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EVALUATION OF THE CONDITIONS OF CONVENTIONAL AND BT CORN SEEDS AFTER TREATMENT WITH INSECTICIDES AND STORAGE

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ABSTRACT

Seed treatment with insecticide is a preventive form and one of the modern concepts for controlling soil pests in the initial phase of the crop. To use this technology, it is necessary to know the influence of these products in relation to the physiological and sanitary quality of the treated seeds. The objective of this study was to evaluate the effect of the main insecticides used in seed treatment, recommended for corn crops, or the combination of these for the treatment of conventional corn seeds and BT (Yieldgard), on germination, vigor and sanitary quality of seeds in different storage periods of treated seeds. Conventional and transgenic seeds were evaluated, which received 6 treatments with insecticides. These were evaluated by germination, chill, emency and health tests at 0, 7, 14, 21 and 28 days after seed treatment. The experimental design was completely randomized blocks and the data were analyzed through deviance analysis, followed by a test to compare means. It was found that theseeds treated with the different insecticides showed adequate percentages of germination and emergence, that the conventional hybrid presented better performance than its genetically modified version in all the tests carried out and that the mixture of products such as Imidacloprid + thiodicarb + Fipronil reduced the incidence of the pathogens *Fusarium moniliforme* and *Aspergillus spp*.

Keywords: Insecticides. Storage. Physiological quality. Sanitary quality.

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INTRODUCTION

In the process of seed production and marketing, one of the main factors that the producer must be aware of is the preservation of seed quality throughout the storage period. If there is no special attention at this stage, all the efforts spent on production may not be rewarded. Care for seed quality should be maintained at least until sowing time (Carvalho 1992).

Such precautions avoid, for example, the presence of pests during seed storage, especially those of the Coleoptera and Lepidoptera orders, which can lead to losses of around 20% of the stored product (Carvalho, 1978; Carvalho & Nakagawa, 1988). In addition to quantitative losses, pest attacks on seeds can cause losses in germination power and vigor (Barney et al., 1991).

The preventive use of insecticides or insecticide mixtures in seed treatment has been proposed as an alternative to avoid possible losses resulting from the actions of insects, soil pests and shoots, which can attack seeds and young plants (Silva, 1998). This practice, when properly performed, makes it possible to reduce the number of foliar applications, which often need to be started soon after seedling emergence (Menten, 1991).

The insecticides used in seed treatment differ from others applied in traditional spraying, due to their systemic action on the plant. When in contact with the soil, they detach from the seeds and, due to their low vapor pressure and water solubility, are slowly absorbed by the roots, giving the plant an adequate period of protection against soil and shoot insects (Silva, 1998).

Although seed treatment is considered one of the most efficient methods of using insecticides (Gassen, 1996), some research results have shown that some products, due to the effect of phytotoxicity, when applied alone or in combination with fungicides, can, in certain situations, cause a reduction in seed germination and seedling survival (Oliveira & Cruz, 1986; Pereira, 1991).

As the treatment of corn seeds with insecticide is routinely performed in the processing unit and due to the lack of information about its effect on processed seeds, it is of great importance to better understand the effects of insecticides available in the market, both in seed quality and in the control of pest insects that attack corn seedlings in their early stages.

Due to the excellent result obtained with the phytosanitary treatment of seeds against insect attack and the importance of using high quality seeds to obtain a crop with an adequate stand, this research aimed to evaluate the effect of the main insecticides used in seed treatment, recommended for corn crop, or the combination of these for the treatment of conventional corn seeds and BT (Yieldgard), in germination, vigor and sanitary quality of the seeds in different storage periods of the treated seeds.

MATERIAL AND METHODS

The research was carried out at the Seed Analysis and Seed Pathology Laboratories, and in the experimental area of the Department of Agriculture of the Federal University of Lavras, Lavras, Minas Gerais.

Seed samples of the simple hybrid DKB390 in its conventional and transgenic versions were used in the 2009/10 harvest. The seeds were packed in multiwall kraft paper bags, where they were stored in a cold chamber (±10°C, ±50% relative humidity) until the experiment was carried out.

The seed samples of each hybrid were divided into six equal portions, weighing about 3 kg each, which received the following treatments according to the manufacturers' recommendations: control (without insecticidal treatment), Imidacloprid + Thiodicarb (Cropstar), Fipronil (Standak) and Thiamethoxan (Cruiser 350 FS), Imidacloprid + thiodicarb + Fipronil (Cropstar + Standak), Thiamethoxan + Fipronil (Cruiser 350 FS + Standak). Each treatment was performed with two replicates and stored in unifoliated paper bags under uncontrolled environmental conditions. A thermohygrograph installed near the seeds was used to evaluate the variations in temperature and relative humidity of the air in the storage environment. Where is this data?

At zero, seven, fourteen, twenty-one and twenty-eight days after insecticide treatment on the seeds, a seed sample was taken from each of the paper bags to perform the germination test, cold test, emergence in bed and sanitary analysis.

The germination test evaluated 4 replicates of 50 seeds per plot, using Germitest paper (CEL 065) in roll form as substrate. Each of the eight sub-samples of 50 seeds were sown on two sheets of Germitest paper towels, moistened with water in a proportion of two and a half times the weight of the dry paper. Then, the seeds were covered with a third leaf, moistened under the same conditions, and wrapped in the form of rolls, stored in germinators at 25^{oC,} where they remained for seven days. Evaluations were made in the room and seventh days after the installation of the trial (Brasil, 2009).

The cold test was conducted according to the criteria described by Barros et al. (1999) and Vieira & Krzyzanowski (1999). For this, plastic trays were used, containing a mixture of sand and soil in a ratio of 2:1. The substrate was moistened to 70% of the field capacity, according to the recommendations of Popinigis (1985). 4 replicates of 50 seeds were evaluated. After sowing, the corn seeds were covered with the same substrate, the trays were protected and randomly arranged in a temperature-controlled chamber (10°C) remaining in these conditions for seven days. Subsequently, they were transferred to an environment with a temperature of around 25°C, where they remained for another seven days. The evaluation was carried out on the fifteenth day after sowing, computing the number of normal seedlings emerged.

The emergence test in bed was carried out with the sowing of 50 seeds and using two replicates for each treatment and each storage season. The mixture of sand and soil was in the ratio of 2:1 (sand:soil). This mixture had its moisture adjusted to 70% of the water saturation capacity. The evaluations were carried out at 7 and 15 days after sowing, accounting only for normal seedlings.

The sanity test was performed according to the modified filter paper method, with freezing (Machado 1988). Five replicates containing 40 seeds per treatment and per storage time were used. These were arranged in Petri dishes on three sheets of filter paper soaked in distilled water and agar (5g/L) and placed at room temperature. The next day, the plates containing the seeds were transferred to the freezer at a temperature of -8°C for 24 hours. Then, the plates returned to the incubation room at 20°C under an alternating regime of 12 hours of light and 12 hours in the dark, for eight days. Ten days after sowing, the presence (or percentage?) of seeds affected by *Fusarium moniliforme* and *Penicillium spp (check) was evaluated* with the aid of a stereoscopic microscope.

The experimental design was completely randomized with two replications, in a 2x6x5 factorial scheme. The response measured in the experimental units was a proportion of seeds of a class (germinated/diseased) in relation to the total seeds. A binomial distribution with a logit linkage function was considered, indicated for proportion-type variables. The effect of the factors studied was evaluated through deviance analysis based on generalized linear models. The binding function employed was logit and the superscatter parameter was estimated based on Pearson's residuals. The statistical model considered for analysis is represented by

$y_{ijkl} \approx Binomial(n, \pi_{ijk})$

$$\ln = \frac{\pi_{ijkl}}{1-\pi_{ijk}} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\lambda_{ijk}$$

where y_{ijkl} it is the proportion of germinated/diseased seeds in *the ijkl-th experimental unit containing* n *seeds,* π_{ijk} it is the probability of germination/disease that is a function of μ, a constant inherent in all observations, α_i the fixed effect of the *i-th* level of the insecticidal factor, β_j is the fixed effect *j-th* level of the type of seed factor, γ_k, the linear time effect $\alpha\beta_{ij}$, $\alpha\gamma_{ik}$, $\beta\gamma_{jk}$ and $\alpha\beta\lambda_{ijk}$ are the double and triple combinations between the levels of the factors. The index *l* represents the repetitions of each combination between levels of the factors (treatments).

The chi-square tests corresponding to the reduction of sequential deviance for each term of the model were applied, according to the order of the terms of equation 1. After deviance analysis, multiple hypothesis comparison tests were performed on the differences between levels of the factors. The FDR (false dicovery rate) criterion was applied to control the type I error rate caused by the multiple hypothesis comparison procedure. For all tests, a nominal significance level of 5% was considered.

The analyses were carried out with the aid of the R application (R Development Core Team, 2010) and through resources available in the contrast (Kuhn et. al, 2010) and multcomp (Hothorn et. al, 2008) supplementary packages.

RESULTS AND DISCUSSION

Table 1 shows that when comparing the chemical treatments applied to conventional and transgenic seeds, it is verified that the products Imidacloprid + thiodicarb, Thiamethoxam and Fipronil showed similar behavior in the germination and cold tests. For the emergency test, treatment with Fipronil was superior to the others, followed by treatments with Thiamethoxam and Imidacloprid + thiodicarb, respectively. Regardless of the type of seed, transgenic or conventional, a reduction in their physiological quality was observed when two commercial insecticides were combined. The mixture Imidacloprid + thiodicarb + Fipronil obtained the worst estimates of germination, emergence and vigor; therefore, the recommendation of this treatment should be made with caution.

Differences in the performance of insecticides used in seed treatment have been frequently cited in the literature. Dan et al. (2010) evaluated the effect of insecticides on the physiological quality of soybean seeds. The authors found that there was no difference in the germination and emergence tests between the active ingredients thiamethoxam and fipronil when compared to the control without treatment. The same was not observed for Imidacloprid + thiodicarb, which presented worse performance than the insecticides mentioned in both the germination test and the cold test.

In this study, the controls showed higher averages of germination, vigor and emergence than the other treatments (Table 1), indicating interference of the active ingredients used on the physiological quality of the seeds. This reduction in seed viability and vigor can be attributed to possible damage to the mitochondrial membrane, which promotes a decrease in aerobic respiration and ATP production and ethanol additions, which are important indicators of respiration intensity and energy availability for the germination process (Reedy and Knapp, 1990; Horri and Shetty, 2007). Thus, in addition to the loss of cell compartmentalization, the disintegration of the membrane system, caused by some external factor, promotes uncontrolled metabolism and the exchange of water and solutes between cells and the external environment, determining the decrease in seed viability (Dan et al., 2010).

Despite the interference of insecticides in the physiological quality, seed treatment should always be recommended, because in addition to the phytotoxicity effect having been small, other factors that interfere in the final stand of plants were not considered in this work.

It should be emphasized that despite the differences observed in the performance of insecticides and types of seeds, most treatments showed adequate germination levels (>85%) and, therefore, are within the accepted standards for the commercialization of maize seeds (Brasil, 2003).

When comparing the effect of the type of seed, conventional and transgenic, through the results of the tests performed on the controls, it was verified that there was a significant difference between them for all tests, and for the transgenic the averages were lower (Table 1). These results may have occurred due to two factors – difference in physiological quality between the evaluated lots and/or interference of the introduced gene in the physiological quality of the hybrid under study. Since the lot used in this work was obtained in the 2009/10 harvest and that theoretically has a high physiological potential, it is more likely that the gene introduced in the hybrid is the main factor to interfere in the results obtained. This result was also observed in conventional and transgenic soybean cultivars. According to Bertolin (2008), cultivar Conquista obtained better physiological quality of its seeds in relation to its genetically modified version (Valiosa RR). Similar results were obtained by Harper (1997); Don Huber and Gordon cited by Yamada (2007). Due to this, the averages of the transgenic seeds treated with insecticides in the different tests were also lower than those of the conventional seeds treated.

Table 1. Averages obtained in the germination (TG), cold test (TF) and emergence in bed (TE) tests in conventional and transgenic corn seeds. UFLA, Lavras, MG, 2010

Insecticides2	Conventional1			Transgenic1		
	TG	Team Fight	YOU	TG	Team Fight	YOU
Imid+thio	97.30a	42.20a	94.04c	75,25c	36.59bc	54.30c
Imid+thio+ Fp	92.60b	42.00b	89.24d	62.50d	35,84c	55.91c
Thiamethoxan	98.40a	46.70a	96.25bc	90.91ab	35.95bc	83.90b
Thia + FP	96.31ab	43.05b	95.57bc	90.52b	36.70bc	83.19b
Fipronil	98.15a	45.95a	97.47ab	94.21ab	38.00b	91.75a
Witness	97.69a	45.55a	98.60a	94.47a	41.45a	89.64a

1Averages followed by the same lowercase letter in the column do not differ from each other, at a 5% probability. [2] Imid (Imidacloprid), Thio (Thiodicarb), Fp (Fipronil), Thia (Thiamethoxan).

No significant difference was found for the seed-type:storage time split in the germination and cold tests. For the emergence test, a difference was observed in the behavior of the seeds over the storage time (Table 2). Conventional seeds have a higher percentage of emerged and normal plants at all times evaluated.

Table 2. Averages related to emergence tests in bed performed on conventional and transgenic maize seeds, at different storage times. UFLA, Lavras, MG, 2010.

Treatments	Times1				
	0d	7d	14d	21d	28d
Conventional	94.92a	95.50a	96.02a	96.48a	86.89a
Transgenic	77.94b	78.76b	79.76b	80.62b	81.46b

1Averages followed by the same lowercase letter in the column do not differ from each other, at a 5% probability.

When performing the germination, cold and emergence tests at 5 different times, a significant difference was found between the times tested only for the cold and emergency tests (Table 3). For these two tests, the controls showed equal or superior performance to the treatments that used insecticides. Seeds treated with Fipronil showed superior behavior to other insecticide treatments, at all times evaluated. In the seeds where the mixtures were used Imidacloprid + thiodicarb+Fipronil and Imidacloprid + thiodicarb+Thiamethoxam was found to lower average vigor and emergence over time, again reinforcing that the use of this mixture should be avoided.

Table 3. Averages related to cold and emergence tests performed on conventional and transgenic maize seeds, at different storage times. UFLA, Lavras, MG, 2010.

Treatments2	Cold Test1				
	0d	7d	14d	21d	28d
Imid+thio	42.41ab	41,62bc	40.82bc	40.04cd	39.25cd
Imid+thio + Fp	39.97b	39.42d	38.87d	38.33e	37.79d
Thiamethoxan	40.00b	40,61cd	41.22bc	41.83ab	42.45ab
Thia + Fipronil	40.58b	40,21cd	39.83cd	39,46de	39,08cd
Fipronil	43.23a	42.58ab	41.92b	41.27bc	40.62bc
Witness	43.57a	43.53a	43.49a	43.45a	43.41a
Treatments	**Emergency Testing1**				
	0d	**7d**	**14d**	**21d**	**28d**
Imid+thio	70.26b	76,18c	81,24c	85.43c	88.82b
Imid+thio + Fp	70.89b	73.76c	76.44d	78.92d	81.21c
Thiamethoxan	92.73a	92.39b	92.04b	91.67b	91.29b
Thia + Fipronil	93.53a	92.44b	91.18b	89.73b	88.08b
Fipronil	93.39a	94.48ab	95.39a	96.16a	96.81a
Witness	95.04a	95.61a	96.11a	96.56a	96.96a

1Averages followed by the same lowercase letter in the column do not differ from each other, at a 5% probability. 2 Imid (Imidacloprid), Thio (Thiodicarb), Fp (Fipronil), Thia (Thiamethoxan).

In the health test, the incidence of pathogens (*Fusarium moniliforme* and *Aspergillus spp*) in transgenic and conventional seeds, treated or not with different insecticides and evaluated after 0, 7, 14, 21 and 28 days of treatment. Comparing the behavior of treated conventional seeds and their control, it is noted that the use of insecticides reduced the presence of *Fusarium moniliforme*, where the combination Imidacloprid + thiodicarb+Fipronil was the most efficient in this purpose (Table 4). When the behavior of the treated transgenic seeds and their controls is considered, it is verified that the combination of the products Imidacloprid + thiodicarb and Fipronil also enabled a lower incidence of the pathogen. However, unlike what happened with conventional seeds, the witnesses were not the most affected by the *Fusarium moniliforme*, that is, the use of insecticide did not always reduce the incidence of this pathogen.

Table 4. Average incidence of *Fusarium moniliforme* (%) as a function of different chemical treatments performed on conventional and transgenic maize seeds, at different storage times. UFLA, Lavras, MG, 2010.

Treatments2	**Conventional1**				
	0d	**7d**	**14d**	**21d**	**28d**
Imid+thio	13.45ab	12.52bcd	11.65bcd	10.82bcd	10.05bc
Imid+thio + Fp	7.00b	7.00d	7.00d	7.00d	7.00c
Thiamethoxan	16.41ab	16.80ab	17.19ab	17.60ab	18.01ab
Thia + Fipronil	7.28b	8.97cd	11.01cd	13.44bc	16.30abc
Fipronil	16.59ab	14.15abc	12.02bc	10.17cd	8.58bc
Witness	20.70a	20.95a	21,20a	21.45a	21.70a
Treatments	**GMOs1**				
	0d	**7d**	**14d**	**21d**	**28d**
Imid+thio	38a	32.47a	27.38a	22.82ab	18.83b
Imid+thio + Fp	19,86bc	18.59c	17.39b	16.24b	15.16b
Thiamethoxan	32.69ab	30.46a	28.31a	26.25ab	24.29ab
Thia + Fipronil	32.06ab	28.53ab	25.24ab	22.22ab	19.46b
Fipronil	37.03a	33.19a	29.56a	26.17ab	23.04ab
Witness	15.98c	19,95bc	24.63ab	29.99a	35.96a

1Averages followed by the same lowercase letter in the column do not differ from each other, at a 5% probability. 2 Imid (Imidacloprid), Thio (Thiodicarb), Fp (Fipronil), Thia (Thiamethoxan).

Regarding the incidence of *Aspergillus spp*, it is noted that transgenic seeds were more affected by this pathogen than conventional seeds, regardless of the time the seeds were stored (Table 5). In conventional seeds, the incidence of *Aspergillus spp* increased as the treated seed was stored, the opposite behavior was observed in transgenic seeds. In all times evaluated, seeds treated with Imidacloprid + thiodicarb]+Fipronil were less affected by the pathogen in question. On the other hand, conventional and transgenic seeds that were not treated (controls) and treated with Thiamethoxam were the most affected.

Table 5. Average incidence of *Aspergillus spp* (%) as a function of different chemical treatments performed on conventional and transgenic maize seeds, at different storage times. UFLA, Lavras, MG, 2010.

Seed type	**Times1**				
	0d	**7d**	**14d**	**21d**	**28d**
Conventional	1.69b	2.26b	3.04b	4.06b	5.41b
Transgenic	8.39a	7.89a	7.42a	6.98a	6.56a
Insecticides2	**Times1**				
	0d	**7d**	**14d**	**21d**	**28d**
Imid+thio	2.24b	2.81c	3.52b	4.41b	5.50b
Imid+thio + Fp	0.78b	0.89c	1.02c	1.16c	1.32c
Thiamethoxan	9.69a	11.34a	13.24a	15.39a	17.83a
Thia + Fipronil	1.91b	2.60c	3.53b	4.78b	6.44b
Fipronil	8.65a	6.68b	5.13b	3.93b	3.00bc
Witness	9.89a	11.06a	12.33a	13.74a	15,27a

Insecticides	**Conventional1**	**GMOs1**
Imid+thio	1.53bc	7.86b
Imid+thio + Fp	0.83c	1.24c
Thiamethoxan	11.57a	15.09a
Thia + Fipronil	1.15bc	10.25ab
Fipronil	3.58b	7.28b
Witness	11.28a	13.46a

1Averages followed by the same lowercase letter in the column do not differ from each other, at a 5% probability. 2 Imid (Imidacloprid), Thio (Thiodicarb), Fp (Fipronil), Thia (Thiamethoxan).

CONCLUSIONS

The seeds treated with the different insecticides showed adequate germination and emergence percentages.

The conventional hybrid showed better performance than the genetically modified hybrid in all the tests performed.

The mixture of products such as Imidacloprid + thiodicarb + Fipronil reduced the incidence of pathogens *Fusarium moniliforme* and *Aspergillus spp*.

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