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INFLUENCE OF ROTATIONAL SPEED ON INSECTICIDE APPLICATION TO CORN SEEDS: QUANTIFICATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

INFLUÊNCIA DA VELOCIDADE DE ROTAÇÃO NA APLICAÇÃO DE INSETICIDA EM SEMENTES DE MILHO: QUANTIFICAÇÃO POR CROMATOGRAFIA LÍQUIDA DE ALTA EFICIÊNCIA (CLAE)

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ABSTRACT

Industrial maize seed treatment is an important strategy for protecting plants during the early stages of development, and its effectiveness depends on the adequate distribution and retention of applied chemical products. This study evaluated the influence of different atomizer disc rotational speeds on thiamethoxam recovery in maize seeds using high-performance liquid chromatography (HPLC). The experiment was conducted in a completely randomized design with four replicates, considering different rotational speed conditions of the industrial seed treatment equipment. Insecticide quantification was performed after solid-phase extraction using C18 cartridges and chromatographic analysis with a diode-array detector. The industry-standard treatment condition presented a mean recovery of 97.4%, remaining within the acceptable range (80–120%). However, the alternative rotational speeds resulted in substantially lower numerical recovery values, ranging from 13.1% to 18.2%. The highest recovery among the alternative conditions was observed at 179 rpm. The results demonstrate that changes in atomizer disc rotational speed may compromise the retention of the active ingredient on seeds, highlighting the importance of appropriate equipment adjustment during industrial seed treatment. It is concluded that the industry-standard operating condition provided the highest thiamethoxam recovery among the evaluated conditions.

Keywords: *Zea mays*; thiamethoxam; seed treatment; atomizer disc; high-performance liquid chromatography

RESUMO

O tratamento industrial de sementes de milho constitui uma estratégia importante para a proteção das plantas durante os estádios iniciais de desenvolvimento, sendo sua eficiência

dependente da adequada distribuição e retenção dos produtos químicos aplicados. Este estudo avaliou a influência de diferentes velocidades de rotação do disco atomizador sobre a recuperação do ingrediente ativo tiametoxam em sementes de milho, utilizando cromatografia líquida de alta eficiência (CLAE). O experimento foi conduzido em delineamento inteiramente casualizado, com quatro repetições, considerando diferentes condições de rotação do equipamento de tratamento industrial de sementes. A quantificação do inseticida foi realizada após extração em fase sólida, utilizando cartuchos C18 e análise cromatográfica com detector de arranjo de diodos. A condição industrial padrão apresentou recuperação média de 97,4%, permanecendo dentro da faixa considerada aceitável (80–120%). Entretanto, as velocidades alternativas resultaram em recuperações significativamente inferiores em termos numéricos, variando entre 13,1% e 18,2%. A maior recuperação entre as condições alternativas foi observada na rotação de 179 rpm. Os resultados demonstram que a alteração da velocidade de rotação pode comprometer a retenção do ingrediente ativo nas sementes, evidenciando a importância da regulagem adequada dos equipamentos de tratamento industrial. Conclui-se que a condição industrial padrão proporcionou a maior recuperação de tiametoxam entre as condições avaliadas.
Palavras-chave: *Zea mays*; tiametoxam; tratamento de sementes; disco atomizador; cromatografia líquida

1. INTRODUCTION

Thiamethoxam (TMX) is a systemic insecticide belonging to the neonicotinoid class, characterized by its activity against insect pests in various crops. Considered a second-generation neonicotinoid, this active ingredient is widely used in agriculture, including seed

treatment, a practice that protects plants during the early stages of development (Forti *et al.*, 2024; Guo *et al.*, 2024). However, the effectiveness of its use depends not only on its insecticidal properties but also on the precision and uniformity of its application, particularly in industrial seed treatment systems (Godói *et al.*, 2023).

Seed quality is one of the main determinants of crop establishment and performance, influencing seedling emergence, early plant development, and the yield potential of crops. Seed quality encompasses genetic, physical, physiological, and sanitary attributes that determine the suitability of seed lots for sowing and their performance under field conditions (Corbineau *et al.*, 2023; Javed *et al.*, 2022; Marcos Filho, 2015). In this context, seed treatment represents an important strategy for reducing damage caused by pests and phytopathogens during the early stages of crop development. This practice may involve the application of chemical products, biological agents, inoculants, biostimulants, and micronutrients, depending on agronomic requirements and crop characteristics (Medeiros *et al.*, 2026; Menten; Moraes, 2010; Reis *et al.*, 2026).

In maize (*Zea mays* L.), insecticide seed treatment is particularly important because seedlings are vulnerable to pest attacks during crop establishment. Damage occurring during this period can compromise stand uniformity, reduce plant vigor, and impair early crop development (Ding *et al.*, 2018; Moraes *et al.*, 2022). In this context, industrial seed treatment (IST) offers advantages related to operational standardization, control of application parameters, and operational safety compared with seed treatment performed directly on farms (on-farm treatment) (Camerini *et al.*, 2024; Pedrini *et al.*, 2020). However, achieving effective treatment depends on the

interaction among spray solution properties, seed characteristics, and the operating conditions of the equipment used.

Among the operational parameters that may influence treatment quality, the rotational speed of the atomizer disc, seed exposure time, and spray application rate are particularly relevant (Wongsuk *et al.*, 2024). The interaction among these variables affects droplet formation and distribution, product deposition on the seed surface, and coating uniformity. Inadequate operating conditions may promote heterogeneous distribution of the active ingredient, increase variability among seeds, and contribute to losses during processing, transportation, and handling. Consequently, the amount of insecticide initially applied may differ from the amount effectively retained in the treated seed lot, with potential implications for agronomic efficacy and the rational use of agricultural inputs (Freiberg *et al.*, 2017; Paulikienė *et al.*, 2025; Taylor *et al.*, 2021).

Optimizing atomizer disc rotational speed is therefore an important aspect of improving industrial seed treatment. Establishing appropriate operating conditions may enhance product distribution, reduce losses, and improve process reproducibility (Shelest *et al.*, 2023). However, the evaluation of treatment quality should not be restricted to visual inspection of the coating, since the external appearance of seeds does not directly indicate the amount of active ingredient present in the treated seeds. Therefore, chemical quantification is necessary to determine the insecticide content recovered from the seeds and investigate potential differences associated with processing conditions, such as treatment speed (Balram *et al.*, 2025; Medeiros *et al.*, 2023).

High-performance liquid chromatography (HPLC) is an analytical technique applicable to the quantitative determination of compounds in complex matrices, provided that appropriate extraction, separation, detection, and analytical method validation conditions are established (Gonçalves *et al.*, 2013; Walker *et al.*, 2022). In the context of seed treatment, HPLC can be used to determine the concentrations of insecticides and other molecules extracted from samples and compare the results obtained under different operating conditions. When combined with appropriate sample preparation procedures and analytical validation criteria, this technique can provide reliable results for assessing the active ingredient content retained in seeds, contributing to industrial process quality control. It can also help evaluate the effects of rotational speed on treatment outcomes, thereby increasing the reliability and consistency of the product delivered to farmers (European Commission, 2021; Huang *et al.*, 2019; Petrović *et al.*, 2023; Skoog *et al.*, 2014).

Despite technological advances in industrial seed treatment, understanding the influence of atomizer disc rotational speed on the amount of thiamethoxam retained in maize seeds remains relevant to improving processing conditions. Evaluating this relationship through quantitative analytical methods makes it possible to investigate whether changes in rotational speed are associated with differences in the insecticide content recovered from seeds, providing a basis for equipment adjustment and production line standardization. This approach may contribute to application standardization and more efficient use of plant protection products, without assuming that increasing rotational speed necessarily results in greater active ingredient retention.

This study aimed to evaluate the influence of different atomizer disc rotational speeds during industrial maize seed treatment on the thiamethoxam content retained in the seeds, using high-performance liquid chromatography (HPLC) for quantitative analysis.

2. METHODS

2.1. Experimental Location

The experiment was conducted between April and July 2026 at the Seed Processing Unit (SPU) of Sempre Agtech Ltda., located in the municipality of Santa Helena de Goiás, Goiás State, Brazil.

2.2. Hybrid Maize Seeds

Approximately 300 kg of SX3569VGU hybrid maize seeds were used in the experiment. Initially, the seeds were physically standardized using a flat-hole sieve with a thickness of 18 and an aperture size of 18/64 of an inch (approximately 7.14 mm) to standardize seed size and reduce physical variability of the experimental material before insecticide treatment.

2.3. Chemical Formulation And Insecticide Class

The chemical product used in the experiment was Cruiser® 600 FS, a systemic insecticide formulated for seed treatment, containing thiamethoxam as the active ingredient at a concentration of 600.0 g L^{-1}. The product belongs to the neonicotinoid chemical class and is classified in Group 4A according to the Insecticide Resistance Action Committee (IRAC), comprising nicotinic acetylcholine receptor (nAChR) competitive modulators. The formulation type is FS (Flowable Concentrate for Seed Treatment).

During the seed treatment process, in addition to thiamethoxam, the seeds were also treated with the fungicide Acronis® UBS at a rate of 1.0 mL kg^{-1} of seeds. This fungicide contains pyraclostrobin (50 g L^{-1}) and thiophanate-methyl (450 g L^{-1}) as active ingredients. Additionally, the polymer Disc L-450 Incotec® was incorporated at a rate of 2.0 mL kg^{-1} of seeds to assist in coating formation and product adhesion to the seed surface.

The insecticide was applied at a constant rate of 3.5 mL kg^{-1} of seeds across all treatments, ensuring a standardized application rate and allowing the effect of atomizer disc rotational speed on thiamethoxam retention on the seeds to be evaluated.

2.4. Batch Seed Treatment Equipment

The chemical treatment of the seeds was performed using a batch seed treatment (BST) machine, model LS B-18, manufactured by LS do Brazil. The equipment consists of an atomizer disc and a treatment chamber, allowing control of the operating parameters, particularly atomizer disc rotational speed and cycle time. Before the experimental treatments were performed, the equipment was calibrated to establish the quantity of seeds processed per batch, aiming to ensure treatment uniformity and chemical treatment quality.

2.5. Experimental Design

The experimental design adopted was a completely randomized design (CRD), consisting of five treatments defined by different rotational speeds of the BST atomizer disc: 70% (89 rpm), 90% (115 rpm), 130% (166 rpm), 140% (179 rpm), and 170% (196 rpm), with four

replicates per treatment, totaling 20 experimental units. The standard industrial seed treatment procedure is described in (Table 1).

Table 1. Industrial seed treatment with Thiamethoxam – industry standard.

Product Injection Time (s)	Sprayer Speed (Hz)	Bowl Homogenization Speed (rpm)	Bowl Homogenization Time (s)	Bowl Discharge Rotation Speed (rpm)	Bow Discha Time
20	80	100	14	130	5

⚠ Esta tabela possui muitas colunas e foi cortada para impressão. Para visualizá-la completa, acesse o artigo original em: https://revistatopicos.com.br/artigos/influence-of-rotational-speed-on-insecticide-application-to-corn-seeds-quantification-by-high-performance-liquid-chromatography-hplc?noblockage

Source: Authors, 2026.

Each experimental unit consisted of a 5 kg sample of treated corn seeds, corresponding to each atomizer disc rotational speed. The insecticide spray application rate was kept constant across all treatments to isolate the effect of atomizer disc rotational speed on insecticide retention. The product used contained thiamethoxam as the active ingredient at a concentration of 600.0 g L^{-1}.

2.6. Solid-Phase Extraction (SPE) And HPLC-DAD Analysis Of Thiamethoxam In Treated Seeds

2.6.1. Extraction Of Thiamethoxam From Corn Seeds

The extraction of thiamethoxam from corn seeds was performed using homogeneous samples consisting of 10 g of seeds. The seeds were transferred to 250 mL beakers for extraction, and 100 mL of HPLC-grade acetonitrile was added as the extraction solvent. The samples were subjected to mechanical agitation on a magnetic stirring platform (Ecom Brasil, Mod. 316, Brazil) at 180 rpm for 15 min to facilitate the transfer of thiamethoxam from the seed surfaces into the solvent. Following agitation, the resulting extracts were collected and subjected to solid-phase extraction (SPE) using C18 cartridges to remove potential matrix interferences and improve sample cleanliness prior to chromatographic analysis.

2.6.2. Solid-Phase Extraction (SPE)

Solid-phase extraction was performed using C18 cartridges in a 12-position SPE manifold (Phenomenex, USA) connected to a vacuum pump (Tecnal, Mod. TE-058, Brazil). The extraction procedure was optimized using a control sample fortified with thiamethoxam, considering the cartridge type, sample pH, sample volume, and elution solvent volume.

The C18 cartridges were initially conditioned with a methanol–ultrapure water mixture (50:50, *v/v*), followed by 3 mL of ultrapure water adjusted to pH 2.0. The pH of the aqueous sample was adjusted to 2.0 using phosphoric acid. Subsequently, a 100 mL aliquot of the fortified control sample was passed through the cartridge at an approximate flow rate of 5 mL min^{-1} under reduced pressure.

After sample loading, 3 mL of ultrapure water was passed through the cartridge to remove residual matrix components. The cartridge

was then dried under vacuum for 15 min. The retained thiamethoxam was eluted using two successive 1.0 mL aliquots of dichloromethane–methanol (65:35, *v/v*), with a contact time of 1 min after the addition of each aliquot.

The eluates were combined and evaporated to dryness under a nitrogen stream. The residue was reconstituted in 500 µL of the chromatographic mobile phase before injection into the HPLC-DAD system. Based on the initial sample volume of 100 mL and the final reconstitution volume of 0.5 mL, the nominal concentration factor was 200-fold, assuming complete analyte recovery.

2.6.3. Chromatographic Analysis

Thiamethoxam was quantified using a Varian HPLC system (USA) equipped with a ProStar 210 pump and a ProStar 335 diode-array detector. Data acquisition and processing were performed using Star Workstation software (version 6.0). Separation was carried out using a Synergi 4 µm Fusion RP-80 analytical column (250 × 4.6 mm, 4 µm particle size) equipped with a Phenomenex guard column (4 × 3 mm; USA).

The mobile phase consisted of acetonitrile and ultrapure water produced using a Milli-Q purification system (60:40, *v/v*), with the pH adjusted to 3.0 using phosphoric acid. The flow rate was 1.0 mL min^{-1}, the injection volume was 20 µL, and detection was performed at 255 nm.

2.6.4. Analytical Standard And Quantification

A thiamethoxam analytical reference standard (Pestanal®, Sigma-Aldrich, St. Louis, MO, USA; molecular formula $C_8H_{10}ClN_5O_3S$) was used for calibration. A stock solution of 1,000 mg L^{-1} was prepared in acetonitrile and stored at −16 °C until analysis. Working standard solutions were prepared by diluting the stock solution immediately before chromatographic analysis.

Thiamethoxam concentrations were quantified using an analytical calibration curve, which demonstrated excellent linearity (R^2 = 0.997). The results were used to compare insecticide deposition among seeds treated at different atomizer disc rotational speeds and to assess deviations from the target application dose. Results were expressed as milligrams of thiamethoxam per seed (mg $seed^{-1}$) or milliliters of commercial formulation per 100 kg of seeds (mL 100 kg^{-1}), as appropriate, considering the active ingredient concentration in the commercial formulation and the experimentally determined extraction recovery.

2.7. Statistical Analysis

The data obtained from the laboratory analyses were tabulated and subjected to analysis of variance (ANOVA), using the *F*-test to assess the statistical significance of treatment effects. When statistically significant effects were detected, regression analysis was performed to characterize the behavior of insecticide retention as a function of increasing rotational speed of the LS B-18 equipment.

3. RESULTS

Table 2 and Figure 1 presents the results obtained by comparing the standard speed with different operating speeds and their respective

recovery rates in corn seeds treated with Thiamethoxam, as determined by HPLC analysis. The factory-standard treatment resulted in the highest thiamethoxam recovery compared with the other rotational speeds tested. Among the alternative speeds, the 140% setting (179 rpm) showed a statistically significant difference, exhibiting higher insecticide recovery than the other rotation speeds, yet still below the acceptable range for the HPLC analytical standard (80–120% of the analyte).

Table 2. Quantification of the active ingredient Thiamethoxam by High-Performance Liquid Chromatography (HPLC).

Speeds*	Active ingredient analyzed	Species	Formulation	Average Recovery	Rema
Standard	Thiamethoxam	Corn	Acronis UBS 1.0 mL + Cruiser 600 FS 3.5 mL + Disc L-450 2.0 mL	97.4 ± 0.01a	Idea recov range (120%

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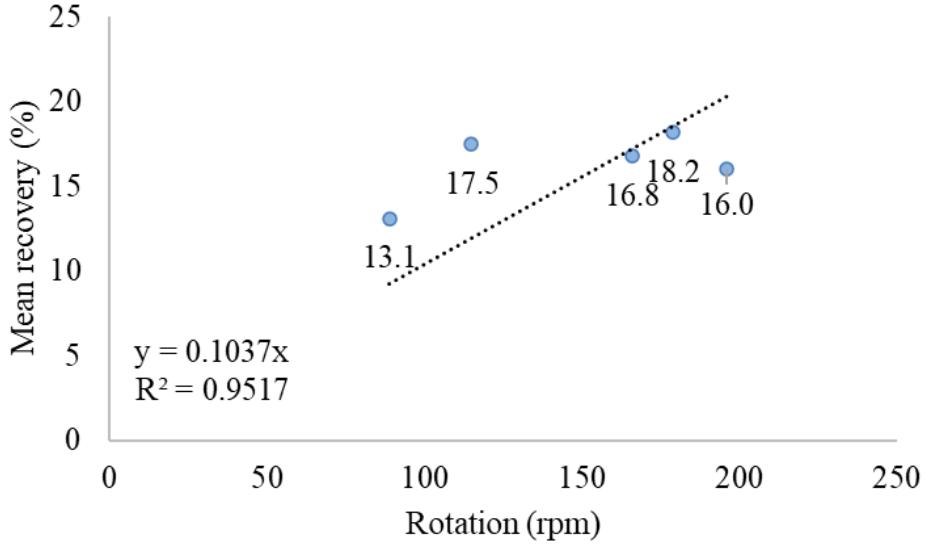
Note: *Speed percentage (%) followed by rotational speed expressed in revolutions per minute (rpm). Values are expressed as mean ± standard deviation (SD) of four replicates (n = 4). Treatment effects were assessed by analysis of variance (ANOVA) using the *F*-test. Different lowercase letters within the Average Recovery column

indicate statistically significant differences among treatments according to the *F*-test. Source: Authors, 2026.

Figure 1. Mean recovery (%) of the active ingredient Thiamethoxam in corn seeds as a function of the rotation speed (rpm) used during industrial batch seed treatment.



Source: Authors, 2026.

4. DISCUSSION

The results revealed a substantial difference in thiamethoxam recovery between the industry-standard treatment and the alternative atomizer disc rotational speeds evaluated. The industry-standard condition presented a mean recovery of 97.4 ± 0.01%, which was within the acceptable range of 80–120%. In contrast, the alternative rotational speeds resulted in considerably lower recovery values, ranging from 13.1% to 18.2%. These values represent reductions of approximately 81.3% to 86.5% relative to the industry-standard condition, indicating that the operating conditions adopted in the alternative treatments were not adequate to ensure comparable recovery of the active ingredient in the treated seeds.

One possible explanation for this difference is the influence of atomizer disc rotational speed on the physical characteristics of spray formation and the subsequent distribution of the chemical formulation within the treatment chamber. Rotational speed directly affects the centrifugal force acting on the liquid during atomization, potentially modifying droplet size, spray distribution, and the trajectory of droplets toward the seed surface. Experimental investigations have demonstrated that increasing rotational speed can reduce droplet diameter by increasing centrifugal forces and promoting liquid film fragmentation at the disc edge (Chen *et al.*, 2024).

Furthermore, the efficiency of seed coating depends on the interaction between atomizer operating conditions, liquid distribution, and the adhesion properties of the formulation (Afzal *et al.*, 2020). Consequently, operating the atomizer outside its appropriate adjustment range may compromise product deposition efficiency and reduce the amount of active ingredient retained by the seeds.

At lower rotational speeds, insufficient centrifugal energy may result in less effective liquid dispersion, potentially producing larger droplets and a less homogeneous spray pattern. This condition may favor localized deposition, uneven coating, and reduced contact between the formulation and the seed surface. Conversely, excessive rotational speeds may promote the formation of smaller droplets, potentially increasing their susceptibility to air movement and deposition on internal equipment surfaces or other areas of the treatment chamber. Chen *et al.* (2024) demonstrated that rotational speed significantly influences droplet size and spray formation patterns in spinning disc atomizers.

In addition, Afzal *et al.* (2020) highlighted that atomizer disc rotational speed, mixing conditions, and binder adhesion properties are important factors affecting the uniformity and final characteristics of seed coatings. Similarly, Medeiros *et al.* (2023) demonstrated that coating uniformity is positively associated with the effectiveness of active ingredient application in industrially treated maize seeds. These mechanisms are plausible explanations for the low recovery values; however, droplet size distribution, spray pattern, and product losses within the equipment were not directly measured in the present study.

The results also indicate that increasing rotational speed did not produce a consistent increase in thiamethoxam recovery. Although the treatment at 179 rpm presented the highest recovery among the alternative conditions 18.2%, the values obtained at 166 and 196 rpm were lower, at 16.8% and 16.0%, respectively. This variation suggests that the relationship between rotational speed and active ingredient retention may not be linear. Indeed, Chen *et al.* (2024) demonstrated that droplet formation and size distribution depend not only on rotational speed but also on liquid flow rate and surface tension.

Similarly, Afzal *et al.* (2020) emphasized that uniform seed coating results from the interaction between atomization, formulation properties, and mixing conditions during processing. Therefore, treatment efficiency may depend on the interaction between atomization conditions, liquid flow, seed movement, and the operating parameters of the treatment chamber. Consequently, rotational speed should be considered as one component of an integrated seed treatment process rather than an isolated parameter.

The importance of controlling application conditions is consistent with the principles discussed in studies on industrial seed treatment, which emphasize the need for appropriate equipment adjustment and uniform product distribution to ensure treatment quality (Medeiros *et al.*, 2023; Taylor *et al.*, 2021). In this context, chemical quantification provides information that cannot be obtained exclusively through visual assessment of seed coating, since the external appearance of treated seeds does not necessarily reflect the actual amount of active ingredient retained on their surfaces.

Another relevant aspect concerns the analytical determination of thiamethoxam. The calibration curve obtained in this study showed a coefficient of determination of $R^2 = 0.997$, indicating a strong linear relationship between analytical response and concentration. Nevertheless, calibration linearity alone does not establish the complete accuracy or recovery performance of the analytical procedure. Consequently, the low values observed in the alternative treatments should be interpreted considering both the possibility of reduced insecticide deposition and potential losses during sample extraction and preparation. Additional analytical recovery tests using fortified seed samples would help distinguish process-related losses from limitations associated with the analytical procedure.

Overall, the results suggest that the alternative rotational speeds evaluated were associated with substantially lower thiamethoxam recovery than the industry-standard condition. However, because the study did not directly measure droplet characteristics, deposition uniformity, or losses within the equipment, the specific mechanisms responsible for this difference cannot be established conclusively. Further investigations combining chemical quantification with spray characterization, equipment surface

residue analysis, and analytical recovery validation are necessary to clarify the effects of atomizer disc rotational speed on insecticide retention during industrial corn seed treatment.

5. CONCLUSION

The results demonstrated that atomizer disc rotational speed was associated with substantial differences in thiamethoxam recovery in industrially treated maize seeds. The industry-standard operating condition provided the highest recovery (97.4%), remaining within the acceptable range of 80–120%, whereas all alternative rotational speeds resulted in considerably lower recovery values, ranging from 13.1% to 18.2%.

Among the alternative conditions, the rotational speed of 179 rpm presented the highest recovery; however, none of the evaluated alternative settings approached the performance obtained under the industry-standard condition. These findings indicate that modifying atomizer disc rotational speed without considering the integrated operating conditions of the treatment equipment may compromise insecticide deposition and retention on maize seeds.

Therefore, maintaining appropriate equipment calibration and operating parameters is essential for improving the consistency and chemical quality of industrial seed treatment. Further investigations should incorporate spray characterization, assessment of product residues on internal equipment surfaces, and analytical recovery validation to distinguish processing losses from potential limitations associated with the extraction procedure.

Overall, the study demonstrates the importance of maintaining standardized industrial operating conditions to ensure adequate

thiamethoxam recovery and provides relevant information for improving quality control in industrial maize seed treatment processes.

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