

Alternative substrates for the germination test with treated soybean seeds

Substratos alternativos para o teste de germinação com sementes de soja tratadas

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ABSTRACT

The present study had as the aim to analyze the performance of soybean seeds treated different products on the germination test using the substrates described by the “Rules for seed analysis” and alternative substrates. Two steps were carried out, in the first the products (fungicides and insecticides) were used individually and in the second in combinations (fungicide + insecticide, with or without polymer). Seeds were treated and sown on paper rolls, sand, vermiculite between paper rolls and sand between paper rolls. The variables evaluated were: first count, abnormal seedlings and germination. In the first step, imidacloprid+thiodicarb presented as the most problematic treatment in the paper substrate, with improved results in sand and in the alternative substrates proposed. It is possible to observe the low influence of the polymer in the results obtained in the second step. Lower results were observed for seeds treated with fludioxonil+metalaxyl-M+thiabendazole + imidacloprid+thiodicarb. Lower percentages of normal seedlings in the first count and germination, and higher percentage of abnormal seedlings, were observed in the paper substrate when the products were used individually. Sand was superior to the paper substrate, and vermiculite between paper stands out as a good alternative.

Keywords: *Glycine max*, seed quality, seed treatment, Rules for Seed Analysis (RAS)

RESUMO

O presente estudo teve como objetivo avaliar o desempenho de sementes de soja tratadas com diferentes produtos no teste de germinação utilizando os substratos descritos pelas Regras para Análise de Sementes e substratos alternativos. Realizaram-se duas etapas, na primeira os produtos (fungicidas e inseticidas) foram utilizados individualmente e na segunda em combinações (fungicida+inseticida, com ou sem polímero). As sementes foram tratadas e semeadas nos substratos rolo de papel, areia, vermiculita entre papel e areia entre papel. As variáveis avaliadas foram: primeira contagem, plântulas anormais e germinação. Numa primeira etapa, imidacloprido+tiodicarbe apresentou-se como o tratamento mais problemático no substrato papel, com melhores resultados na areia e nos substratos alternativos propostos. É possível constatar baixa influência do polímero nos resultados obtidos na segunda etapa. Resultados inferiores foram observados em fludioxonil+metalaxil-M+tiabendazol + imidacloprido+tiodicarbe quando a mistura de produtos foi utilizada para o tratamento das sementes. Menores porcentagens de plântulas normais em primeira contagem e germinação final, e maior porcentagem de plântulas anormais, foram encontradas no substrato papel quando utilizados produtos isoladamente. A areia foi superior ao substrato papel, e a vermiculita entre papel aparece como uma boa alternativa.

Palavras-chave: *Glycine max*, qualidade de sementes, tratamento de sementes, Regras para Análise de Sementes (RAS)

1 INTRODUCTION

On the global agribusiness, Brazil is presenting a significant participation in the supply and demand of products of the soybean agroindustry complex, which performs a fundamental role in the development of several regions of the Country (Lazzarotto & Hirakuri, 2009).

To maintain the different technologies invested in the sector and to reach farmers satisfactorily, achieving high indices of production and yield, some parameters for seed commercialization were established over the years, such as the Seed Analysis Bulletin. In Brazil, the rules that govern the methodology of the tests required in this bulletin are met in the “Rules for Seed Analysis” (RAS), where, for the germination test on soybean seeds, paper rolls and sowing between sand are recommended as the substrate, the temperatures of 20-30°C (Alternated), 25°C or 30°C, performing counting at the five and eight days (Brasil, 2009), wherein any combination of these temperatures and substrates may be used.

Seeds are considered as the main input in the productive process and seed quality is indispensable to the implementation of a crop (Dan et al., 2011). Furthermore, seed quality is not only linked to physiological and genetic conditions, which involve metabolic and productive aspects, respectively, but also physical attributes, that are related to the conditions of the entire production process, and sanitary, which is the guarantee of the propagation of healthy plants and free of pathogens (Peske & Barros, 2006).

Considering the last attribute of seed quality, the preventive use of insecticides and fungicides on seed treatment is being widely adopted, due to the increase of defense conditions of the seeds and the future plant, thus enabling a greater growth potential and initial stand of the crop in the field (Dan et al., 2010a). Seed treatment is used on more than 90% of Brazilian soybean seeds (Nunes, 2016), and the Industrial Seed Treatment (IST), when performed at the Seed Processing Unit (SBU), is already a step of seed processing, which is gaining ground in the soybean seed market, wherein about 40% of the seeds are treated using this method (Abati et al., 2013).

Given the above, it is clear that most soybean seeds marketed nationally are based in the emission of the Seed Analysis Bulletin using the germination test with seeds treated

with fungicides, insecticides, polymers and other products, as well as the mixture of them, and that this test was standardized for seeds without any treatment, in which laboratory results often do not match real field performance. Therefore, the present study had as the aim to analyze the performance of soybean seeds treated with different products available in the market, as well as the association of them, on the germination test using the substrates described by the RAS and alternative substrates.

2 MATERIAL AND METHODS

The experiments were performed on the Seed Analysis Laboratory at the Department of Phytotechny of the “Eliseu Maciel” College of Agronomy – FAEM/UFPeL.

The present work was divided into two stages, in the first the commercial products (fungicides and insecticides, registered for seed treatment) were used isolated, contemplating the following chemical treatments (CT): CT0-seeds without treatment; CT1-fludioxonil+metalaxyl-M+thiabendazole (fungicide); CT2-bifenthrin+imidacloprid (insecticide); CT3-cyantraniliprole (insecticide); CT4-imidacloprid+thiodicarb (insecticide); CT5-thiamethoxam (insecticide); CT6-chlorantraniliprole (insecticide); CT7-fipronil+pyraclostrobin+thiophanate-methyl (fungicide and insecticide).

The treatments were performed using a seed treating machine, model TRATEC LAB (MECMAQ[®], Piracicaba – Brazil), designed for research purposes, with up to 2 kg of capacity. The products were used accordingly to the supplier instructions, always using the maximum limit. The volume used in the two steps was of 13 mL kg⁻¹ of seeds, based on preliminary tests which aimed for satisfactory seed coating, like the observed on the IST, for the quantity of seeds used.

The substrates used were paper rolls and sand, standardized and described by RAS, and vermiculite or sand between paper, used as alternatives to the standardized tests, considering possible occurrences of phytotoxicity in the paper roll and the difficulty of using sand in the laboratory routine. For the germination test in paper rolls, 50 seeds were distributed in each roll, composed of three sheets of *germitest* paper, moistened with 2.5 times the weight of the dry paper using distilled water, the sum of four paper rolls composed a statistical repetition, composed of 200 seeds. For the germination test on sand, plastic trays (7 cm x 21 cm x 29.5 cm) with four liters capacity, filled with 2 Kg of clean sand, with average particle size, moistened with 330 mL of distilled water (according to the water retention test, where an amount of 165 mL kg⁻¹ of sand was

determined). Each tray received 50 seeds, which composed a statistical repetition. To test the alternative substrates vermiculite between paper and sand between paper, the preparation of the paper was carried out using the same procedure used for the standard paper rolls, however, with the difference that the vermiculite or sand were deposited over two paper sheets, distributing 50 mL of moistened average class vermiculite or moistened sand. To moisten the vermiculite, the substrate was placed in a bucket with distilled water for 16 hours, when the excess water was removed. The moisten sand used was prepared using the method described for the plastic trays, adding 165 mL of distilled water kg of sand⁻¹. For both substrates, 50 seeds were sown in each roll, wherein the set of four rolls composed a statistical repetition.

After preparation, rolls and trays were disposed in a germinator of the chamber type, with a water blade for moisture maintenance, in the conditions established by the RAS (25±1°C, with 12 light hours day⁻¹ for eight days).

This first step was performed in a completely randomized design using an 8x4 factorial scheme with four repetitions. The factor A corresponded to the chemical treatments (CT0, CT1, CT2, CT3, CT4, CT5, CT6 and CT7), and the factor B to the substrates (paper, sand, vermiculite between paper and sand between paper). Each repetition was composed of four rolls or one tray (for sand as substrate).

In the second step, the process previously described was repeated, using the products mentioned, however, different combinations were tested according to the classes (fungicide and insecticide), thus forming the following chemical treatments (CT): CT0-seeds without treatment; CT1.2-fludioxonil+metalaxyl-M+thiabendazole + bifenthrin+imidacloprid; CT1.3-fludioxonil+metalaxyl-M+thiabendazole + cyantraniliprole; CT1.4-fludioxonil+metalaxyl-M+Thiabendazole + imidacloprid+thiodicarb; CT1.5-fludioxonil+metalaxyl-M+thiabendazole + thiamethoxam; CT1.6-fludioxonil+metalaxyl-M+thiabendazole + chlorantraniliprole. In addition to the combination of products, the use of polymer was added as a third factor.

Thus, the second step was performed under a completely randomized design in a 6x4x2 factorial scheme with four repetitions. The factor A corresponded to the treatments (CT0, CT1.2, CT1.3, CT1.4, CT1.5 and CT1.6), the factor B to the substrates (paper, sand, vermiculite between paper and sand between paper), and factor C to the use of polymer (with or without polymer). Each repetition was composed of four rolls or one tray (for sand as substrate)

In the two steps, the variables evaluated were: first count, abnormal seedlings and germination. Data obtained were analyzed for normality by the Shapiro Wilk test, for homoscedasticity by the Hartley test, and the independence of the residues by graphical analysis. Data were submitted to the analysis of variance using the F test ($P < 0.05$). When significant differences were verified, the adequate tests were performed.

3 RESULTS AND DISCUSSION

The statistical analysis of the first step indicated the interaction of the factors chemical treatment and substrate for all variables. For the first count (Table 1), the greater percentages of normal seedlings are observed in CT0 for all substrates, with significant variation between the different treatments. Considering the factor substrates, for all treatments, the paper presented the lower percentages, differing from sand in all treatments, but not differing, in some cases, from the vermiculite between paper (CT3 and CT7) and from the sand between paper (CT2, CT3 and CT0).

Table 1: First count (%) for seedlings derived from treated seeds subjected to the germination test according to the Rules for Seed Analysis (RAS) and in alternative substrates. FAEM/UFPEL, Capão do Leão/RS, 2019

Chemical treatment	Substrate							
	Paper		Sand		Vermiculite between paper		Sand between paper	
CT0	86	Ab ^L	94	ABa	93	Aa	91	Aab
CT1	77	BCb	87	Ba	90	ABa	85	BCa
CT2	77	BCc	96	Aa	88	ABb	81	Cc
CT3	86	Ab	98	Aa	90	ABb	89	ABb
CT4	71	Cb	95	Aa	88	Ba	86	ABa
CT5	80	ABCb	91	ABa	90	ABa	89	ABa
CT6	79	ABCc	97	Aa	91	ABb	90	Ab
CT7	85	ABa	96	Aa	90	ABbc	91	Aab

^LAverages followed by the same uppercase letter in the column and lowercase letter in the line, do not differ by the Tukey test ($p \leq 0.05$).

For the variable abnormal seedlings (Table 2), besides the absence of variation in CT0 between substrates, the greater percentages were observed for paper as substrate, which did not differ from sand between paper in the treatments CT1, CT2 and CT3, where variations were verified between treatments, differing from the substrates sand and vermiculite between paper, that did not present significant variation between the treatments used.

Table 2: Abnormal seedlings (%) derived from treated seeds subjected to the germination test according to the Rules for Seed Analysis (RAS) and in alternative substrates. FAEM/UFPEL, Capão do Leão/RS, 2019

Chemical treatment	Substrate							
	Paper		Sand		Vermiculite between paper		Sand between paper	
CT0	8	Ba ^{1/}	10	Aa	4	Aa	8	ABa
CT1	14	ABa	3	Ac	6	Abc	9	ABab
CT2	13	ABa	2	Ab	4	Ab	12	Aa
CT3	10	Ba	2	Ab	4	Ab	8	ABa
CT4	20	Aa	1	Ac	6	Abc	10	ABb
CT5	14	ABa	1	Ac	5	Abc	8	ABb
CT6	14	ABa	3	Ab	5	Ab	6	Bb
CT7	10	Ba	2	Ab	5	Ab	5	Bb

^{1/}Averages followed by the same uppercase letter in the column and lowercase letter in the line, do not differ by the Tukey test ($p \leq 0.05$).

Table 3 presents the results of the variable germination, the variation between treatments used is consistent, except for sand as substrate, where there were no significant differences between treatments. Furthermore, there was no significant difference for the factor substrate in treatments CT5 and CT0, while for other treatments, lower germination percentages were observed for paper as substrate, which did not differ from sand between paper in treatments CT1, CT2 and CT3.

Table 3: Seedling germination (%) of treated seeds subjected to the germination test according to the Rules for Seed Analysis (RAS) and in alternative substrates. FAEM/UFPEL, Capão do Leão/RS, 2019

Chemical treatment	Substrate							
	Paper		Sand		Vermiculite between paper		Sand between paper	
CT0	92	Aa ^{1/}	89	Aa	97	Aa	92	Aa
CT1	86	ABb	96	Aa	94	ABa	91	ABab
CT2	87	ABc	98	Aa	93	Bb	86	Bc
CT3	90	Ab	98	Aa	96	Aa	92	Ab
CT4	80	Bc	98	Aa	94	ABab	90	ABb
CT5	86	ABa	90	Aa	95	ABa	92	Aa
CT6	86	ABb	97	Aa	95	ABa	93	Aa
CT7	90	Ab	98	Aa	95	ABa	95	Aa

^{1/}Averages followed by the same uppercase letter in the column and lowercase letter in the line, do not differ by the Tukey test ($p \leq 0.05$).

Beyond the obvious inferiority of the paper substrate and superiority of sand as substrate, which is comparable to the alternative substrates tested, especially vermiculite between paper, CT4 (imidacloprid+thiodicarb), was the treatment whose difference for the untreated seeds in the paper substrate was more intense, with significant improvement in the results using sand, in the proposed alternative substrates. Some studies have already reported low percentages of germination and greater percentages of abnormal seedlings on tests of physiological quality in paper rolls using soybean seeds treated with this insecticide (Dan et al., 2012; Dan et al., 2010b), as well as in different levels of quality

of soybean seeds, with or without addition of fungicide (TUNES et al., 2020), while when the germination test is performed using sand, the treatment did not differ from the control (Benitez, 2014).

It is worth mentioning, that seeds after being treated with chemicals become unfit for human or animal consumption and, when inadequate for seed commercialization due to low germination percentages, must be discarded on appropriate locations, in order to prevent any type of contamination (Bobek & Kiihl, 2016).

On the second step, the statistical analysis revealed a triple interaction for the factors in the variable first count, and double interactions for the variables abnormal seedlings (except for polymer x substrate) and germination. In the first count (Table 4), there was significant variation between treatments for all substrates, with or without the use of polymer. Generally, the paper substrate presented lower percentages of normal seedlings (except in CT1.5 with or without polymer), not differing from sand (CT1.2, CT1.5 and CT1.6), vermiculite between paper (CT1.2, CT1.3, CT1.4, CT1.5 and CT1.6), and sand between paper (CT0, CT1.5 and CT1.6) when the polymer was not used, and from sand (CT1.3 and CT1.5), vermiculite between paper (CT1.2, CT1.3, CT1.5 and CT1.6) and sand between paper (CT0, CT1.2, CT1.3, CT1.5 and CT1.6) when the polymer was used. The use of the polymer was significant for CT1.2 using sand as substrate, for CT1.5 with vermiculite between paper as substrate and for CT1.2 and CT1.5 with sand between paper as substrate, with the increase of the percentage of normal seedlings in the first count using sand and sand between paper (CT1.5).

Table 4: First count (%) for seedlings of treated seeds, with or without polymer, subjected to the germination test according to the Rules for Seed Analysis (RAS) and in alternative substrates. FAEM/UFPel, Capão do Leão/RS, 2019

Chemical treatment	Substrate							
	Paper		Sand		Vermiculite between paper		Sand between paper	
	Without polymer							
CT0	88	ABb ^{LNS}	97	Aa ^{NS}	94	Aa ^{NS}	92	Aab ^{NS}
CT1.2	84	Bb ^{NS}	89	ABab*	84	Bb ^{NS}	92	Aa*
CT1.3	86	ABb ^{NS}	95	ABa ^{NS}	92	Aab ^{NS}	94	Aa ^{NS}
CT1.4	71	Cb ^{NS}	87	Ba ^{NS}	81	Bab ^{NS}	86	Ba ^{NS}
CT1.5	93	Aab ^{NS}	91	ABb ^{NS}	98	Aa*	93	Aab*
CT1.6	91	ABa ^{NS}	96	ABa ^{NS}	94	Aa ^{NS}	91	Aa ^{NS}
	With polymer							
CT0	88	Ab	96	ABa	94	Aa	92	Aab
CT1.2	83	Bb	98	Aa	86	Bb	86	Bb
CT1.3	88	Aa	88	Ba	93	Aa	94	Aa
CT1.4	77	Cb	90	ABa	85	Ba	88	Ba
CT1.5	92	Aa	92	ABa	91	Aa	95	Aa
CT1.6	90	Ab	96	ABa	93	Aab	93	Aab

^LAverages followed by the same uppercase letter in the column and lowercase letter in the line, do not differ by the Tukey test ($p \leq 0.05$). * and ^{NS}, significant and not significant, respectively, by the t test ($p \leq 0.05$) comparing the use of polymer.

For the variable abnormal seedlings (Table 5), in the interaction treatment x substrate, on all treatments, except for CT1.2, where the vermiculite between paper, sand between paper and paper rolls did not differ, and for CT1.6, where the substrates did not differ, the paper presented the greater number of abnormal seedlings and the treatments varied statistically for all substrates. In the interaction treatment x polymer, CT1.4 presented the greater percentage of abnormal seedlings, without or with polymer, not differing from CT1.2 in the last condition. There was no statistical difference between seeds treated with or without polymer for any of the treatments evaluated.

Table 5: Abnormal seedlings (%) derived from treated seeds, with or without polymer, subjected to the germination test according to the Rules for Seed Analysis (RAS) and in alternative substrates. FAEM/UFPEL, Capão do Leão/RS, 2019

Chemical treatment	Substrate							
	Paper		Sand		Vermiculite between paper		Sand between paper	
CT0	6	BCa ^U	2	ABb	2	Cb	2	Bb
CT1.2	8	Ba	1	Bb	7	Ba	6	Aa
CT1.3	7	BCa	3	ABb	2	Cb	2	Bb
CT1.4	16	Aa	5	Ac	10	Ab	7	Abc
CT1.5	5	Ca	2	ABb	2	Cb	2	Bb
CT1.6	6	BCa	2	ABa	3	Ca	2	Ba
	Polymer							
	Without				With			
CT0	3		B ^{NS}		3		C	
CT1.2	5		B ^{NS}		6		AB	
CT1.3	4		B ^{NS}		4		BC	
CT1.4	10		A ^{NS}		8		A	
CT1.5	3		B ^{NS}		3		C	
CT1.6	3		B ^{NS}		4		BC	

^UAverages followed by the same uppercase letter in the column and lowercase letter in the line, do not differ by the Tukey test ($p \leq 0.05$). ^{NS} not significant by the t test ($p \leq 0.05$) comparing the use of polymer.

The variable germination (Table 6), in the interaction treatment x substrate presented considerable variation between treatments on all substrates, except for sand, which did not differ, maintaining lower percentages in the paper substrate, that did not differ from sand in CT1.5, and from vermiculite between paper in CT1.2 and CT1.6. In the interaction treatment x polymer, there were no differences due to the use of polymer, however, between treatments, CT1.4 presented lower percentages and CT1.6 the greater, with or without the polymer. For the interaction substrate x polymer, the paper substrate presented lower percentages of germination with or without polymer, differing from the others, and there was no effect of the polymer according to the t test.

Table 6: Seedling germination (%) of treated seeds, with or without polymer, subjected to the germination test according to the Rules for Seed Analysis (RAS) and in alternative substrates. FAEM/UFPeI, Capão do Leão/RS, 2019

Chemical treatment	Substrate							
	Paper		Sand		Vermiculite between paper		Sand between paper	
CT0	92	ABb ^L	97	Aa	98	Aa	97	Aa
CT1.2	89	Bc	97	Aa	90	Bbc	93	Bab
CT1.3	92	ABb	96	Aa	97	Aa	98	Aa
CT1.4	80	Cb	93	Aa	89	Ba	92	Ba
CT1.5	94	Ab	96	Aab	98	Aa	98	Aa
CT1.6	94	Ab	98	Aa	96	Aab	98	Aa
	Polymer							
	Without				With			
CT0	96		AB ^{NS}		96		AB	
CT1.2	92		B ^{NS}		93		BC	
CT1.3	95		AB ^{NS}		96		AB	
CT1.4	86		C ^{NS}		91		C	
CT1.5	96		A ^{NS}		96		AB	
CT1.6	97		A ^{NS}		97		A	
	Substrate							
Polymer	Paper		Sand		Vermiculite between paper		Sand between paper	
Sem	87	b ^{NS}	95	a ^{NS}	96	a ^{NS}	94	a ^{NS}
Com	92	b	97	a	96	a	95	a

^LAverages followed by the same uppercase letter in the column and lowercase letter in the line, do not differ by the Tukey test ($p \leq 0.05$). ^{NS} not significant by the t test ($p \leq 0.05$) comparing the use of polymer.

In this step, where the mixture of fungicides with insecticides, with or without polymer, was performed, the use of the polymer caused little influence in the results obtained, a fact which corroborates the results obtained by Bays et al. (2007), according to these authors, the values of germination and first count presented little variation for soybean seeds with or without polymer. Fagundes (2016), working with different polymers, also observed that there were no significant differences between rice seed treatments. Generally, the polymers used for seed treatment are biodegradable molecules, derived from materials found in nature (Roy et al., 2014), which are aimed to improve the distribution and adherence of the active ingredient and to protect fungicides and insecticides on the seed surface (Kunkur et al., 2007). Furthermore, some authors even consider the association and compatibility of polymers with fungicides beneficial, resulting in higher germination percentages in soybean seeds (Verma & Verma, 2014).

For the treatments, inferior results were observed for CT1.4, which combines the fungicide fludioxonil+metalaxyl-M+thiabendazole and the insecticide imidacloprid+thiodicarb, differing from CT1.5 (fludioxonil+metalaxyl-M+thiabendazole + thiamethoxam) and CT1.6 (fludioxonil+metalaxyl-M+thiabendazole + chlorantraniliprole), which presented similar results to the control (without treatment).

According to some authors, the technologies used for seed coating allow the use of mixtures (fungicides and insecticides) in the same formulation, aiming a wider protection against insects and diseases (Juliatti, 2010; Menten, 2010). Studies using the mixture of fludioxonil+metalaxyl-M+thiabendazole + thiamethoxam did not present significant differences in the germination percentage and in seedling emergence in sand beds, compared to the control treatment (Rocha et al., 2017). Furthermore, according to Horii & Shetty (2007), which evaluated insecticides, the thiamethoxam can affect the pentose phosphate pathway, stimulating reserve hydrolysis and increasing energy availability for germination and seedling emergence. Other authors also reported the stimulating effect of thiamethoxam on the physiological performance of black oat seeds (Almeida et al., 2012) and the better expression of rice seed germination and vigor during storage (Borges et al., 2014).

4 CONCLUSION

The paper substrate has the lowest percentages of normal seedlings at first count and final germination and the highest percentage of abnormal seedlings when used alone.

Between the recommended substrates, sand is considered the best, and between alternative substrates, vermiculite between paper can be recommended.

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