0, 45, 90 and 135 days, all with a completely randomized design, with four replicates. The treatments were arranged in a 12 x 2 (A x B) factorial scheme, in which factor A consisted of the application of seed treatment and factor B consisted of initial vigor levels. The physiological potential of the seeds was determined by means of the following evaluations: first germination count, seed germination, seedling emergence in the sand substrate, accelerated aging with NaCl-saturated solution, vigor index and average seedling length (Vigor-S®). Storage of seeds under uncontrolled conditions causes greater deterioration, and this process can be intensified by previous chemical treatment of the seeds. Among the products studied, the use of drying powder alone was the treatment that caused the greatest losses in the different situations studied. To ensure the maintenance of the physiological potential of soybean seeds, treatment should be carried out as close as possible to sowing.

Index terms: germination, *Glycine max* (L.) Merrill, seed treatment, vigor.

Resumo: O objetivo deste estudo foi avaliar o efeito isolado e a combinação de produtos no tratamento químico, sobre o potencial fisiológico de sementes de soja com diferentes níveis de vigor, em períodos de armazenamento distintos. O estudo foi dividido em quatro experimentos, com diferentes tempos de armazenamento: 0, 45, 90 e 135 dias todos com delineamento inteiramente casualizado, com quatro repetições. Os tratamentos foram dispostos em esquema fatorial 12 x 2 (A x B), em que o fator A foi composto pela aplicação do tratamento de sementes e o fator B pelos níveis de vigor inicial. O potencial fisiológico das sementes foi determinado por meio: primeira contagem de germinação, germinação, emergência de plântulas em areia, envelhecimento acelerado com solução saturada com NaCl, índice de vigor e comprimento médio de plântulas (Vigor-S®). O armazenamento de sementes em condições não controladas ocasiona maior deterioração das sementes e este processo pode ser intensificado pelo prévio tratamento químico das sementes. Dentre os produtos estudados, o uso de pó-secante de maneira isolada foi o tratamento que ocasionou maiores perdas nas diferentes situações estudadas. Para garantir a manutenção do potencial fisiológico das sementes de soja, recomenda-se que o tratamento seja realizado o mais próximo possível da semeadura.

Termos para indexação: germinação, *Glycine max* (L.) Merrill, tratamento de sementes, vigor.

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INTRODUCTION

Obtaining high yields depends on the use of high-quality seeds, combined with the use of management techniques. Soybean crop (*Glycine max* (L.) Merrill), for instance, stands out as the commodity that most employs technology in the field, and seed treatment represents about 1.5% of the production cost (Richetti and Goulart, 2018; Silva and Dobashi, 2021).

Seed treatment is an important tool in the control of seed-borne pathogens, besides ensuring the establishment of an appropriate stand, under adverse climate and soil conditions (Pereira et al., 2011). To ensure this protection to seeds, the market offers a diversity of products for application via seed treatment, and various combinations of insecticides, fungicides, polymers and drying powders are used (França-Neto et al., 2016).

The literature reports numerous studies aimed at evaluating the effect of the application of chemicals in seed treatment (Brzezinski et al., 2017; Camilo et al., 2017; Pereira et al., 2018; Santos et al., 2021). However, few studies have sought to assess the isolated effects and the interaction between phytosanitary products at different levels of initial vigor.

Understanding not only the interaction between these products and their isolated effects, but also about the logistics used in seed treatment is essential to ensure the maintenance of seed quality, as seeds are often treated in processing units or cooperatives with vigor levels very close to the acceptable limit and stored for a few months under uncontrolled conditions until sowing (Abati et al., 2013; Carvalho et al., 2020).

In the present study, the aim was to evaluate the isolated effect and the combination of products in chemical treatment on the physiological potential of soybean seeds with different vigor levels, after several storage periods.

MATERIAL AND METHODS

The research was conducted at the Seed Laboratory of the main campus of the *Universidade Estadual de Maringá* (UEM), in Maringá, PR, Brazil, and at the Brazilian Agricultural Research Corporation (Embrapa Soybean), in Londrina, PR, Brazil.

Soybean seeds of the cultivar TMG 7062 IPRO were used, represented by two commercial lots, with two vigor levels: one of high (86%) and the other of low (74%) initial vigor, determined by the tetrazolium test (França-Neto et al., 2018). The seeds were separated and treated, according to Table 1.

For the treatment of seeds, plastic bags with capacity for 3 kg were used. The treatments were applied to the seeds, which were shaken in plastic bags until complete homogenization. After treatment, the seeds were placed in the shade at a temperature of approximately 24 °C for 20 minutes for the product to dry.

After treatment, the study was divided into four experiments, with different storage periods: 0 (experiment I), 45 (experiment II), 90 (experiment III) and 135 (experiment IV) days.

All experiments were conducted in a completely randomized design, with four replicates, and the treatments were arranged in a 12 x 2 (A x B) factorial scheme. Factor A consisted of the application of seed treatment involving fungicides, insecticides, polymers and drying powder, according to Table 1, and factor B consisted of the initial vigor levels (high and low). All factors were considered as fixed.

Each experiment was conducted for 45 days, aiming to monitor the physiological potential of the seeds treated differently in each experiment. The seeds remained stored in Kraft paper bags in a laboratory environment, with an average temperature of 25 °C ± 5 °C and relative air humidity of 60% ± 25% throughout the experimental period.

The physiological potential of the seeds was evaluated in the four experiments, using the following variables: first count of the germination test, germination, seedling emergence in sand substrate (Brasil, 2009), and accelerated aging with NaCl-saturated solution (Marcos-Filho et al., 2020), all with four replications of 50 seeds.

For the emergence test in sand substrate, seeds were sown in trays, kept in a greenhouse under scheduled irrigation equivalent to 5 mm of water.day^{-1}, at approximately 25 °C and relative humidity of 60%, and the evaluation was carried out at 14 days after sowing (Krzyzanowski et al., 2020).

Table 1. Description of chemicals used and their respective doses.

Treatment	Description	Commercial dose[1]
T1	Imidacloprid + thiodicarb	300
T2	Fipronil + thiophanate methyl + pyraclostrobin	200
T3	Polymer	200
T4	Drying powder[2]	200*
T5	Imidacloprid + thiodicarb + polymer	300 + 200
T6	Imidacloprid + thiodicarb + drying powder[2]	300 + 200*
T7	Fipronil + thiophanate methyl + pyraclostrobin + polymer	200 + 200
T8	Fipronil + thiophanate methyl + pyraclostrobin + drying powder[2]	200 + 200*
T9	Polymer + drying powder[2]	200 + 200*
T10	Imidacloprid + thiodicarb + polymer + drying powder[2]	300 + 200 + 200*
T11	Fipronil + thiophanate methyl + pyraclostrobin + polymer + drying powder[2]	200 + 200 + 200*
T12	Control	-

[1]Dose of commercial product: mL.100 kg^{-1} of seeds. [2]Talkum gloss®*g. 100 kg^{-1} of seeds. For all treatments, the volume of solution was 600 mL. 100 kg^{-1} of seeds (recommended dose + water).

In addition, the Automated Seed Vigor Analysis System (Vigor-S®) was employed to obtain the vigor index and average seedling length, using four replications of 20 seeds each, with evaluation performed at 3 days after planting according to Rodrigues et al. (2020).

The data of the analyzed variables were subjected to the basic assumptions of the analysis of variance by the Lilliefors test ($p \leq 0.05$) and Bartlett's sphericity test ($p < 0.05$). To meet the basic assumptions, the variables first germination count, accelerated aging with NaCl-saturated solution and average seedling length (experiment II), germination, vigor index and average seedling length (experiment III) and first germination count, germination and vigor index (experiment IV) were transformed to square root of X + 1. Then, the data of each experiment were subjected to analysis of variance, and the means were compared by Scott-Knott test ($p \leq 0.05$).

To better characterize the effect of the treatments on the physiological potential of the seeds, multivariate analysis of principal components was performed for each of the experiments. For this, the data of the different response variables were standardized, by transforming them so that the means were equal to zero and the variance was equal to one (Barbosa et al., 2013). This transformation was performed to avoid overestimating or underestimating the weight of a studied variable in the final result, due to differences in measurement scale.

The principal component analysis (PCA) was calculated using the n x p matrix, in which "n" is the number of treatments (treatments = 12) and "p" is the mean result for the variables first germination count, germination, seedling emergence in sand, accelerated aging with NaCl-saturated solution, vigor index and average seedling length (variables = 6).

From the correlation matrix, the eigenvalues (representative values of the retained variability for each new component) and eigenvectors (linear combination of the evaluated parameters) were calculated. The results were expressed by the two-dimensional graph (biplot). The statistical program R® version 3.6.3 (R Core Team, 2018) was used.

RESULT AND DISCUSSION

The data from the four experiments met the basic assumptions of the analysis of variance ($p \leq 0.05$). Through the analysis of variance, it is possible to infer that there were significant differences ($p \leq 0.05$) for all response variables evaluated in the experiments.

In relation to the first germination count, in the four experiments, generally the highest percentages were observed for high-vigor seeds (Table 2), indicating that the first germination count test is efficient in separating seed lots with different levels of initial vigor (Nakagawa, 1999).

On the other hand, when comparing the influence of seed treatments on this variable, it is observed that, for seeds of high initial vigor, all treatments used led to percentages lower than those of the control (Table 2).

For seeds of low initial vigor, the treatments showed different behaviors in each experiment (Table 2). This result is justified, because low-vigor seeds show lower speed in metabolic processes, with an uneven initial development (Marcos-Filho, 2015).

For the germination variable, it is possible to observe, in experiment I (0 days of storage), that the treatments imidacloprid + thiodicarb + polymer (T5), fipronil + thiophanate methyl + pyraclostrobin + polymer (T7), fipronil + thiophanate methyl + pyraclostrobin + drying powder (T8), polymer + drying powder (T9), imidacloprid + thiodicarb + polymer + drying powder (T10) and fipronil + thiophanate methyl + pyraclostrobin + polymer + drying powder (T11), promoted results equivalent to those of the control and were superior to the other treatments (Table 3).

For experiment II (45 days of storage), there was no significant difference between treatments in the germination variable; for experiment III, in seeds of high initial vigor, there was a reduction in germination percentage with the application of the treatments drying powder (T4), imidacloprid + thiodicarb + polymer (T5) and fipronil + thiophanate methyl + pyraclostrobin + drying powder (T8). For experiment IV (135 days of storage), the treatments imidacloprid + thiodicarb (T1), fipronil + thiophanate methyl + pyraclostrobin + polymer (T7) and polymer + drying powder (T9) promoted results equivalent to those of the control.

For seeds with low initial vigor, it was observed that a higher number of treatments led to reduced germination percentage (T3, T4 and T11), compared to the control (Table 3).

Table 2. Means obtained in the first germination count (%) in experiments I (0 days of storage), II (45 days of storage), III (90 days of storage) and IV (135 days of storage).

Treat.	0 days				45 days				90 days				135 days			
	High		Low		High		Low		High		Low		High		Low	
T1	55	Ba	56	Aa	55	Ba	51	Aa	53	Ba	46	Aa	49	Ba	38	Bb
T2	56	Ba	56	Aa	52	Ba	53	Aa	52	Ba	38	Ab	42	Ca	37	Cb
T3	57	Ba	43	Bb	57	Ba	43	Bb	45	Ba	41	Aa	44	Ca	34	Cb
T4	53	Ba	47	Ba	52	Ba	41	Bb	45	Ba	40	Aa	43	Ca	36	Cb
T5	56	Ba	56	Aa	52	Ba	44	Bb	51	Ba	43	Ab	51	Ba	43	Bb
T6	49	Ba	49	Ba	49	Ba	49	Aa	47	Ba	43	Aa	46	Ba	43	Bb
T7	55	Ba	56	Aa	55	Ba	50	Aa	55	Ba	49	Aa	38	Ca	41	Cb
T8	50	Ba	56	Aa	48	Ba	54	Aa	46	Ba	47	Aa	46	Ba	43	Bb
T9	58	Ba	54	Aa	54	Ba	45	Bb	49	Ba	43	Aa	49	Ba	43	Bb
T10	56	Ba	55	Aa	56	Ba	46	Bb	51	Ba	39	Ab	46	Ca	36	Cb
T11	59	Ba	44	Bb	56	Ba	44	Bb	52	Ba	39	Ab	49	Ba	41	Bb
T12	68	Aa	61	Aa	69	Aa	52	Ab	69	Aa	46	Ab	67	Aa	43	Ab
Mean	56		53		54		48		51		43		47		40	
CV (%)	9.22				4.74				12.04				7.05			

*Means followed by the same uppercase letters in the column and lowercase letters in the row, within each experiment, do not differ from each other by the Scott-Knott test, at 5% probability level. CV: coefficient of variation.

Table 3. Means obtained in the germination test (%) in experiments I (0 days of storage), II (45 days of storage), III (90 days of storage) and IV (135 days of storage).

Treat.	0 days				45 days				90 days				135 days			
	High		Low		High		Low		High		Low		High		Low	
T1	73	Ba	74	Ba	71	Aa	71	Aa	70	Aa	69	Aa	68	Aa	60	Aa
T2	77	Ba	74	Ba	75	Aa	69	Aa	74	Aa	60	Ba	63	Ba	58	Aa
T3	78	Ba	69	Ba	77	Aa	66	Aa	73	Aa	62	Ba	62	Ba	57	Aa
T4	69	Ba	64	Ba	68	Aa	63	Aa	63	Ba	62	Ba	60	Ba	55	Aa
T5	88	Aa	80	Aa	71	Aa	70	Aa	65	Ba	63	Ba	60	Ba	60	Aa
T6	77	Ba	72	Ba	76	Aa	70	Aa	73	Aa	58	Bb	62	Ba	57	Aa
T7	81	Aa	76	Aa	73	Aa	71	Aa	71	Aa	69	Aa	69	Aa	57	Ab
T8	85	Aa	81	Aa	71	Aa	73	Aa	64	Bb	72	Aa	63	Ba	65	Aa
T9	76	Ba	83	Aa	75	Aa	69	Aa	70	Aa	66	Aa	69	Aa	58	Ab
T10	79	Ba	80	Aa	76	Aa	70	Aa	71	Aa	60	Bb	58	Ba	59	Aa
T11	83	Aa	72	Ba	78	Aa	68	Aa	75	Aa	62	Bb	59	Ba	61	Aa
T12	85	Aa	77	Aa	81	Aa	73	Aa	80	Aa	66	Ab	80	Aa	63	Ab
Mean	78		75		74		69		70		64		64		59	
CV (%)	8.26				9.57				3.66				5.83			

*Means followed by the same uppercase letters in the column and lowercase letters in the row, within each experiment, do not differ from each other by the Scott-Knott test, at 5% probability level. CV: coefficient of variation.

It was verified, in general, that the germination percentage is reduced with the advance of storage, due to seed deterioration. This is related to the uncontrolled storage conditions, accentuated by the use of products for the chemical treatment of seeds. This behavior was also observed by Matera et al. (2018) and Pereira et al. (2018), who recommend performing the treatment of seeds with a maximum of 45 to 60 days before the beginning of sowing, aiming to minimize possible phytotoxic effects of the active ingredients on the seeds.

It is possible to observe that the commercial products used (fungicide, insecticide, drying powder and polymers) reduced the physiological potential of the seeds, compared to the control (T12). Similar results were observed by Matera et al. (2018), who indicated that seed treatment based on imidacloprid + thiodicarb and fipronil + thiophanate methyl + pyraclostrobin may affect the germination of soybean seeds, even when they are analyzed soon after treatment.

The same was pointed out by Carvalho et al. (2020), who observed adverse effects on the initial development of seedlings after application of fungicides and insecticides on soybean seeds, over 60 days of storage, indicating that the harmful effects may be associated with the active ingredients used.

In addition, the treatment with drying powder (T4) caused considerable reductions in the germination potential of the seeds. These results are similar to those found by Abati et al. (2018), in which the use of drying powder reduced the physiological potential of soybean seeds.

This result may be related to the composition of the drying powder (titanium dioxide and natural talc chloride), which despite being hydrophilic in nature, can cause damage related to imbibition, leading to problems in seed germination (Clifton, 1985; Toledo et al., 2010).

For seedling emergence in sand, seeds with high initial vigor generally showed better performance in the four experiments (Table 4). According to Marcos-Filho (2015), this can be justified, because seed lots with high initial quality show lower fluctuation in the percentage of emerged seedlings.

Table 4. Means obtained in the emergence test in sand substrate (%) in experiments I (0 days of storage), II (45 days of storage), III (90 days of storage) and IV (135 days of storage).

Treat.	0 days				45 days				90 days				135 days			
	High		Low		High		Low		High		Low		High		Low	
T1	85	Ba	73	Cb	83	Ba	71	Bb	82	Ba	65	Cb	82	Ca	64	Db
T2	88	Ba	76	Cb	87	Ba	76	Bb	87	Aa	72	Cb	87	Ba	72	Cb
T3	86	Ba	81	Ba	81	Ba	78	Ba	79	Ba	76	Ba	78	Ca	75	Ca
T4	89	Ba	76	Cb	86	Ba	74	Bb	84	Ba	73	Cb	80	Ca	72	Cb
T5	84	Ba	79	Ba	76	Ba	78	Ba	78	Ba	77	Ba	78	Ca	74	Ca
T6	85	Ba	86	Aa	78	Ba	82	Aa	76	Ba	76	Ba	76	Ca	76	Ca
T7	83	Ba	76	Cb	83	Ba	70	Bb	82	Ba	67	Cb	80	Ca	66	Db
T8	84	Ba	87	Aa	84	Ba	87	Aa	83	Ba	77	Ba	82	Ca	73	Cb
T9	88	Ba	87	Aa	86	Ba	87	Aa	84	Ba	77	Ba	80	Ca	80	Ba
T10	87	Ba	80	Bb	85	Ba	79	Ba	83	Ba	73	Cb	80	Ca	70	Cb
T11	94	Aa	72	Cb	94	Aa	69	Bb	91	Aa	69	Cb	84	Ca	73	Cb
T12	97	Aa	88	Ab	97	Aa	87	Aa	96	Aa	87	Ab	94	Aa	87	Ab
Mean	87		80		85		78		84		74		82		73	
CV (%)	4.58				9.20				8.02				6.21			

*Means followed by the same uppercase letters in the column and lowercase letters in the row, within each experiment, do not differ from each other by the Scott-Knott test, at 5% probability level. CV: coefficient of variation.

In experiments I (0 days of storage) and II (45 days of storage), similar effects were observed; for seeds of high initial vigor, the best results were observed with fipronil + thiophanate methyl + pyraclostrobin + polymer + drying powder (T11) and in the control (T12), according to Table 4. For seeds of low initial vigor, the treatments imidacloprid + thiodicarb + drying powder (T6), fipronil + thiophanate methyl + pyraclostrobin + drying powder (T8), polymer + drying powder (T9) and control (T12) showed the best percentages of seedling emergence.

In experiments III (90 days of storage) and IV (135 days of storage), it was possible to verify that, for seeds of high initial vigor, the application of fipronil + thiophanate methyl + pyraclostrobin (T2), together with the control (T12), showed better performance for seedling emergence in sand substrate. For seeds of low initial vigor, it was found that those without the addition of products (T12) showed better results (Table 4). This behavior may have been observed because, according to studies by Marcos-Filho (2015), seeds treated and stored for long periods may show reduction in their potential for emergence.

In general, it was observed that, with the advance of deterioration, the test of seedling emergence in sand allowed a greater distinction of the treatments employed, especially in experiments III (90 days of storage) and IV (135 days of storage).

Regarding the accelerated aging test with NaCl-saturated solution, it was found that for high-vigor seeds, the treatments polymer (T3) and drying powder (T4) caused deleterious effects in stress situations in experiments I (0 days of storage) to III (90 days of storage), with no difference between the treatments tested in experiment IV (135 days of storage). When comparing seeds of different levels of initial vigor, better results were generally observed in seeds of high initial vigor (Table 5).

For the vigor index variable, seeds with high initial vigor showed better performance in the four experiments, indicating that this tool leads to results compatible with those of traditional tests (Table 6). Similar results were found by Silva and Cicero (2014).

Table 5. Means obtained in the accelerated aging test (%) in experiments I (0 days of storage), II (45 days of storage), III (90 days of storage) and IV (135 days of storage).

Treat.	0 days				45 days				90 days				135 days			
	High		Low		High		Low		High		Low		High		Low	
T1	58	Aa	48	Ab	58	Aa	47	Ab	56	Aa	46	Ab	54	Aa	44	Aa
T2	61	Aa	43	Ab	59	Aa	42	Ab	57	Aa	42	Ab	47	Aa	41	Aa
T3	52	Ba	50	Aa	52	Ba	49	Aa	48	Ba	45	Aa	49	Aa	42	Aa
T4	52	Ba	47	Aa	51	Ba	49	Aa	50	Ba	44	Aa	49	Aa	38	Ab
T5	55	Ba	50	Aa	55	Ba	48	Aa	54	Aa	47	Aa	48	Aa	45	Aa
T6	63	Aa	48	Ab	62	Aa	47	Ab	49	Ba	46	Aa	48	Aa	43	Aa
T7	62	Aa	49	Ab	64	Aa	46	Ab	60	Aa	48	Ab	58	Aa	48	Aa
T8	63	Aa	54	Ab	62	Aa	44	Ab	54	Aa	44	Ab	53	Aa	44	Aa
T9	63	Aa	47	Ab	60	Aa	47	Ab	58	Aa	44	Ab	55	Aa	44	Ab
T10	64	Aa	45	Ab	61	Aa	44	Ab	58	Aa	44	Ab	56	Aa	41	Ab
T11	59	Aa	50	Ab	58	Aa	49	Ab	55	Aa	46	Ab	50	Aa	45	Aa
T12	67	Aa	51	Ab	64	Aa	49	Ab	64	Aa	48	Ab	61	Aa	46	Ab
Mean	60		48		59		47		55		45		52		43	
CV (%)	10.88				5.66				11.6				15.86			

*Means followed by the same uppercase letters in the column and lowercase letters in the row, within each experiment, do not differ from each other by the Scott-Knott test, at 5% probability level. CV: coefficient of variation.

Table 6. Means obtained for the vigor index in experiments I (0 days of storage), II (45 days of storage), III (90 days of storage) and IV (135 days of storage).

Treat.	0 days				45 days				90 days				135 days			
	High		Low		High		Low		High		Low		High		Low	
T1	467	Ca	367	Bb	413	Ba	281	Bb	326	Ca	238	Bb	297	Ba	215	Bb
T2	469	Ca	249	Cb	470	Aa	243	Bb	417	Ba	282	Bb	417	Aa	138	Cb
T3	435	Ca	190	Db	460	Aa	166	Cb	306	Ca	154	Cb	369	Aa	122	Cb
T4	416	Ca	333	Bb	408	Ba	267	Bb	376	Ca	146	Cb	357	Aa	162	Cb
T5	455	Ca	369	Bb	440	Ba	309	Bb	416	Ba	253	Bb	370	Aa	224	Bb
T6	442	Ca	439	Aa	498	Aa	361	Ab	514	Aa	391	Ab	389	Aa	333	Aa
T7	505	Ba	390	Ab	488	Aa	414	Ab	466	Aa	320	Ab	403	Aa	179	Cb
T8	462	Ca	367	Bb	473	Aa	367	Ab	498	Aa	355	Ab	425	Aa	235	Bb
T9	516	Ba	403	Ab	480	Aa	386	Ab	424	Ba	281	Bb	445	Aa	321	Ab
T10	470	Ca	323	Bb	472	Aa	311	Bb	503	Aa	268	Bb	414	Aa	279	Ab
T11	522	Ba	297	Cb	480	Aa	310	Bb	316	Ca	289	Ba	287	Ba	227	Ba
T12	598	Aa	325	Bb	516	Aa	274	Bb	427	Ba	343	Ab	430	Aa	286	Ab
Mean	480		338		466		307		416		277		383		227	
CV (%)	10.88				10.48				7.84				8.37			

*Means followed by the same uppercase letters in the column and lowercase letters in the row, within each experiment, do not differ from each other by the Scott-Knott test, at 5% probability level. CV: coefficient of variation.

Based on the results, it was verified that for experiment I (0 days of storage), using seeds with initial high vigor, the control (T12) was classified as more vigorous, followed by the treatments fipronil + thiophanate methyl + pyraclostrobin + polymer (T7), polymer + drying powder (T9) and fipronil + thiophanate methyl + pyraclostrobin + polymer + drying powder (T11). For seeds with low initial vigor, the best results were observed with imidacloprid + thiodicarb + drying powder (T6), fipronil + thiophanate methyl + pyraclostrobin + polymer (T7) and polymer + drying powder (T9), according to Table 6.

For experiment II (45 days of storage), with seeds of initial high vigor, all treatments showed good classification, except for the treatment imidacloprid + thiodicarb (T1), drying powder (T4) and imidacloprid + thiodicarb + polymer (T5); on the other hand, for seeds of low initial vigor, the response was very similar to that observed in experiment I (0 days of storage) (Table 6).

In experiment III (90 days of storage), the best classification for seeds of initial high vigor was obtained in the treatments imidacloprid + thiodicarb + drying powder (T6), fipronil + thiophanate methyl + pyraclostrobin + polymer (T7), fipronil + thiophanate methyl + pyraclostrobin + drying powder (T8) and imidacloprid + thiodicarb + polymer + drying powder (T10); however, seeds of low vigor showed a behavior similar to that of high-vigor seeds (Table 6).

In the fourth experiment (135 days of storage), there was a considerable reduction in seed vigor. For high-vigor seeds, the treatments with the highest reductions were imidacloprid + thiodicarb (T1) and fipronil + thiophanate methyl + pyraclostrobin + polymer + drying powder (T11), whereas for low-vigor seeds, a greater number of treatments showed a lower vigor index than the control (T12), according to Table 6.

Reduction of seed vigor due to the application of the combination of products has also been observed by other authors (Lanferdini et al., 2017; Pereira et al., 2018; Matera et al., 2018; Santos et al., 2021), indicating that the intensity of seed deterioration can be boosted by the application of chemicals under uncontrolled storage conditions. For seedling length, obtained by Vigor-S® software, seeds with high initial vigor showed the best results in all experiments (Table 7).

Table 7. Means obtained for seedling length in experiments I (0 days of storage), II (45 days of storage), III (90 days of storage) and IV (135 days of storage).

Treat.	0 days				45 days				90 days				135 days			
	High		Low		High		Low		High		Low		High		Low	
T1	5.0	Ba	3.5	Bb	4.0	Ca	2.8	Cb	3.3	Da	2.0	Cb	2.8	Da	1.6	Bb
T2	4.6	Ca	2.2	Db	4.3	Ca	2.2	Db	4.1	Ca	2.4	Bb	3.9	Ba	2.5	Ab
T3	4.8	Ba	2.9	Cb	5.2	Aa	2.4	Db	4.0	Ca	1.9	Cb	3.6	Ca	1.7	Bb
T4	3.9	Ca	1.6	Db	4.6	Ba	1.6	Eb	4.3	Ca	1.4	Db	3.5	Ca	1.7	Bb
T5	4.9	Ba	3.9	Ab	4.7	Ba	2.9	Cb	4.5	Ba	2.5	Bb	3.9	Ba	2.0	Bb
T6	4.7	Ba	4.5	Aa	4.9	Aa	3.3	Bb	5.1	Aa	3.3	Ab	4.1	Ba	2.8	Ab
T7	6.5	Aa	4.3	Ab	5.4	Aa	4.4	Ab	4.5	Ba	3.0	Ab	3.7	Ba	2.7	Ab
T8	4.9	Ba	3.5	Bb	5.2	Aa	4.0	Ab	4.6	Ba	3.6	Ab	4.2	Ba	2.5	Ab
T9	5.5	Ba	4.4	Ab	4.7	Ba	4.0	Ab	4.6	Ba	2.7	Bb	3.9	Ba	2.9	Ab
T10	5.3	Ba	3.5	Bb	4.8	Ba	3.2	Bb	4.8	Ba	3.1	Ab	4.0	Ba	2.6	Ab
T11	5.2	Ba	3.0	Cb	5.0	Aa	3.0	Cb	4.6	Ba	2.5	Bb	4.3	Ba	1.9	Bb
T12	6.4	Aa	3.2	Bb	5.4	Aa	2.6	Db	5.2	Aa	2.4	Bb	4.9	Aa	2.1	Bb
Mean	5.1		3.4		4.8		3.0		4.5		2.6		3.9		2.3	
CV (%)	11.05				4.32				4.20				12.83			

*Means followed by the same uppercase letters in the column and lowercase letters in the row, within each experiment, do not differ from each other by the Scott-Knott test, at 5% probability level. CV: coefficient of variation.

For the four experiments, in seeds with high initial vigor, the treatments imidacloprid + thiodicarb (T1), fipronil + thiophanate methyl + pyraclostrobin (T2), imidacloprid + thiodicarb + polymer (T5), polymer + drying powder (T9) and imidacloprid + thiodicarb + polymer + drying powder (T10) led to shorter seedling length, compared to the control. For seeds of low initial vigor, there was a distinct behavior between treatments.

In general, the treatment with drying powder (T4) was the one that promoted the greatest significant reduction in the growth of seedlings, grown from seeds of low initial vigor, similar to the results found by Abati et al. (2018), who evaluated the effect of seed treatment, with and without application of drying powder, on the physiological potential of soybean seeds, and observed that the use of drying powder can lead to problems in germination speed, reducing the physiological potential of soybean seeds.

For the first experiment, 0 days of storage (Figure 1), the linear combination of the parameters generated a new linear component, the principal component 1 (PC1), which represented 62.4% of the total variability of the data for high-vigor seeds, and a second component, principal component 2 (PC2), representing 17.8% of the variability, totaling 80.2% of accumulated variability.

When analyzing the principal components of Figure 1A, it was observed that for PC1 the drying powder (T4) led to the lowest estimates, compromising the first germination count, vigor index and average seedling length, as they had higher weights for this component; on the other hand, the best result was observed for the control (T12). For the principal component 2, it was found that the drying powder (T4) negatively affected the physiological potential of the seeds, especially seedling emergence. On the other hand, higher percentages of seedling emergence were obtained in the treatment with fipronil + thiophanate methyl + pyraclostrobin + polymer (T7).

For seeds of low initial vigor, the principal components (PC1 and PC2) totaled 67% of accumulated variability. It was observed that, for PC1, again the treatment with drying powder (T4) obtained the lowest estimates, driven by germination percentage, vigor index and average seedling length. The best results were observed for the treatments

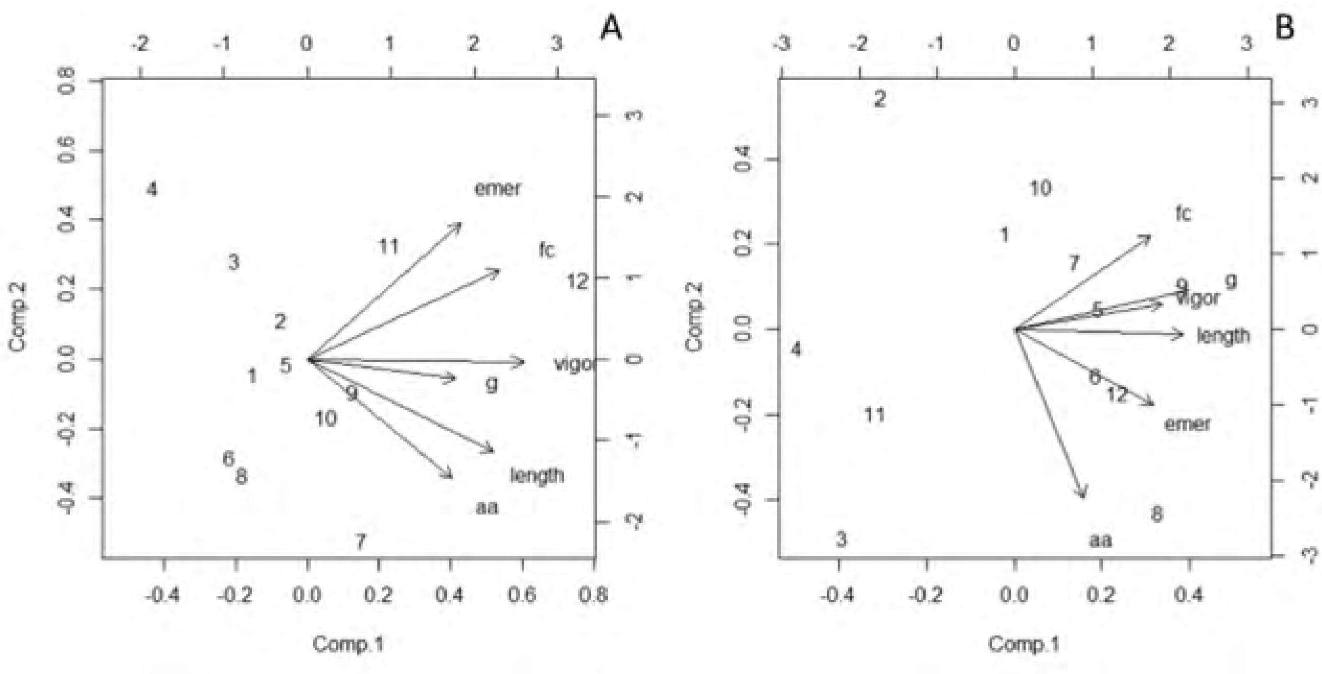

Figure 1. Principal component analysis for experiment I in soybean seeds. A - Seeds with high initial vigor; B - Seeds with low initial vigor; emer (emergence); fc (first count); g (germination); aa (accelerated aging).

fipronil + thiophanate methyl + pyraclostrobin + drying powder (T8) and polymer + drying powder (T9). For PC2, the T2 treatment showed lower performance, especially due to accelerated aging, as this variable aided the treatments polymer (T3) and fipronil + thiophanate methyl + pyraclostrobin + drying powder (T8), as observed in Figure 1B.

For experiment II (45 days of storage), for seeds of high initial vigor, the linear combination of parameters generated PC1 with variability of 57.4% and PC2 with variability of 18.8%, totaling 76.2% of accumulated variability (Figure 2).

When analyzing each principal component separately, it was possible to notice that, for PC1, the treatment with drying powder (T4) remains the one with the lowest estimates, due to the weights observed for the variables first germination count, germination and vigor index; on the other hand, the best results were observed for T12, according to Figure 2A.

For seeds of low initial vigor, the principal components (PC1 and PC2) totaled 71.4% of accumulated variability. When analyzing the components separately, it was observed that PC1 followed the same trend already observed previously, in which T4 obtained the lowest estimates, driven by the low values of the first germination count, germination and average seedling length. For PC2, the treatment with fipronil + thiophanate methyl + pyraclostrobin (T2) showed lower performance due to the low vigor index. On the other hand, the treatments polymer + drying powder (T9) and fipronil + thiophanate methyl + pyraclostrobin + polymer (T7) showed better results (Figure 2B).

In experiment III (90 days of storage), for high-vigor seeds, the linear combination of parameters generated PC1 with variability of 51.3% and PC2 with variability of 26.8%, totaling 78.1% accumulated variability (Figure 3).

For seeds of high initial vigor, the principal components of Figure 3A showed that, for PC1, the treatments polymer (T3) and drying powder (T4) obtained the lowest estimates, with higher weights for the first germination count, germination, emergence and accelerated aging. For PC2, the treatment with imidacloprid + thiodicarb (T1) was harmful to the variables vigor index and average seedling length, while the treatment with imidacloprid + thiodicarb + drying powder (T6) obtained the best results.

For seeds of low initial vigor, the principal components PC1 and PC2 totaled 68.3% of accumulated variability; for principal component 1, the drying powder (T4) obtained the lowest estimates, since the first germination count and

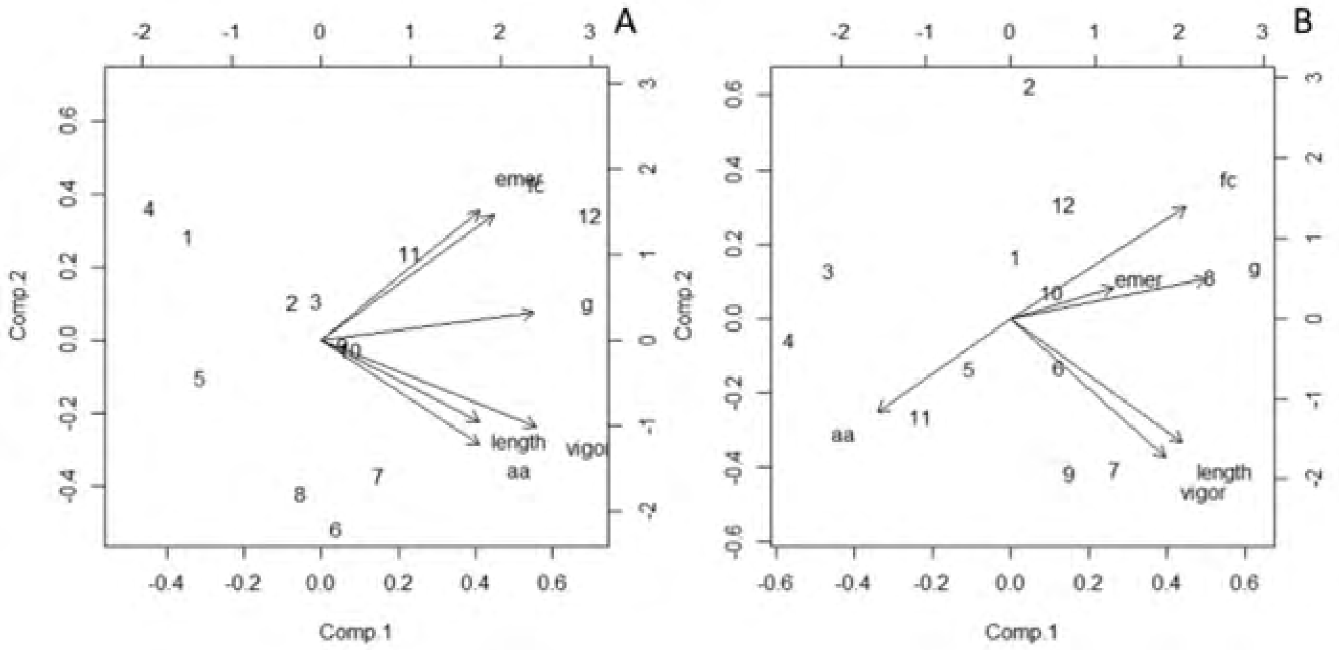

Figure 2. Principal component analysis for experiment II in soybean seeds. A - Seeds with high initial vigor; B - Seeds with low initial vigor; emer (emergence); fc (first count); g (germination); aa (accelerated aging).

the vigor index had higher weights. The best results were observed for fipronil + thiophanate methyl + pyraclostrobin + polymer (T7), fipronil + thiophanate methyl + pyraclostrobin + drying powder (T8) and the control (T12), as shown in Figure 3B.

In experiment IV (135 days of storage), for high-vigor seeds, there was variability of 54.5% for PC1 and 17.7% for PC2, totaling an accumulated variability of 72.2% (Figure 4).

For seeds of high initial vigor, in the principal component 1, the treatment drying powder (T4) obtained lower estimates, with higher weights for the first germination count, germination, emergence, accelerated aging and average seedling length; however, the best result was observed for the control (T12). For the principal component 2, the treatment with imidacloprid + thiodicarb (T1) showed lower performance, with a very low vigor index, whereas the treatment with fipronil + thiophanate methyl + pyraclostrobin + drying powder (T8) obtained better results for vigor index (Figure 4A).

For seeds of low initial vigor, the principal components showed that there was variability of 47.5% for PC1 and 20.1% for PC2, totaling 67.6%. For the principal component 1, again the drying powder (T4) obtained the lowest estimates, with the highest weights for the first germination count and vigor index. As already observed in the other experiments, the best results were obtained in the control (T12), according to Figure 4B.

Thus, the principal component analysis in the four experiments showed that, in general, 76.6% of the variability was retained in the combination of the first two components, corroborating studies conducted by Rencher (2002), who indicates that the sum of the principal components 1 and 2 should total approximately 70% of the total variance of the data.

In general, it was observed that, regardless of the storage period studied, seeds without treatment showed the best results, while seeds treated with drying powder, in isolation, had considerable reductions in their physiological potential, a result possibly associated with a phytotoxic effect of the drying powder in isolated effect (Abati et al., 2018).

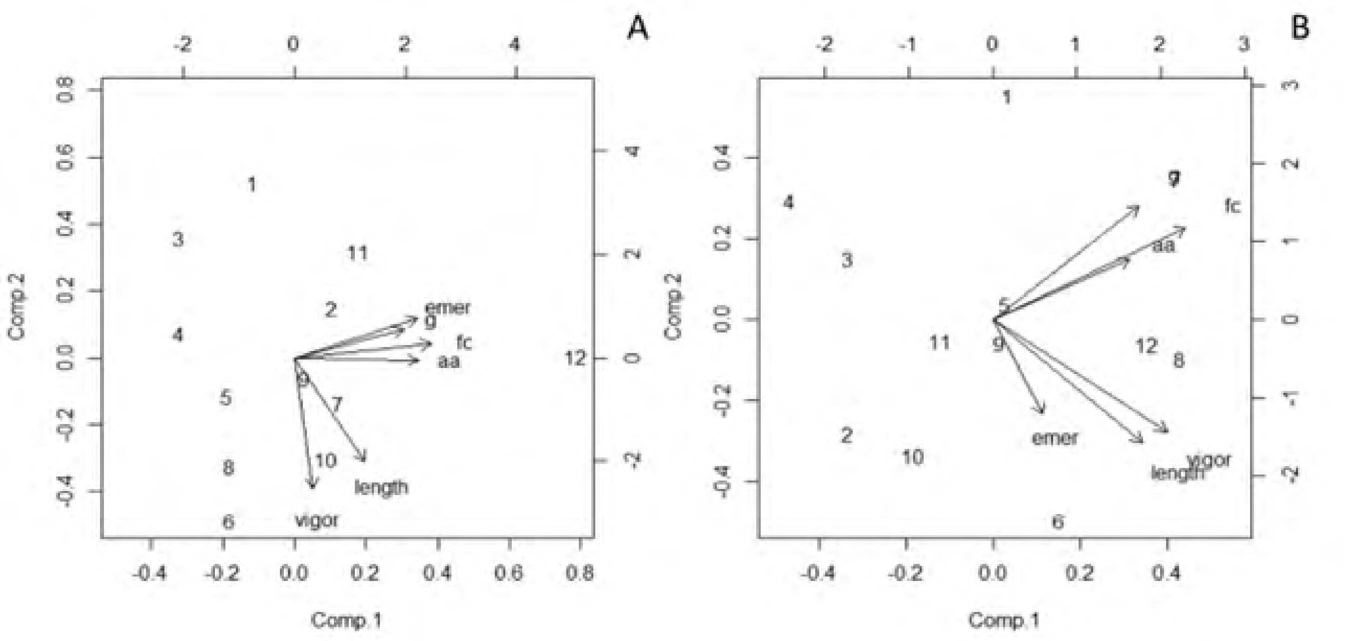

Figure 3. Principal component analysis for experiment III in soybean seeds. A - Seeds with high initial vigor; B - Seeds with low initial vigor; emer (emergence); fc (first count); g (germination); aa (accelerated aging).

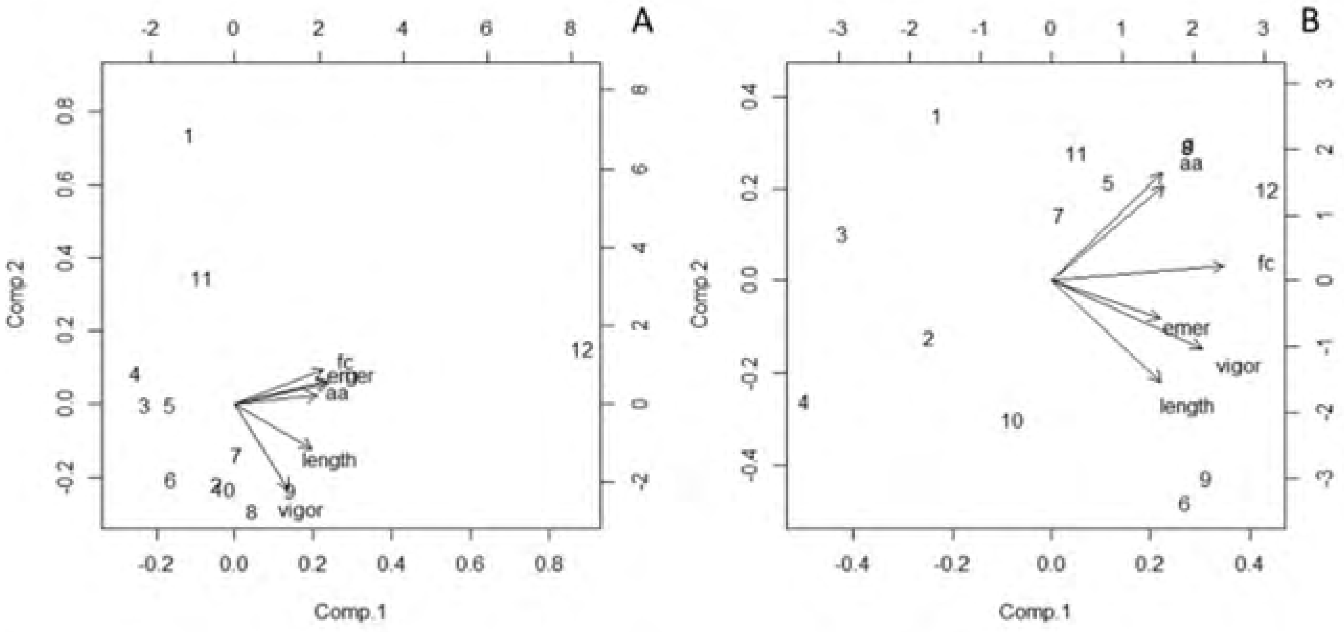

Figure 4. Principal component analysis for experiment IV in soybean seeds. A - Seeds with high initial vigor; B - Seeds with low initial vigor; emer (emergence); fc (first count); g (germination); aa (accelerated aging).

CONCLUSIONS

Storage of seeds under uncontrolled conditions causes greater deterioration, and this process can be intensified by the previous chemical treatment of the seeds, especially with the mixture fipronil + thiophanate methyl + pyraclostrobin + drying powder.

Among the products studied, the use of drying powder, in isolation, was the treatment that caused greater losses in the different situations studied.

To ensure the physiological quality of soybean seeds, the treatment should be carried out as close as possible to sowing.

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