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Physiological quality of soybean seeds submitted to chemical treatment and storage times

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ABSTRACT

Early treatment of seeds, using new combinations of chemicals has been increasingly adopted, however, its effects on seed quality during storage are still not completely understood. The aim was to evaluate the physiological seed quality of two soybean cultivars treated with different combinations of chemical products during storage. The experiment was arranged in a completely randomized design, with four replications in a factorial 7x5 for each cultivar, with seven seed treatments and five evaluated periods (times) during storage (0, 60, 120, 180 and 240 days). The treatments were: T1) fipronil + pyraclostrobin + methyl thiophanate; T2) imidacloprid + thiodicarb + carbendazin + thiram; T3) abamectin + mefenoxam + fludioxonil + thiamethoxam + thiabendazole; T4) carbendazin + thiram; T5) fludioxonil + mefenoxam + thiabendazole; T6) carboxin + thiram and T7) control (no treated seeds). The physiological seed quality was evaluated by the following tests: germination, accelerated aging, seedling emergence in sand, and shoot and root length of seedlings. Based on the results observed in the cultivar BRS 360 RR, all treatments evaluated, except for T6, maintained seed germination above 80%, for up to 120 days. The physiological quality of soybean seeds decreased during storage in all treatments (including the control). The treatment with the insecticides imidacloprid + thiodicarb associated with the fungicides carbendazin + thiram markedly reduces the physiological quality of the tested soybean cultivars seeds.

KEYWORDS: Fungicides. Germination. *Glycine max* (L.) Merrill. Inseticides. Vigor.

INTRODUCTION

Seed treatments consist of the application of processes and substances that preserve or refine the performance, allowing crops to express their genetic potential by favoring seedling emergence and through the control of pathogens and pests. In addition, they include the application of compounds that can have some bio-active effect, affecting the plant metabolism and increasing the crop yield potential (BALARDIN et al., 2011; CONCEIÇÃO et al., 2014).

Chemical treatments used to be applied before sowing, both on the farm as well as in at the resale stage, using specific machines. However, with the nation-wide development of agriculture, seed companies came to adopt techniques that maximize crop yield, e.g., an industrial process of seed treatment, in which the seeds are treated in the proper processing stage and later bagged and stored until sowing (BRZEZINSKI et al., 2015; ABATI et al., 2018).

The industrial seed treatment process associate equipment and the use of innovative techniques such as the use of new formulations containing fungicides, insecticides and nematicides in the same treatment, as well as its carrier, which can maximize the efficiency of the products, help protect operators and avoid contamination of the environment (BRZEZINSKI et al., 2017).

However, an anticipation of the chemical treatment can reduce seed vigor and even germination during storage, due to the possible phytotoxic effect some active ingredients of the products can have on the seeds (PEREIRA et al., 2018; ABATI et al., 2020). This phytotoxic effect alters the seed quality, reduces germination, vigor and seedling emergence and impairs plant establishment and crop yield (FERREIRA et al., 2016).

Given the above, the study of new combinations of formulations applied in the industrial seed treatment as well as their effect on seed quality during storage is imperative. Thus, the purpose of this study was to evaluate the physiological seed quality of two soybean cultivars (BRS 360 RR and BRS 284) treated with different combinations of chemical products (inseticide, fungicide and nematicide) during the storage period (0, 60, 120, 180 and 240 days).

MATERIAL AND METHODS

The experiment was carried out at the Seed and Grain Technology Center of the Empresa Brasileira de Pesquisa Agropecuária - Embrapa Soja, in Londrina, PR, Brazil (23°11' S; 51°11'W; 620 m), at Seed Physiology, Technology and Pathology Laboratories.

The experiment was a completely randomized in a factorial 7x5 design, with four replications. The factors consisted of seven soybean seed treatments with chemicals recommended for the crop (Table 1) and five evaluations during storage (0, 60, 120, 180 and 240 days).

Table 1 - Active ingredients, commercial products and rates used for the seed treatment of the soybean cultivars BRS 360 RR and BRS 284

Treatments	Active ingredient (a.i.)	Commercial name	Type of product[1]	Commercial product dose[2]	Water rate[3]
1	fipronil + pyraclostrobin + methyl thiophanate	Standak Top®	I + F + F	200	400
2	imidacloprid + thiodicarb + carbendazin + thiram	Cropstar® + Derosal Plus®	I + I + F + F	300+200	100
3	abamectin + tiametoxan + fludioxonil + mefenoxam + thiabendazole	Avicta Completo (Avicta 500 FS® + Cruiser® 350 FS + Maxim Advanced®)	N + I + F + F + F	125+200+100	175
4	carbendazin + thiram	Derosal Plus®	F + F	200	400
5	fludioxonil + mefenoxam + thiabendazole	Maxim Advanced®	F + F + F	100	500
6	carboxin + thiram	Vitavax-Thiram 200 SC®	F + F	250	350
7	Control	-	-	-	-

[1]Product type: I - Inseticide; F - Fungicide and N - Nematicide; [2]Commercial product dose: mL 100 kg^{-1} of seeds; [3]Water rate: mL 100 kg^{-1} of seeds; Maximum solution volume: 600 mL 100 kg^{-1} of seeds.

Source: Prepared by the author (2020).

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BRS 360 RR and BRS 284 soybean cultivars were analyzed separately. BRS 360 RR is a genetically modified cultivar tolerant of the herbicide glyphosate and with an indeterminate growth habit; while BRS 284 is a conventional soybean cultivar with an indeterminate type of growth. A seed lot for each cultivar was selected according to the results of the tetrazolium test. This test was applied to investigate the vigor and viability, to ensure a similar initial quality of the seed lots.

The tetrazolium test was conducted with two subsamples of 50 seeds per replication distributed on germitest paper, moistened with distilled water, for 16 h in an incubator chamber, at 25 °C. Tetrazolium solution (2, 3, 5-triphenyltetrazolium chloride) was used at a concentration of 0.075% (FRANÇA NETO; KRZYZANOWSKI, 2018). The seeds were individually assessed and classified according to criteria proposed by França Neto e Krzyzanowski (2018). The potential vigor and viability were expressed in percentage. The data showed vigor of 93 to 92% and viability of 99 and 98%, respectively, for the cultivars BRS 360 RR and BRS 284.

The chemical seed treatment was performed in polyethylene plastic bags using a solution volume of 600 mL 100 kg^{-1} seed (product + water), except in the control treatment. The treated seeds were packed in cardboard boxes and stored for 240 days under natural environmental conditions, a condition corresponding to the reality of most structures adopted in seed storage.

To determine the physiological seed quality, the following evaluations were performed:

Germination: the test was performed with two subsamples of 50 seeds per replications, with a total of 400 seeds per treatment. The seeds were distributed in rolls of germitest paper towel, moistened with water at a rate of 2.5 times the dry paper mass. The prepared rolls were placed in a germination chamber at 25 °C. The seedlings were evaluated eight days later, according to the rules for seed testing (BRASIL, 2009), taking only normal seedlings into account.

Accelerated aging: in plastic incubator boxes (gerbox) with mesh, containing 40 mL of water at the bottom, the seeds were arranged in a uniform layer on the surface of this inner screen, and then, maintained in an incubator at 41 °C for 48 h

(MARCOS-FILHO, 1999). Thereafter, the germination test was conducted according to the Rules for Seed Analysis (BRASIL, 2009).

Seedling emergence in sand: a total of 400 seeds per treatment were divided into one subsample of 100 seeds per replication and the seeds sown in a greenhouse in plastic trays containing sand. Moisture was maintained by irrigation, as required by the seedlings. The final number of normal emerged seedlings was counted on the 10th day and the results expressed in percentage.

Shoot and root length of seedlings: five subsamples of 20 seeds per replications were used, totaling 400 seeds per treatment, distributed on rolls of germitest paper towels moistened with distilled water ata rate of 2.5 times the dry paper mass, and maintained in a germinator at 25 °C for five days (NAKAGAWA, 1999). Subsequently, the shoot and primary root length considered normal were determined with a millimeter ruler and the results expressed in cm.

Analysis of variance was performed and the treatment means were compared by Tukey's test at 0.05 probability. Regression analysis was performed on the basis of storage time. The computer program System for Analysis of Variance – SISVAR was used for the analyses (FERREIRA, 2011).

RESULTS AND DISCUSSION

The data of BRS 360 RR indicated no significant effect of interaction between treatments and storage times were detected for the traits seedling emergence in sand and shoot and root length. For this cultivar, no isolated effect of the chemical treatment was observed on the variable seedling emergence. For BRS 284 however, no interaction was observed between germination, accelerated aging and root length, neither an isolated treatment effect on seedling emergence in sand (Table 2).

Table 2 - Summary of analysis of variance (mean squares) for the physiological quality characteristics evaluated in two soybean cultivars (BRS 360 RR and BRS 284), depending on the industrial seed treatment and evaluation times

BRS 360 RR						
		Mean Squares				
F.V.	GL	G	AA	EMA	SL	SR
Treat. (T)	6	358*	740*	3,6^{ns}	4.3*	13.3*
Period (P)	4	2075*	27080*	50.9*	24.0*	77.9*
T x P	24	45.7*	1648*	4.3^{ns}	1.2^{ns}	1.3^{ns}
Error	70	11.5	31.1	2.7	1.0	2.7
Mean		84	48	96	7.3	13.5
BRS 284						
		Average Squares				
F.V.	GL	G	AA	EMA	SL	SR
Treat. (T)	6	136*	73.6*	26.8^{ns}	1.4*	6.9*
Period (P)	4	14112*	144460*	4268*	23.9*	254*
T x P	24	58.1^{ns}	30.1^{ns}	40.0*	0.79*	2.1^{ns}
Error	70	49.5	26.5	23.1	0.46	2.6
Mean		52	21	78	5.0	8.7

^{ns}, not significant and *, significant at 5% probability, by the F test. F.V.: source of variation; T: treatment; P: storage periods; G: germination test; AA: accelerated aging; EMA: seedling emergence; SL: shoot length; RL: root length and SDW: shoot dry weight.

Source: Prepared by the author (2020).

For the variable germination of cultivar BRS 360 RR, the results (Table 3) show that there was no difference between the seed treatments in the initial assessments (before storage) and 60 days later. During these periods, the germination rate of soybean seeds was adequate in all treatments, with percentages above 80%, i.e., the minimum threshold required for seed marketing (MAPA, 2013). In tests with soybean seeds treated with the insecticides thiamethoxam, fipronil, imidacloprid, imidacloprid + thiodicarb, carbofuran and acephate, and stored for 45 days, Dan et al. (2010) found a similar result in the initial evaluation of the germination test.

After 120 days of storage, it was found that germination was not reduced only in treatment 1 (fipronil + pyraclostrobin + methyl thiophanate) and 4 (carbendazin + thiram), compared with the control (Table 3). In the third storage period of 180 days, germination decreased in all chemically treated seeds, but by the end of the storage period (240 days), only treatment 4 did not differ significantly from the control.

Table 3 - Effect of different chemical seed treatments on germination (%), accelerated aging (%) and shoot and root length of seedlings (cm), derived from soybean seeds of the cultivar BRS 360 RR, at different evaluation times during storage

	Storage periods (days)					
Treatments[1]	0	60	120	180	240	Mean
			Germination			
1	95 A	92 A	86 ABC	75 BC	72 B	84
2	96 A	90 A	83 BC	69 C	62 C	80
3	95 A	90 A	81 BC	73 C	66 BC	81
4	97 A	93 A	87 AB	82 B	82 A	88
5	97 A	88 A	81 BC	75 BC	71 B	83
6	96 A	90 A	78 C	68 C	67 BC	80
7	98 A	96 A	92 A	91 A	89 A	93
CV (%)			4.02			
			Accelerated aging			
1	88 AB	76 ABC	65 AB	12 ABC	10 A	50
2	79 B	62 C	33 C	3 C	3 A	36
3	83 AB	68 BC	61 B	13 ABC	5 A	46
4	94 A	84 A	59 B	20 A	11 A	54
5	91 AB	72 ABC	62 B	17 ABC	10 A	50
6	90 AB	67 BC	53 B	8 BC	2 A	44
7	95 A	80 A	77 A	23 A	13 A	58
CV (%)			11.53			
			Shoot length			
1	8.76	8.26	8.10	7.61	6.00	7.75 A
2	7.98	7.10	5.92	5.82	5.20	6.40 B
3	9.43	8.04	6.90	7.30	5.50	7.50 AB
4	7.71	6.84	7.15	7.44	6.29	7.09 AB
5	6.54	7.86	7.57	7.34	5.26	6.91 AB
6	9.26	7.78	7.75	6.74	5.38	7.38 AB
7	10.29	8.18	8.31	7.77	5.50	8.01 A
CV (%)			13.74			
			Root length			
1	17.69	15.15	14.45	14.51	11.56	14.67 AB
2	15.47	12.94	12.36	11.55	9.89	12.44 C
3	16.33	13.94	12.66	12.95	11.05	13.39 ABC
4	16.02	13.71	12.98	12.36	11.73	13.36 ABC
5	14.20	13.32	14.01	13.38	10.78	13.14 BC
6	16.75	13.73	13.21	11.60	9.83	13.02 BC
7	18.46	14.63	15.67	13.83	12.80	15.08 A
CV (%)			12.31			

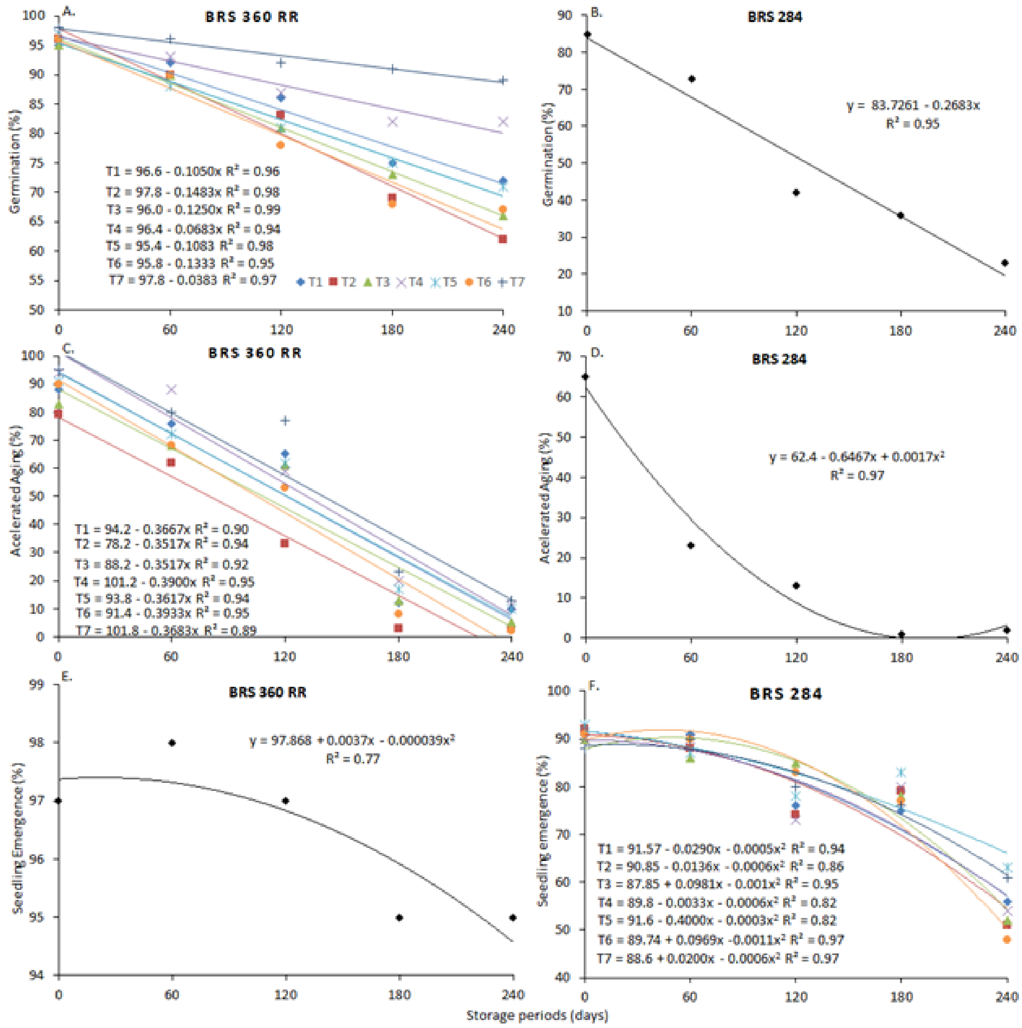
*Means followed by the same letter in a column, did not differ from each other by the Tukey test at 0.05 probability. [1]Treatments: 1 - Fipronil + pyraclostrobin + methyl thiophanate; 2 - Imidacloprid + thiodicarb + carbendazin + thiram; 3 - Abamectin + tiametoxan + fludioxonil + mefenoxam + thiabendazole; 4 - Carbendazin + thiram; 5 -Fludioxonil + mefenoxam + thiabendazole; 6 - Carboxin + thiram; 7 - Control (without treatment).

Source: Prepared by the author (2020).

Regarding the effect of storage periods, a decrease in germination was found during seed storage in all treatments (Figure 1A). However, in the treatments 4 and

7 (control), germination decreased at lower rates, respectively. In the other treatments, germination was reduced to an extent that would hamperseed marketing.

Figure 1 - Effect of chemical seed treatment and storage periods on germination, accelerated aging and seedling emergence in sand, in both soybean cultivars: BRS 360 RR and BRS 284



Source: Prepared by the author (2020).

For cultivar BRS 284, germination was significantly reduced in treatment 2 (carbendazin + thiram + imidacloprid + thiodicarb), compared to the control (Table 4). This difference was possibly due to the association of the insecticides (imidacloprid + thiodicarb) with the fungicides (carbendazin + thiram), because separately, these fungicides (treatment 4) had no negative effect on germination.

Table 4 - Effect of chemical seed treatment and storage periods on germination (%), accelerated aging (%) seedling emergence in sand (%) and shoot and root length of seedlings (cm) of the soybean cultivar BRS 284

Treatments[1]	Storage periods (days)					
	0	60	120	180	240	Mean
			Germination			
1	86	73	46	33	24	52 AB
2	85	64	36	28	23	47 B
3	83	73	33	34	24	50 AB
4	85	82	37	41	25	54 AB
5	86	71	42	36	23	52 AB
6	79	68	50	35	24	51 AB
7	87	80	49	44	21	57 A
CV (%)			13.58			
			Accelerated aging			
1	66	19	20	1	0	21 AB
2	65	19	10	0	0	19 AB
3	62	16	9	0	0	18 B
4	73	30	15	3	1	24 A
5	57	21	10	1	6	19 AB
6	63	27	11	0	1	20 AB
7	65	25	13	1	3	22 AB
CV (%)			25.10			
			Seedling emergence in sand			
1	91 A	91 A	76 AB	75 A	56 AB	78
2	92 A	88 A	74 AB	79 A	51 AB	77
3	90 A	86 A	85 A	78 A	52 AB	78
4	90 A	90 A	73 B	80 A	54 AB	77
5	93 A	87 A	78 AB	83 A	63 A	81
6	91 A	89 A	83 AB	77 A	48 B	77
7	88 A	90 A	80 AB	76 A	61 A	79
CV (%)			6.15			
			Shoot length			
1	5.84 B	6.00 A	4.96 A	4.28 A	3.42 A	4.90
2	5.76 B	4.52 A	4.60 A	4.08 A	3.45 A	4.48
3	5.85 B	5.86 A	4.67 A	4.10 A	3.99 A	4.89
4	6.49 B	5.78 A	4.79 A	4.61 A	3.94 A	5.12
5	8.42 A	5.21 A	5.64 A	4.48 A	3.56 A	5.46
6	6.75 AB	5.29 A	5.65 A	4.09 A	3.54 A	5.06
7	5.99 B	5.78 A	5.50 A	4.72 A	3.74 A	5.15
CV (%)			13.58			
			Root length			
1	13.77	9.58	8.08	5.65	4.71	8.36 AB
2	12.73	6.55	7.32	5.91	4.68	7.44 B
3	15.31	9.52	8.60	5.58	6.77	9.15 AB
4	14.11	9.08	8.22	6.85	5.04	8.66 AB
5	16.18	8.74	8.81	5.69	4.96	8.87 AB
6	14.57	8.41	10.44	7.87	5.81	9.42 A
7	13.76	10.41	9.63	7.12	5.44	9.27 A
CV (%)			18.42			

*Means followed by the same letter in a column did not differ from each other by the Tukey test, at 0.05 probability. [1]Treatments: 1 - Fipronil + pyraclostrobin + methyl thiophanate; 2 - Imidacloprid + thiodicarb + carbendazin + thiram; 3 - Abamectin + tiametoxan + fludioxonil + mefenoxam + thiabendazole; 4 - Carbendazin + thiram; 5 - Fludioxonil + mefenoxam + thiabendazole; 6 - Carboxin + thiram; 7 - Control (without treatment).

Source: Prepared by the author (2020).

These results are similar to those of Pereira et al. (2011), who found that the performance of soybean seeds treated with the fungicides carbendazin + thiram was not affected. However, Dan et al. (2010) stated that soybean seed treatment with the insecticides imidacloprid + thiodicarb impaired germination during storage (45 days).

These results corroborate findings of Munkvold et al. (2006), who stated that the active ingredients of insecticides may be detrimental to seed quality due to a phytotoxic effect. These effects can be observed during germination, with an abnormal seedling development, characterized mainly by the thickening and shortening of the hypocotyl.

The germination percentage for BRS 284 decreased with storage time (Figure 1B). This decline can be related to the natural process of seed deterioration. This reduction may be associated with factors such as a high seed damage rate by moisture and bedbugs, inadequate management of storage conditions and chemical treatments. This observation supports the findings of Ludwig et al. (2011), who observed a greater decrease in germination of soybean seeds treated with fungicide, insecticide and amino acids, separately and/or together during storage. In another paper, Vanin et al. (2011) reported that the storage of insecticide-treated sorghum seeds also reduced the germination percentage.

In terms of seed vigor, measured by the accelerated aging test, the effect of the interaction between factors was greater number of abnormal seedlings to grow from seeds of cultivar BRS 360 RR in treatment 2 (imidacloprid + thiodicarb + carbendazin + thiram), and consequently, lower vigor than in the control (Table 3). After 60 and 120 days of storage, treatment 7 (control) and treatment 1 (fipronil + pyraclostrobin + methyl thiophanate) did not affect seed germination. However, after 180 days, seed vigor decreased markedly in all treatments.

With regard to the age effect, there was a negative linear fit in seed vigor in all treatments (Figure 1C). The same trend was observed by Cardoso et al. (2004) in the accelerated aging test with soybean seeds treated with fungicide difeconazole, where seed vigor was reduced during a cold storage period. In another study, Mbofung et al. (2013) reported similar results in seeds of 24 soybean cultivars treated with fungicide and insecticide, in different storage systems.

The accelerated aging data for cultivar BRS 284 showed that in the first evaluation all seeds already had only median vigor. In seeds of treatment 3 (abamectin + thiamethoxam + fludioxonil + mefenoxam + thiabendazole), the vigor percentage was lower, but diferent only from treatment 4 (carbendazin + thiram) (Table 4). For the effect of storage period (Figure 1D), our results were in agreement with those of De Moraes Dan et al. (2013), who found that in soybean seeds treated with the insecticide thiamethoxam, seed vigor was drastically reduced by a storage period of 90 days. In another study, Piccinin et al. (2013) also found that soybean seeds treated with the insecticides fipronil and thiamethoxam affected the vigor of seeds exposed to storage for 180 days under natural conditions.

In the emergence test on sand the percentage of normal seedlings of cultivar BRS 360 RR decreased as seed storage time increased (Figure 1E). Krohn and Malavasi (2004) found reductions for this variable in fungicide-treated soybean seeds stored under environmental conditions for 150 days.

The chemical treatments did not affect seedling emergence BRS 284 until 60 days of storage (Table 4). However, from this period on, emergence was reduced in all treatments (Figure 1F). In the last storage period (240 days), the germination percentage in treatment 6 (carboxin + thiram) was lower than in the control, but not different from the other chemical treatments (Table 4). According to Marcos-Filho (2015), the seedling emergence test is vital, since the climatic conditions at the experimental location are rather varied and the success of the initial plant establishment, and consequently of the crop, will be analyzed.

With regard to the seedling development, the effect for treatment and storage times onshoot length of cultivar BRS 360 RR was isolated. The seedling shoot length was shorter in treatment 2 (imidacloprid + thiodicarb + carbendazin + thiram) than in the control (Table 3). In relation to the storage periods, the data indicated a decrease in shoot growth during storage (Figure 2A).

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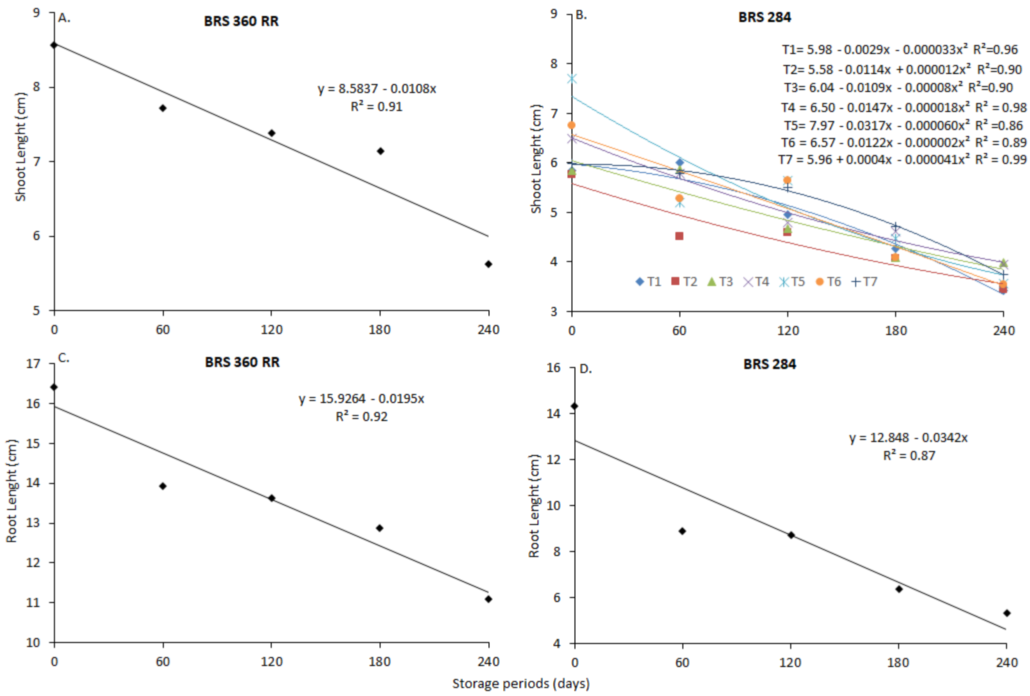
Figure 2 - Effect of chemical seed treatment and storage periods on the plant development (shoot and root length) from both soybean cultivars: BRS 360 RR and BRS 284



Source: Prepared by the author (2020).

It was also found that the treatments 2, 5 and 6 stunted root development in cultivar BRS 360 RR, compared to the control (Table 3). These results contradict Vanin et al. (2011), who found greater root mass development after applying an imidacloprid - thiodicarb mixture in the sorghum seed treatment. A linear decrease was stated for the effect of storage periods (Figure 2).

For BRS 284, the interaction between factors in the first evaluation demonstrated that treatments 5 and 6 increased shoot length, compared to the control, but after 60 days, no difference was detected (Table 4). The data showed a decline in this variable during the storage periods (Figure 2B). In relation to the root development, the seed treatment with the active ingredients imidacloprid + thiodicarb + carbendazin + thiram was harmful (Table 4). Also, a negative linear response to increasing storage periods was found (Figure 2C and D).

Based on the results, the cultivars responded differently to the chemical treatments and storage due to their genetic characteristics, so the selection of seed lots with high physiological quality is essential (germination and vigor), due to the effect of the storage period, which may impair the seed quality when associated to the anticipated treatment. When choosing products for the seed treatment, those containing insecticides in their formulations should be treated with particular caution due to the possible phytotoxic effect.

CONCLUSIONS

The physiological quality of soybean seeds decreased during storage in all treatments (including the control).

The treatment with the insecticides imidacloprid + thiodicarb associated with the fungicides carbendazin + thiram markedly reduces the physiological seed quality of the tested soybean cultivars.

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Qualidade fisiológica de sementes de soja submetidas ao tratamento químico e épocas de armazenamento

RESUMO

O tratamento antecipado de sementes, utilizando novas combinações de produtos químicos vem sendo adotado crescentemente, contudo, seus efeitos sobre a qualidade das sementes durante o armazenamento ainda não estão totalmente esclarecidos. O objetivo do estudo foi avaliar ao longo do período de armazenamento a qualidade fisiológica de sementes de duas cultivares de soja, tratadas com diferentes combinações de produtos químicos. O delineamento experimental foi inteiramente casualizado, com quatro repetições, em esquema fatorial 7x5, sendo, sete tratamentos químicos de sementes e cinco épocas de armazenamento (0, 60, 120, 180 e 240 dias). Os tratamentos foram: T1) fipronil + piraclostrobina + tiofanato metílico; T2) imidacloprido + tiodicarbe + carbendazin + thiram; T3) abamectina + tiametoxan + fludioxonil + mefenoxam + thiabendazole; T4) carbendazin + thiram; T5) fludioxonil + mefenoxam + thiabendazole; T6) carboxin + thiram e T7) testemunha (sem tratamento). A qualidade fisiológica das sementes foi avaliada pelos seguintes testes: germinação, envelhecimento acelerado, emergência de plântulas em areia e comprimento de parte aérea e raiz de plântulas. Com base nos resultados observado na cultivar BRS 360 RR, todos os tratamentos avaliados, com exceção do T6, mantiveram a germinação das sementes acima de 80%, por até 120 dias. A qualidade fisiológica das sementes de soja diminui ao longo do armazenamento, em todos os tratamentos (incluindo a testemunha). O tratamento com os inseticidas imidacloprido + tiodicarbe associados aos fungicidas carbendazin + thiram reduz acentuadamente a qualidade fisiológica de sementes das cultivares de soja testadas.

PALAVRAS-CHAVE: Fungicidas. Germinação. *Glycine max* (L.) Merrill. Inseticidas. Vigor.

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