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Quality of sweet corn seeds treated before and after storage

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Abstract
This work aims to evaluate the effect of industrial treatment with different chemicals and also seed sizes on the physiological behavior of sweet corn in addition to evaluating the content of active ingredients of insecticides before and three months after storage. Four lots of a single sweet corn cross hybrid (L1, L2, L3 and L4) and different sieves (S1, S2 and S3) were carried out in two experiments. Experiment 1: industrial seed treatment, T1 = untreated seeds (control), T2 = Maxim Advanced®, T3 = Cruiser® 350 FS and T4 = Poncho®. Experiment 2: quantification of the content of active ingredients, commercial products Cruiser® 350 FS and Poncho and the distribution efficiency was measured by HPLC-UV. The results showed that industrial seed treatment with Maxim Advanced®, Cruiser® 350 FS and Poncho® can be carried out three months before sowing without damaging sweet corn seeds. HPLC is efficient to quantify the content of active ingredients in sweet corn seeds treated with Cruiser® 350 FS and Poncho®. The industrial seed treatment was calibrated for each batch, which is justified by the different amounts of active ingredient between batches and seed sizes.
Keywords: *Zea mays* L.; high performance liquid chromatography; chemical residues.

Qualidade de sementes de milho doce tratadas antes e após armazenamento

Resumo
Este trabalho tem por objetivo avaliar efeito do tratamento industrial com diferentes produtos químicos e também tamanhos de sementes sobre o comportamento fisiológico do milho doce além de avaliar o teor de ingredientes ativos dos inseticidas antes e três meses após o armazenamento. Quatro lotes de um único híbrido cruzado de milho doce (L1, L2, L3 e L4) e diferentes peneiras (S1, S2 e S3) foram realizados dois experimentos. Experimento 1: tratamento industrial de sementes, T1 = sementes não tratadas (controle), T2 = Maxim Advanced®, T3 = Cruiser® 350 FS e T4 = Poncho®. Experimento 2: quantificação do teor de princípios ativos, produtos comerciais Cruiser® 350 FS e Poncho e a eficiência de distribuição foi medida por HPLC-UV. Os resultados mostraram que o tratamento de sementes industriais com Maxim Advanced®, Cruiser® 350 FS e Poncho® pode ser realizado três meses antes da semeadura sem danificar as sementes de milho doce. A HPLC é eficiente para quantificar o conteúdo de ingredientes ativos em sementes de milho doce tratadas com Cruiser® 350 FS e Poncho®. O tratamento industrial de sementes foi calibrado para cada lote, o que se justifica pelas diferentes quantidades de ingrediente ativo entre os lotes e os tamanhos das sementes.
Palavras-chave: *Zea mays* L.; cromatografia líquida de alta eficiência; resíduos químicos.

Introduction

Sweet corn is a specialty corn type that presents one or more recessive genes which alter sugar concentration, leading to high sugar and low starch contents (TEIXEIRA *et al*., 2017), thin pericarp and soft texture which increase acceptance for human consumption (CLEMENTE, 2017). Nevertheless, these characteristics make the maintenance of physiological quality challenging throughout storage due to rapid loss of viability and damage by insects and fungi which drastically reduce seed quality (ARAÚJO *et al*., 2016).

According to Kwiatkowski and Clemente (2017), the postharvest management is an important factor for maintaining sweetcorn seed quality due to the high perishability caused by a high metabolic activity. Therefore, adequate temperature, moisture and treatment are crucial for seed quality maintenance during sweet corn storage. Seed treatments often achieve better results for pest and disease control and reduce the number of chemical products applied after germination (SUTTON, 2017).

A practical concern of seed treatment is that the dosage of active ingredient can vary according to the seed lot, which can cause negative effects for industrial seed treatment as insufficient dosage and consequent inefficiency of control or, alternatively, an excessive dosage and phytotoxicity. Additionally, sweet corn seeds present quite irregular shape, therefore, it is necessary to check treated seed lots to guarantee high-quality seeds containing the appropriate dose of the chosen ingredient.

The High-Performance Liquid Chromatography (HPLC) is a method widely used in the pharmaceutical industry due to its high precision and technical reliability, being employed in the agricultural sector as a tool for separation and analysis, identification, and quantification of active compounds, including chemical ingredients applied on seeds (Hayes *et al*., 2015), which are present in low amounts in the mixture. Chen *et al*. (2015) observed that different HPLC methods such as HPLC-UV, HPLC-MS and HPLC-ECD presented good reproducibility, precision, accuracy, and recovery, and could be used for quantitative analysis of active components in *Fructuspsoraleae* seeds. Additionally, Mauldin *et al*. (2016) were able to quantify chlorpyrifos residues on black oil sunflower seeds using HPLC-UV. Thus, chromatography has a substantial importance for industrial seed treatment by enabling a precise quantification of residues present on seeds.

Therefore, this work aimed to evaluate the effect of industrial treatment with different pesticides and seed sizes on the physiological performance of sweet corn seeds, as well as on the content of active ingredients of insecticides before and three months after storage.

Material and Methods

The experiments were performed using four sweet corn seed lots (L1, L2, L3 and L4)of a single-cross hybrid provided by Monsanto do Brasil LTDA.L1 seeds were classified into three sieve sizes: S1, S2 and S3; L2 and L3 into sieve S1 and L4 into sieve S2. S1seeds consisted of seeds which passed through oblong hole sieves 13/64" x ¾" and were retained in round hole sieves of 21/64" x ¾". Seeds S2 and S3 correspond to seeds which passed through oblong hole sieves 12,5 and 12/64" x ¾" and were retained in round hole sieves 19 and 16/64" x ¾", respectively. The physical and physiological profile of the seed lots is presented in Table 1.

Table 1. Initial physical and physiological profile of sweetcorn lots used for seed treatment

Treatment	Lot/Sieve					
	L1/S1	L1/S2	L1/S3	L2/S1	L3/S1	L4/S2
1000-seeds weight (g)	187.75	168.81	147.55	173.50	171.07	153.95
Germination (%)	88	91	95	78	73	91

The work was divided into two experiments:

Experiment 1: evaluation of seed physiological quality after treatment with pesticides and three months after storage. The application was performed by industrial seed treatment using a batch modular coater, BMC Manual (*Bayer Seed Growth™*). The cycle used was of 20 seconds per batch for each 1 kg sample.

Four treatments were applied to the seeds: T1: untreated seed (control); T2: Cruiser® 350 FS – (Insecticide - Syngenta); T3: Poncho® - (Insecticide - Bayer Crop Science) and T4: Maxim Advanced® (Fungicide - Syngenta). The active ingredients, doses and concentration of each active ingredient are described in Table 2. Additionally, each seed treatment received the polymer Disco AG L322, in a ratio of 500 mL 100 kg of seeds^{-1}.

Table 2. Chemical products used to perform sweetcorn seed treatment.

ID	Active Ingredient	Product Name	Use	Dose[1]	Concentration[2]
1	Control	-	-	-	-
2	Metalaxyl-M + Fludioxonil + Thiabendazole	Maxim Advanced®	Fungicide	100**	20 + 25 + 150
3	Thiamethoxam	Cruiser® 350FS	Insecticide	120*	350
4	Clothianidin	Poncho®	Insecticide	70*	600

[1]Dose of commercial product: *mL 60,000 seeds^{-1} and **mL 100 kg of seeds^{-1}.
[2]Concentration of the active ingredient (g L^{-1}). The doses used for Cruiser® 350 Sand Poncho® correspond to 0.7 mg of active ingredient seed^{-1}.

After treatment seeds were evaluated for water content according to Brasil (2009) to check the necessity of drying (Table 3), which was not needed for any of the treatments since the differences were below the maximum acceptable amplitude which is from 1 to 2% (MARCOS FILHO, 2015a). Furthermore, seed water content was lower than 13% in all treatments, which is suitable for the preservation of corn seeds according to several studies (CAMARGO; CARVALHO, 2018).

Table 3. Water content (%) of sweetcorn seed lots submitted to different treatments.

Treatment	Lot/Sieve					
	L1/S1	L1/S2	L1/S3	L2/S1	L3/S1	L4/S2
	%					
Control	12.56	12.39	12.57	12.50	12.25	12.37
Maxim Advanced®	12.54	12.55	12.65	12.35	12.68	12.29
Cruiser® 350 FS	12.53	12.40	12.74	12.52	12.42	12.12
Poncho®	12.54	12.35	12.77	12.42	12.50	12.26
Maximum: 12.77% Minimum: 12.12% Average: 12.47%						

Seed samples of 1 kg from each treatment were placed into multilayer paper bags, similar of the used by the industry for storage and commercialization. A portion of each seed lot treated was stored in laboratory for three months at room temperature and relative humidity. The average temperature and relative humidity during this period were 11.5 °C and 65%, respectively.

Seeds were evaluated for germination and seedling emergence. The germination test was performed according to Brasil (2009). Seedling emergence was performed in raised beds, with a mixture of sand and clay soil, 2:1 ratio. Four repetitions of 50 seeds for each treatment were sown at 2.5 to 3.0 cm depth, spaced 2 cm from each other. The evaluation was performed 14 days after sowing. Germination and seedling emergence evaluations were also performed three months after storage.

Experiment 2: the amount of active ingredient in treated seeds was evaluated using High Performance Liquid Chromatography (HPLC). The sweet corn seed lots and sieves were the previously described and three treatments: untreated seed (control); Cruiser® 350 FS and Poncho®.

Samples of 30 seeds by each treatment were taken randomly and used to determine the amount of active ingredient, which was extracted using 25 mL of methanol. Two analytical replicates were used for each experimental unit. Each tube containing the mixture (seeds + methanol) was placed on an orbital shaker at 350 rpm. After two hours, the supernatant was filtered in a specific filter with 0.45 µm and transferred to vials which were, posteriorly, placed in the chromatographer, model *Alliance* (Waters Corporation, Milford MA, USA).The chromatographer was equipped with a detector at the wavelength of 270 nm and a C-18 4.6 x 250mm column. The mobile phase consisted of acetonitrile and ultrapure water using a 25:75 ratio, flow of 1.2 mL min^{-1} and injection volume 5 µL.

The quantification of each active ingredient was performed using the software *Empower™ 3* (Waters Corporation, Milford MA, USA) that assisted the comparison of peak areas against the calibration curve, obtained using analytical standards whose purities were above 99%. Afterwards, the average and standard deviation of the two analytical replicates was calculated. Results are presented in mg active ingredient seed.

Data obtained in the experiments were tested for normality and subjected to analysis of variance, means were compared by Tukey test at the 5% probability level. All statistical analyses were performed using SISVAR (FERREIRA, 2018).

Results and Discussion

There were no significant differences in the percentage of germination of sweet corn seeds between treatments, regardless of the seed lot and the tested sieves, as can be seen in table 4. According to Normative Instruction Nº. 45 of September 17, 2013 (BRAZIL, 2015), the minimum acceptable germination for commercialization of sweet corn seeds is 70%. Therefore, these results demonstrate the absence of harmful effects of chemical treatments on this variable.

Table 4. Germination (%) of sweetcorn seed lots submitted to different treatments.

Treatment	Lot/Sieve					
	L1/S1	L1/S2	L1/S3	L2/S1	L3/S1	L4/S2
	%					
Control	88 a*	91 a	95 a	78 a	73 a	91 a
Maxim Advanced®	87 a	90 a	93 a	72 a	71 a	88 a
Cruiser® 350 FS	86 a	90 a	94 a	73 a	76 a	85 a
Poncho®	87 a	92 a	91 a	70 a	70 a	85 a
Mean	87	91	93	73	71	87
CV (%)	4.82	3.16	2.81	9.54	7.02	5.21

*Means followed by the same letter, in the column, do not differ statistically between each other at the 5% probability level by Tukey test.

Seed vigor leads to improved seedling emergence or greater stability and there are various techniques for vigor assessment as electrical conductivity, tetrazolium and tests that evaluate seedling growth (MARCOS FILHO, 2015b), such as the seedling emergence test. The best emergence results for the seed lot L1/S1 were observed in the control and seeds treated Maxim Advanced® and Cruiser® 350 FS, while for L1/S2, seeds treated with Maxim Advanced® shown better results than the others (Table 5). For L2/S1, the best treatment for sweet corn seeds was Poncho®, followed by Cruiser® 350 FS and there was no difference between the control and Maxim Advanced®. For the lots L1/S1, L1/S2 and L1/S3 the mean emergence was above 90% and for the lots L2/S1, L3/S1 and L4/S2 the mean remained between 69 and 84%.

Table 5. Seedling emergence (%) of sweet corn seed lots submitted to different treatments

Treatment	Lot/Sieve					
	L1/C1	L1/C2	L1/C3	L2/C1	L3/C1	L4/C2
	%					
Control	92 a	95 b	95 a	69 c	69 a	84 a
Maxim Advanced®	92 a	99 a	95 a	67 c	72 a	86 a
Cruiser® 350 FS	91 a	91 c	98 a	70 b	72 a	85 a
Poncho®	88 b	92 c	98 a	74 a	65 a	84 a
Mean	90	94	96	70	69	84
CV (%)	2.19	1.16	2.42	1.95	3.39	1.04

*Means followed by the same letter, in the column, do not differ statistically between each other at the 5% probability level by Tukey test.

The active ingredient of Cruiser® 350 FS, has demonstrated positive effects on the physiological performance of carrot, rice, pumpkin, and pearl millet (LEMES *et al*., 2015; CUNHA *et al*., 2016). However, Grisi *et al*. (2019) did not observe differences in germination and vigor, when the active ingredient was used in sunflower seed treatment.

Sweet corn seeds did not show differences in germination after three months of storage, regardless of the lot and sieve tested (Table 6). The lots L2 and L3 presented the worst values of germination, 73% in average, but still above the minimum required standard by MAPA, which is 70% (BRASIL, 2015).

Table 6. Germination (%) of sweetcorn seed lots submitted to different treatments three months after storage

Treatment	Lot/Sieve					
	L1/S1	L1/S2	L1/S3	L2/S1	L3/S1	L4/S2
			%			
Control	89 a	95 a	93 a	74 a	77 a	88 a
Maxim Advanced®	88 a	95 a	94 a	75 a	75 a	89 a
Cruiser® 350 FS	89 a	94 a	94 a	71 a	73 a	91 a
Poncho®	91 a	95 a	95 a	72 a	72 a	87 a
Mean	89	95	94	73	73	89
CV (%)	4.99	2.48	2.83	7.66	7.17	4.13

*Means followed by the same letter, in the column, do not differ statistically between each other at the 5% probability level by Tukey test.

As observed by Magalhães *et al*. (2016), Maxim Advanced® was not phytotoxic to corn seeds stored for up to 360 days under environmental conditions while Avicta Completo® or Cruiser® 350 FS + Maxim Advanced® were phytotoxic 45 days after storage, depending on the hybrid. Furthermore, according to Vazquez *et al*. (2015), seed treatment with Maxim XL (Metalaxil-M+Fludioxonil) does not affect the quality of maize seeds, even when the seeds are evaluated after 35 days of storage. In soybean, Pereira *et al*. (2019) observed that seed treatment with different fungicides, including Fludioxonil, did not interfere with the germination and emergence of seedlings in trays, which corroborates the results obtained in this study, where the quality of sweet corn seed treated with fungicide was maintained. during the storage period (Table 6 and 7).

In contrast, Baldiga Tonin *et al*. (2015) pointed out that quality maintenance in corn seeds treated with insecticides depends on the hybrid, storage conditions and insecticide used, furthermore, the authors observed intensified decrease of germination and vigor in seeds treated with thiamethoxam, the active ingredient contained in Cruiser® 350 FS, due to storage period extension. Additionally, Dan *et al*. (2016) observed seed damage during storage in soybean seeds treated with insecticides and indicated seed treatment close to sowing.

Seedling emergence three months after storage only showed differences between treatments for L1/S1 where seeds treated with Cruiser® 350 FS and Poncho® presented the smaller percentages of emerged seedlings (Table 7). Rosa *et al*. (2015) when evaluated the storability of hybrid corn seeds treated with thiamethoxam, observed that along storage, seed vigor is negatively affected. Similar results were obtained by Dan *et al*. (2016), that verified the decrease of physiological seed quality, caused by insecticides containing the active ingredients thiamethoxam, fipronil, imidacloprid, imidacloprid + thiodicarb, carbofuran and ace hate, which was intensified with the elongation of the storage period of treated seeds. On the other hand, corn seeds with high initial physiological quality, when treated with insecticides, fungicides, and polymers, were stored for six months without damage to the quality (PEREIRA *et al*., 2015).

Table 7. Seedling emergence (%) of sweetcorn seed lots submitted to different treatments three months after storage.

Product	Lot/Sieve					
	L1/S1	L1/S2	L1/S3	L2/S1	L3/S1	L4/S2
	%					
Control	88 a	91 a	94 a	67 a	69 a	81 a
Maxim Advanced®	85 a	88 a	93 a	63 a	60 a	88 a
Cruiser® 350 FS	83 b	94 a	91 a	66 a	69 a	85 a
Poncho®	84 b	87 a	91 a	67 a	64 a	85 a
Mean	85	90	92	65	65	85
CV (%)	2.03	2.63	3.02	4.89	9.9	3.65

*Means followed by the same letter, in the column, do not differ statistically between each other at the 5% probability level by Tukey test.

In this study, in general, there was no damage caused by the different treatments to seed quality. However, it is perceivable when checking the averages of each lot, that larger seeds, obtained in the sieve S1, presented lower percentage of emergence, being this result observed in germination after treatment and three months after storage, which differs from other studies available in the literature that, usually, associate lower germination and/or vigor with small seeds lots (GOLEZANi *et al*., 2015).

The HPLC is a technique of better performance regarding resolution, quantification, and detection in a shorter period of analysis compared to classical liquid chromatography (HAYES *et al*., 2015). In this study, a tolerance limit of ±10% for the content of active ingredient on seeds was considered for the approval of the seed lots. This value was stablished accordingly to values currently used in the seed industry, however, established tolerance limits are not available in the literature. Considering the recommended dose and the concentration of the active ingredient in Cruiser® 350 FS, which is presented in Table 2, each seed requires 0.7±0.07 mg of the active ingredient for approval. Similarly, the recommended dose and concentration for Poncho® treatment is equivalent to the application of 0.7±0.07 mg of the active ingredient seed^{-1}.

Maxim Advanced® was not evaluated since the recommended dose is given by the seed weight and, therefore, will vary according the 1000-seeds weight for each seed lot. Furthermore, this fungicide is composed by three different active ingredients and is used in relatively small amounts (Table 2), wherein considering 60,000 seeds as weighting 10 kg the recommend dose used would be of approximately 10 mL 60,000 seeds^{-1}. Additionally, Maxim Advanced® was not phytotoxic to sweetcorn seeds up to three months of storage (Tables 6 and 7).

The efficiency of distribution measured by HPLC showed high and low quantities of the active ingredients on seeds. According to results found for Cruiser® 350 FS treatment, seed lots L1/S1 and L1/S3 would be disapproved, due to excess or insufficient quantity of the active ingredient for effective seed protection (Table 8). For Poncho® treatment, seed lots L2/S1, L3/S1 and L4/S2 were found with insufficient dosages of the active ingredient as well.

Vieira and Gusmão (2016) reported phytotoxic effects in seeds of *Genipaamericana* L. caused by high doses of fungicides and greater germination uniformity of the same seed lot when treated with low doses. Binsfeld *et al*. (2015), in soybean seeds, observed that Cruiser® 350 FS presents negative effects on seed germination and seedling development for doses above the recommendation.

The efficiency of distribution of the active ingredients three months after storage on sweetcorn seed lots is presented in Table 8. For L1/S1 seeds treated with Poncho® the recommended amount of the product was maintained. Nevertheless, Cruiser® 350 Treatments phytotoxic for the lot L1/S1 and inferior to the recommendation for lots L1/S2 and L1/S3. In corn, Fessel *et al*. (2018) observed that some chemical treatments, with the increase of the dose, generate latent effects unfavorable to seed performance which are intensified with the elongation of storage period.

Table 8. Content of active ingredients (mg seed^{-1}) on sweet corn seed lots submitted to different treatments before and three months after storage

Treatment	Lot/Sieve											
	L1/S1		L1/S2		L1/S3		L2/S1		L3/S1		L4/S2	
	0	3	0	3	0	3	0	3	0	3	0	3
Control	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c
Cruiser® 350 FS	0.85 a	0.81 a	0.66 b	0.61 a	0.44 b	0.41 b	0.72 a	0.66 a	0.74 a	0.74 a	0.71 a	0.68 a
Poncho®	0.75 b	0.69 b	0.67 a	0.58 b	0.66 a	0.56 a	0.60 b	0.56 b	0.43 b	0.40 b	0.57 b	0.58 b
CV (%)	1.79	0.63	1.16	0.80	1.89	2.41	1.78	2.83	1.99	2.31	1.31	3.17

*Means followed by the same letter, in the column, do not differ statistically between each other at the 5% probability level by Tukey test.

The results obtained in this study demonstrate the importance of calibrating industrial seed treatment for each seed lot, due to variations in seed size and weight, which may lead to an under or overdose of the active ingredient, thus, preventing effective seed protection. Furthermore, it is important to mention that the results obtained in this study may have suffered from the variation resultant of the simulated treatment by the batch treating machine which may not be as effective as the industrial seed treatment at the seed processing unit. Usually, the application of seed treatment uses a batch treating machine equipped with a rotary disk atomizer similar the one used in this experiment.

Conclusions

It was possible to observe that the seed treatment can be carried out with the products Maxim Advanced®, Cruiser® 350 FS and Poncho® three months before sowing without harming the physiological performance of sweet corn seeds.

The HPLC technique is efficient in the quantification of active ingredients in sweet corn seeds treated with the insecticides Cruiser® 350 FS and Poncho®.

The industrial seed treatment must be calibrated for each batch of seeds justified by the different amounts of active ingredient between batches of larger and smaller sieves.

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