

Soybean seed (*Glycine max* (L.) Merr) physiological performance treated with phytochemical products in several germination substrates

Desempenho fisiológico de sementes de soja (*Glycine max* (L.) Merr) tratadas com produtos fitoquímicos em vários substratos de germinação

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<https://doi.org/10.19084/rca.19755>

Received/recebido: 2020.03.25

Accepted/aceite: 2020.07.07

ABSTRACT

Since there are no standardized standards for carrying out laboratory tests with chemically treated soybean seeds, the objective was to evaluate the physiological performance of soybean seeds treated by phytochemicals on the standard substrates (paper roll and between sand) indicated by the Rules for Seed Analysis and on substrates classified as alternative (vermiculite between paper and sand between paper). For that, seeds of different soybean cultivars were used treated with fungicides and/or insecticides (Maxim Advanced®, CropStar®, Rocks® and Cruiser® 350 FS), sown on standard substrates and on alternative substrates. The study was carried out in a completely randomized design in a bifactorial scheme, with four replications. The variables first count, abnormal seedlings and germination were evaluated. The cultivars 'Elite IPRO' and '2606 IPRO' showed interaction of factors with lower results regarding the physiological quality of seeds treated with imidacloprid + thiodicarb, while '7161 RR', also with significant interaction, little varied between treatments. '6410 IPRO', '7062 IPRO' and '15630 IPRO' showed evidence of a negative relationship in the presence of the active ingredient imidacloprid with a positive emphasis on the use of the substrate sand instead of paper. The cultivar '7209 IPRO' was more stable, without significant interaction, but with better results for untreated seeds and treated with thiamethoxam, as well as for the substrate sand. It is possible to observe divergences between cultivars and negative effects of products containing imidacloprid, mainly in the paper substrate.

Keywords: *Glycine max*, chemical treatment, Rules for Seed Analysis.

RESUMO

Não havendo normas padronizadas para realização de testes laboratoriais com sementes de soja tratadas quimicamente, objetivou-se avaliar o desempenho fisiológico de sementes de soja tratadas por produtos fitoquímicos nos substratos padrão (rolo de papel e entre areia) indicados pelas Regras para Análise de Sementes e em substratos classificados como alternativos (vermiculita entre papel e areia entre papel). Para isso, foram utilizadas sementes de diferentes cultivares de soja tratadas com fungicidas e/ou inseticidas (Maxim Advanced®, CropStar®, Rocks® e Cruiser® 350 FS), semeadas nos substratos padrões e em substratos alternativos. O estudo foi realizado em delineamento inteiramente casualizado em esquema bifatorial, com quatro repetições. Foram avaliadas as variáveis primeira contagem, plântulas anormais e germinação. As cultivares 'Elite IPRO' e '2606 IPRO' apresentaram interação dos fatores com resultados inferiores quanto à qualidade fisiológica das sementes tratadas com imidacloprido+tiodicarbe, enquanto '7161 RR', também com interação significativa, pouco variou entre tratamentos. '6410 IPRO', '7062 IPRO' e '15630 IPRO' apresentaram indícios de relação negativa na presença do ingrediente ativo imidacloprido com destaque positivo para o uso do substrato areia em detrimento do papel. A cultivar '7209 IPRO' apresentou-se mais estável, sem interação significativa, mas com melhores resultados para sementes não tratadas e tratadas com tiametoxam, assim como para o substrato areia. É possível constatar divergências entre as cultivares e efeitos negativos de produtos contendo imidacloprido, principalmente no substrato papel.

Palavras-chave: *Glycine max*, tratamento químico, Regras para Análise de Sementes.

INTRODUCTION

Soy (*Glycine max* (L.) Merrill) in the world is considered the most important oilseed in terms of international production and commercialization, being the main product of Brazilian agriculture, where it had its first report in 1882, in the State of Bahia, however, the center of origin and domestication is northeast Asia, more precisely in China and surrounding areas (Chung and Singh, 2008). In the 1970s, soybean production in Brazil increased from 1.5 million tons to more than 15 million tons, while the cultivated area grew from 1.3 to 8.8 million hectares only in the Southern Region (EMBRAPA, 2004). In 2010, production harvested reached 68.7 million tons and cultivated area was 23.6 million hectares while estimates were pointing to more than 105 million tons in 2020, this estimate was already reached in the 2018/2019 growing season, when 114.3 million tons were produced (CONAB, 2019).

The remarkable increase in production during the years and across the country was only possible due to plant breeding, since, due to the several environmental variations in which soybean is commonly subjected in Brazil, the interaction among genotype and environment is expected to assume a fundamental role in phenotype expression and, therefore, understanding the genetic diversity and the relationship between improved cultivars is of major importance for soybean breeding (Bertini *et al.*, 2006).

Due to the great ecosystem diversity and types of soil and climates (latitude and altitude), MAPA (Ministry of agriculture, livestock and supply) approved a model of regionalized VCU (Value for Cultivation and Use) tests and for soybean cultivar recommendation in Brazil, which establishes five soybean macro regions and 20 distinct edaphoclimatic regions for research and cultivar recommendation (Carneiro *et al.*, 2014).

Between the several studies performed considering this aspect, some were intended, for example, to investigate the physiological quality of soybean cultivars from different maturity groups (Carvalho *et al.*, 2017), to evaluate the effect of sowing date in the agronomic performance of soybean cultivars

in determined regions, indicating those which are more stable and adapted to each date (Meotti *et al.*, 2012), to evaluate physiological quality and the lignin content of soybean seeds of distinct cultivars subjected to different harvest times (Gris *et al.*, 2010), to verify the contribution of branches and the evolution of the leaf area index in modern soybean cultivars (Zanon *et al.*, 2015), and even to determine the quality of soybean seeds of different cultivars produced under the foliar application of nutrients (Carvalho *et al.*, 2014) and to identify the role of plant arrangement and soybean cultivar type in the resulting interference with competing plants (Bianchi *et al.*, 2010).

Despite the constant search for better performances and greater yields, some factors still limit crop yield, as diseases and pests, a scenario where some technologies are being increasingly used, such as the seed treatment (Freitas, 2011), with treated seeds corresponding to more than 95% of soybean seeds currently used in Brazil, which are treated with fungicides and insecticides, either industrially or on farm (Nunes, 2016).

Therefore, seed chemical treatment, due to the widespread use, combined with the expressive variability of cultivars used in the Country, and considering that some studies have demonstrated that seed laboratory tests are not representing field conditions, the search for alternative substrates is necessary when using treated seeds, considering the existing cultivars. Thus, the present study aimed to evaluate the physiological performance of soybean seeds treated with the phytochemical products Maxim Advanced®, CropStar®, Rocks® e Cruiser® 350 FS, registered for the crop, in the standard substrates roll of paper and between sand, recommended by the Rules for Seed Analysis (RAS) and in alternative substrates vermiculite between paper and sand between paper, using seven soybean cultivars.

MATERIAL AND METHODS

The experiments were performed at the Laboratory of Seed Analysis at the Department of Phytotechnology of the College of Agronomy “Eliseu Maciel” – FAEM, Federal University of Pelotas – UFPel.

Different cultivars available in the market were used, with similar physiological quality, belonging to distinct breeders, such as 'Elite IPRO' (Brasmax), '7062 IPRO' (TMG), '6410 IPRO' (Monsoy), '2606 IPRO' (Bayer), '7161 RR' (TMG), '15630 IPRO' (Syngenta) and '7209 IPRO' (Nidera), thus, seeking greater variability between genotypes.

For seed treatment the mixture of the fungicides fludioxonil+metalaxyl-M+thiabendazole (Maxim Advanced®) and the insecticides imidacloprid+thiodicarb (CropStar®), bifenthrin+imidacloprid (Rocks®) and thiamethoxam (Cruiser® 350 FS) at the doses of 125, 700, 700 and 300 mL 100 kg⁻¹ of seeds, respectively, associated or not, composed the following phytochemical treatment (PT): PT0 – without treatment; PT1 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of imidacloprid+thiodicarb; PT2 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of bifenthrin+imidacloprid; PT3 – 700 mL of imidacloprid+thiodicarb; PT4 – 700 mL of bifenthrin+imidacloprid and PT5 – 300 mL of thiamethoxam per 100 kg⁻¹ of seeds.

The treatments were performed using a seed treating machine, model TRATEC LAB (MECMAQ®, Piracicaba – Brazil) designed for research purposes and with up to 2 kg of capacity, and following the manufacturer's recommendations and using the maximum dose recommended. The spraying volume applied was 13 mL kg⁻¹, aiming for a greater approximation to the industrial treatment, with a satisfactory coverage of seeds.

The tested standard substrates to the germination test were paper rolls and trays filled with sand (standard method by RAS). The alternative substrates were vermiculite between paper and sand between paper. The germination test in paper rolls, vermiculite between paper and sand between paper were performed in a comparable manner, only differing regarding the use of vermiculite and sand among paper sheets, directly in contact with the seeds. Therefore, for each roll, three sheets of *germitest* paper moistened with distilled water at the ratio of 2.5 times the weight of the dry paper were used. To make the rolls with vermiculite or sand, a volume of 50 mL of medium size vermiculite (for vermiculite between paper) or medium size sand (for sand between paper) was

disposed on two paper sheets. The vermiculite was previously moistened in a bucket containing distilled water for approximately 16 hours, removing the excess water for usage. Sand was moistened according to the water retention test, where an amount of 165 mL of water per kg of sand was determined, weighting enough quantity for usage. After all substrates were prepared, 50 seeds were disposed into each roll, where four rolls composed one experimental unit.

For the germination test on sand, trays of approximately four liters (H7 cm x W21 cm x L29.5 cm) were used. Trays were filled with 2 kg of clean sand, with average particle size from 0.05 to 0.8 mm, which was moistened with 330 mL of distilled water (165 mL kg⁻¹ of sand), sowing 50 seeds per tray, where four trays composed one experimental unit.

All paper rolls and trays were kept into germination chambers containing a water blade for moisture maintenance, for eight days (until the final counting), at 25°C±1°C, on a regime of 12 hours of light.

The experiments were performed under a completely randomized design in a factorial 6x4 scheme with four repetitions. The factor A corresponded to the five phytochemical treatments (PT) used (PT0, PT1, PT2, PT3, PT4 and PT5), factor B to the four substrates (paper, sand, vermiculite between paper and sand between paper). Each experimental unit was composed of four rolls or four trays (for the sand substrate).

The variables evaluated were: first count (normal seedlings at five days after sowing), abnormal seedlings (damaged, deformed and/or deteriorated seedlings at the eight days after sowing) and normal seedlings (sum of normal seedlings at the five and eight days after sowing) Data were analyzed for normality by the Shapiro Wilk test; for homoscedasticity using the Hartley test; and for independence of the residues through graphical analysis. Data were subjected to the analysis of variance through the F test ($p < 0.05$). If a statistical significance was observed for a variable, the effect of the chemical treatments and substrates were compared by the Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Of the seven cultivars studied, significant interactions were observed for all variables in three of them ('Elite IPRO', '2606 IPRO' and '7161 RR'), only for one cultivar ('6410 IPRO') this interaction was repeated for the variables first count and abnormal seedlings, not occurring for germination variable, where its factors were significant in isolation. The same happened to the other cultivars ('7062 IPRO', '15630 IPRO' and '7209 IPRO'), which presented significance of their factors in isolation for all variables, except for the germination variable of one of these ('7209 IPRO'), where only the substrate factor was significant.

That said, first dealing with 'Elite IPRO', it is noted that in the paper substrate, there were differences of 16 pp (percentage points) between treatments TQ0 and PT1 in the variables first count and normal seedlings and 15 pp between treatments PT2 and PT3 for abnormal seedlings variables (Table 1).

For '2660 IPRO', PT1 was the treatment which differed the most from untreated seeds (PT0) for the first count in the paper substrate, with 11pp less seedlings, with less expressive differences for the other substrates, while PT3 did not present the same behavior, also presenting low percentages for sand between paper and vermiculite between paper, with similar results extended to the other treatments, except for PT5 and PT0, in the variable abnormal seedlings where, overall, only the sand substrate presented an improvement, with 14pp less abnormalities in PT3, for example, when compared to paper and 9pp if compared to others substrates (Table 2).

Some studies have demonstrated inferior results for the insecticide imidacloprid+thiodicarb associated with fungicides for the seed treatment of different cultivars, with a decrease in the averages for first count of seedlings, germination and accelerated aging when compared to other products, while presenting only slight and tolerable differences for seedling emergence in raised beds (Camilo *et al.*, 2017), which corroborates with the results observed in this study where even harmful treatments in other substrates, especially on paper, did not demonstrate any evidence of reduced seed viability in sand.

Table 1 - First count (%), abnormal seedlings (%) and normal seedlings (%) from seeds of the cultivar 'Elite IPRO' phytochemically treated and subjected to the germination test using the standard methods of the Rules for Seed Analysis (RAS) and alternative substrates

Phyto-chemical Treatment (PT)	First count (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	86 Ab ^{1/2}	92 ABab	94 Aa	91 Aab
PT1	70 Bb	85 Ba	81 Ca	87 ABa
PT2	84 Aa	90 ABa	85 BCa	91 Aa
PT3	72 Bb	96 Aa	89 ABa	87 ABa
PT4	77 ABc	97 Aa	88 ABb	81 Bc
PT5	81 ABb	91 ABa	91 ABa	89 Aa

Phyto-chemical Treatment (PT)	Abnormal seedlings (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	8 Ba	14 Aa	3 Ba	8 Aa
PT1	16 ABa	7 Ab	12 Aab	8 Ab
PT2	7 Ba	5 Aa	5 Ba	6 Aa
PT3	22 Aa	0 Ac	6 Bbc	10 Ab
PT4	15 ABa	2 Ac	7 Bbc	13 Aab
PT5	15 ABa	1 Ac	5 Bbc	8 Aab

Phyto-chemical Treatment (PT)	Normal seedlings (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	92 Aa	86 Aa	97 Aa	92 ABa
PT1	76 Bb	91 Aa	86 Dab	92 ABa
PT2	89 Aa	95 Aa	91 Ca	94 Aa
PT3	79 Bb	99 Aa	94 ABCa	90 ABa
PT4	86 ABb	98 Aa	93 BCa	85 Bb
PT5	86 ABb	95 Aa	96 ABa	92 ABab

^{1/2}Averages followed by the same uppercase letter in the column (comparing treatments in each substrate) and averages followed by the same lowercase letter in the line (comparing substrates in each phytochemical treatment), do not differ between each other by the Tukey test (p<0.05). PT0 - without treatment; PT1 - 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of imidacloprid+thiodicarb; PT2 - 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of bifenthrin+imidacloprid; PT3 - 700 mL of imidacloprid+thiodicarb; PT4 - 700 mL of bifenthrin+imidacloprid and PT5 - 300 mL of thiamethoxam per 100 kg of seeds.

For the cultivar '7161 RR', very subtle differences were observed between chemical treatments and substrates in all variables, demonstrating that depending of the genotype there are no severe damages due to the chemical treatment used, regardless of the substrate (Table 3). Camilo *et al.* (2017), which evaluated the physiological quality of two chemically treated soybean cultivars during storage, observed that the cultivars differentially responded to seed coating with the distinct products tested.

For the cultivar '6410 IPRO' there was interaction between factors for the variables first count and abnormal seedlings, where the sand substrate presented the best results, slightly differing from the other substrates, especially for treatments PT5 and PT0, and except for PT1 and PT3 which presented the smaller percentages in the first count in paper while, also for paper, PT1, PT3 and PT4 presented the greater percentages of abnormal seedlings (Table 4). The treatments PT1, PT3 and PT4 contained the active ingredient imidacloprid in their

composition while PT5, which presented comparable results to the control in this study, is composed by thiamethoxam. Accordingly, Dan *et al.* (2010) reported that seed treatment with insecticides based on imidacloprid significantly reduced germination during storage, suggesting that seed treatment with insecticides should be performed near sowing. Still for this cultivar, there was no interaction between factors for the variable normal seedlings, whereas the main effect of the factors was significant. However, there were no differences between

Table 2 - First count (%), abnormal seedlings (%) and normal seedlings (%) from seeds of the cultivar '2606 IPRO' phytochemically treated and subjected to the germination test using the standard methods of the Rules for Seed Analysis (RAS) and alternative substrates

Phyto-chemical Treatment (PT)	First count (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	91 Ab ^{1/}	99 Aa	91 Ab	91 Ab
PT1	70 Db	88 Ba	83 ABCa	81 Ba
PT2	78 BCDb	94 ABa	85 ABab	85 ABab
PT3	73 CDb	94 ABa	75 Cb	78 Bb
PT4	79 BCb	90 Ba	80 BCb	83 ABb
PT5	85 Aba	95 ABa	86 ABa	91 Aa

Phyto-chemical Treatment (PT)	Abnormal seedlings (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	5 Ba	1 Aa	3 Ba	2 Ca
PT1	16 Aa	3 Ac	8 ABbc	11 Aab
PT2	13 Aa	4 Ab	11 Aab	7 ABab
PT3	17 Aa	3 Ab	12 Aa	12 Aa
PT4	15 Aa	6 Ab	13 Aa	9 Ab
PT5	7 Ba	1 Aa	7 ABa	3 BCa

Phyto-chemical Treatment (PT)	Normal seedlings (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	95 Aa	99 Aa	97 Aa	97 Aa
PT1	84 Cc	95 Aa	92 ABab	87 Bbc
PT2	87 BCb	96 Aa	89 ABab	93 ABab
PT3	83 Cb	96 Aa	88 Bb	87 Bb
PT4	85 Cb	94 Aa	86 Bb	90 Bab
PT5	93 Aba	97 Aa	93 ABa	97 Aa

^{1/} Averages followed by the same uppercase letter in the column (comparing treatments in each substrate) and averages followed by the same lowercase letter in the line (comparing substrates in each phytochemical treatment), do not differ between each other by the Tukey test (p<0.05). PT0 – without treatment; PT1 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of imidacloprid+thiodicarb; PT2 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of bifenthrin+imidacloprid; PT3 – 700 mL of imidacloprid+thiodicarb; PT4 – 700 mL of bifenthrin+imidacloprid and PT5 – 300 mL of thiamethoxam per 100 kg of seeds.

Table 3 - First count (%), abnormal seedlings (%) and normal seedlings germination (%) from seeds of the cultivar '7161 RR' phytochemically treated and subjected to the germination test using the standard methods of the Rules for Seed Analysis (RAS) and alternative substrates

Phyto-chemical Treatment (PT)	First count (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	95 Ab ^{1/}	100 Aa	99 Aab	98 Aab
PT1	89 Aab	99 ABa	90 Cab	87 Bb
PT2	93 Aa	95 Ba	91 BCa	92 ABa
PT3	92 Ab	100 Aa	90 Cb	92 ABb
PT4	94 Aa	97 ABa	93 BCa	94 Aa
PT5	96 Aab	99 ABa	97 ABab	95 Ab

Phyto-chemical Treatment (PT)	Abnormal seedlings (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	3 Aa	0 Ab	1 Bab	1 ABab
PT1	4 Aa	0 Aa	5 Aa	4 Aa
PT2	2 Aa	2 Aa	2 ABa	0 Ba
PT3	3 Aa	0 Ab	5 Aa	4 Aa
PT4	1 Aab	0 Ab	3 ABa	1 ABab
PT5	3 Aa	0 Ab	1 Bab	2 ABab

Phyto-chemical Treatment (PT)	Normal seedlings (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	96 Ab	100 Aa	99 Aa	98 Aab
PT1	96 Aa	100 Aa	95 ABa	95 Aa
PT2	97 Aa	95 Ba	95 ABa	95 Aa
PT3	97 Ab	100 Aa	94 Bc	95 Abc
PT4	99 Aa	97 ABa	95 ABa	97 Aa
PT5	97 Aa	99 ABa	97 ABa	97 Aa

^{1/} Averages followed by the same uppercase letter in the column (comparing treatments in each substrate) and averages followed by the same lowercase letter in the line (comparing substrates in each phytochemical treatment), do not differ between each other by the Tukey test (p<0.05). PT0 – without treatment; PT1 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of imidacloprid+thiodicarb; PT2 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of bifenthrin+imidacloprid; PT3 – 700 mL of imidacloprid+thiodicarb; PT4 – 700 mL of bifenthrin+imidacloprid and PT5 – 300 mL of thiamethoxam per 100 kg of seeds.

chemical treatments, which all presented averages above 90%. High percentages were also observed for all the substrates, where there was statistical significance, with the best result for sand substrate, with 7pp more germinated seedlings compared to paper (Table 4). Some researchers consider that the application of fungicides and/or insecticides may cause a phytotoxic effect on seeds causing, for example, reduced germination (Ludwig *et al.*, 2011), which may be directly related to the substrate used. In this study, some products applied

Table 4 - First count (%), abnormal seedlings (%) and normal seedlings (%) from seeds of the cultivar '6410 IPRO' phytochemically treated and subjected to the germination test using the standard methods of the Rules for Seed Analysis (RAS) and alternative substrates

Phyto-chemical Treatment (PT)	First count (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	92 Aab ¹	95 Aa	93 Aab	89 ABb
PT1	79 BCb	95 Aa	82 Bb	83 ABb
PT2	87 ABb	96 Aa	86 ABb	87 ABb
PT3	77 Cb	93 Aa	84 Bab	81 Bab
PT4	83 ABCb	96 Aa	83 Bb	85 ABb
PT5	91 Ab	95 Aa	92 Aab	91 Ab

Phyto-chemical Treatment (PT)	Abnormal seedlings (%)			
	Substrate			
	Paper	Sand	Vermiculite between paper	Sand between paper
PT0	7 Ba	3 Ab	3 Bb	4 Ab
PT1	12 Aba	2 Ab	7 ABab	7 Aab
PT2	9 Aba	0 Ab	6 ABa	5 Aa
PT3	15 Aa	4 Ab	6 ABb	7 Aab
PT4	13 Aba	1 Ac	9 Aab	5 Abc
PT5	6 Ba	3 Aa	3 Ba	3 Aa

Phytochemical Treatment (PT)	Normal seedlings (%)
PT0	96 A
PT1	93 A
PT2	95 A
PT3	91 A
PT4	93 A
PT5	96 A

Substrate			
Paper	Sand	Vermiculite between paper	Sand between paper
90 c	97 A	94 b	95 ab

¹/Averages followed by the same uppercase letter in the column (comparing phytochemical treatments) and averages followed by the same lowercase letter in the line (comparing substrates), do not differ between each other by the Tukey test (p<0.05). PT0 – without treatment; PT1 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of imidacloprid+thiodicarb; PT2 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of bifenthrin+imidacloprid; PT3 – 700 mL of imidacloprid+thiodicarb; PT4 – 700 mL of bifenthrin+imidacloprid and PT5 – 300 mL of thiamethoxam per 100 kg of seeds.

had better results when sand was used instead of paper, in which the concentration of ingredients in contact with the seedlings possibly increases.

For cultivars '7062 IPRO' and '15630 IPRO', the main effect of the factors was significant, with small variations between chemical treatments when '7062 IPRO' was used, where PT0 (control) presented the best results, differing from PT1, PT3 and PT4 for first count, from PT3 for abnormal seedlings and from PT2, PT3 and PT4 for normal seedlings (Table 5) and, as the previous cultivar, presented evidence of a negative relationship with the active ingredient imidacloprid, present in the chemical treatments highlighted, partially agreeing with the studies that indicated the association of imidacloprid+thiodicarb as harmful to soybean germination and vigor, but considered that the use of imidacloprid isolated was adequate to maintain physiological quality (Dan *et al.*, 2012), which is not supported by the results observed for most cultivars here studied. For substrates, in all variables, sand can be highlighted with the best performance, however, not differing from vermiculite between paper and sand between paper regarding abnormal seedlings and normal seedlings (Table 5).

Table 5 - First count (%), abnormal seedlings (%) and normal seedlings (%) from seeds of the cultivar '7062 IPRO' phytochemically treated and subjected to the germination test using the standard methods from the Rules for Seed Analysis (RAS) and alternative substrates

Phytochemical Treatment (PT)	First count (%)	Abnormal seedlings (%)	Normal seedlings (%)
PT0	94 A ¹	3 B	97 A
PT1	85 B	5 AB	94 AB
PT2	89 AB	4 B	93 B
PT3	85 B	8 A	90 C
PT4	88 B	5 AB	92 BC
PT5	94 A	4 B	95 AB

Substrate			
	First count (%)	Abnormal seedlings (%)	Normal seedlings (%)
Paper	86 b	6 a	93 b
Sand	94 a	3 b	95 a
Vermiculite between paper	88 b	5 ab	94 ab
Sand between paper	89 b	5 ab	94 ab

¹/Averages followed by the same uppercase letter in the column (comparing phytochemical treatments) and averages followed by the same lowercase letter in the column (comparing substrates), do not differ between each other by the Tukey test (p<0.05). PT0 – without treatment; PT1 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of imidacloprid+thiodicarb; PT2 – 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of bifenthrin+imidacloprid; PT3 – 700 mL of imidacloprid+thiodicarb; PT4 – 700 mL of bifenthrin+imidacloprid and PT5 – 300 mL of thiamethoxam per 100 kg of seeds.

On the other hand, the cultivar '15630 IPRO' presented greater variability of results, with low values for the first count and normal seedlings, as well as greater values for abnormalities in PT1 and PT2, differing from the other chemical treatments, except for PT1, which did not differ statistically from PT3 and PT4 for the variables abnormal seedlings and normal seedlings (Table 6). Comparing substrates, paper and vermiculite between paper did not differ between each other and presented the greater values of abnormal seedlings and lower values of normal seedlings (Table 6).

Table 6 - First count (%), abnormal seedlings (%) and normal seedlings (%) from seeds of the cultivar '15630 IPRO' phytochemically treated and subjected to the germination test using the standard methods of the Rules for Seed Analysis (RAS) and alternative substrates

Phytochemical Treatment (PT)	First count (%)	Abnormal seedlings (%)	Normal seedlings (%)
PT0	94 A ^{1/}	3 D	96 A
PT1	77 D	14 AB	85 CD
PT2	73 D	15 A	83 D
PT3	84 BC	9 BC	89 BC
PT4	83 C	9 BC	89 BC
PT5	89 B	6 CD	92 AB
Substrate			
Paper	81 a	12 a	86 b
Sand	85 a	6 b	91 a
Vermiculite between paper	82 a	12 a	88 ab
Sand between paper	86 a	7 ab	91 a

^{1/} Averages followed by the same uppercase letter in the column (comparing phytochemical treatments) and averages followed by the same lowercase letter in the column (comparing substrates), do not differ between each other by the Tukey test ($p < 0.05$). PT0 - without treatment; PT1 - 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of imidacloprid+thiodicarb; PT2 - 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of bifenthrin+imidacloprid; PT3 - 700 mL of imidacloprid+thiodicarb; PT4 - 700 mL of bifenthrin+imidacloprid and PT5 - 300 mL of thiamethoxam per 100 kg of seeds.

Lastly, cultivar '7209 IPRO', presented significance for the main effect of the factors for first count and abnormal seedlings by the F test. However, only PT1 and PT5 differed by the Tukey test for first count and no difference was observed between chemical treatments for abnormal seedlings. For the substrates, paper differed from the others for all variables evaluated, including the variable normal seedlings, where this was the only factor which varied (Table 7).

Table 7 - First count (%), abnormal seedlings (%) and normal seedlings (%) from seeds of the cultivar '7209 IPRO' phytochemically treated and subjected to the germination test using the standard methods of the Rules for Seed Analysis (RAS) and alternative substrates

Phytochemical Treatment (PT)	First count (%)	Abnormal seedlings (%)
PT0	96 AB ^{1/}	2 A
PT1	92 B	4 A
PT2	94 AB	3 A
PT3	94 AB	2 A
PT4	95 AB	3 A
PT5	98 A	2 A
Substrate		
Paper	91 b	5 a
Sand	97 a	1 b
Vermiculite between paper	94 a	2 b
Sand between paper	96 a	2 b
Normal seedlings (%)		
Paper	94 b	
Sand	98 a	
Vermiculite between paper	97 a	
Sand between paper	97 a	

^{1/} Averages followed by the same uppercase letter in the column (comparing phytochemical treatments) and averages followed by the same lowercase letter in the column (comparing substrates), do not differ between each other by the Tukey test ($p < 0.05$). PT0 - without treatment; PT1 - 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of imidacloprid+thiodicarb; PT2 - 125 mL of fludioxonil+metalaxyl-M+thiabendazole + 700 mL of bifenthrin+imidacloprid; PT3 - 700 mL of imidacloprid+thiodicarb; PT4 - 700 mL of bifenthrin+imidacloprid and PT5 - 300 mL of thiamethoxam per 100 kg of seeds.

CONCLUSIONS

The standard substrates indicated by RAS presented non-concordant results in the germination test, with better performances when sand was used for most of the phytochemical products in practically all the seven soybean cultivars tested.

Products containing the active ingredient imidacloprid revealed to induce damages in the initial development of seedlings for most of the studied cultivars, varying according to the substrate used.

The soybean cultivars tested showed divergent responses to the studied variables, regarding the effect of phytochemical treatment on the different substrates used.

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