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Physiological Quality of Treated and Stored Barley Seeds

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Abstract

Industrial seed treatment (IST) is used in some grain crops. However, the products used often impair the quality of seeds, especially insecticides. In the case of barley seeds, investigative studies on the use of IST are scarce. The present study aimed to evaluate the influence of IST with the fungicide (Iprodione) and insecticide (Thiamethoxan) products, mixed or not, on the physiological quality of barley seeds, before and after storage for two months. A completely randomized design was used, in a 4x2 factorial scheme, with four replications. The treatments consisted of four combinations of applications carried out via IST on barley seeds, of chemical pesticides (fungicide/insecticide), alone or in mixtures, as follows: {T1 – fungicide Iprodione (Rovral); T2 – Thiamethoxan insecticide (Cruizer); T3 – mixture of fungicide/insecticide products (Iprodione + Thiamethoxan); T4 – Control)}, submitted to storage for two months, with evaluations carried out at T0 (treated and analyzed seeds) and T1 (treated seeds stored for 60 days). The following tests were carried out: water content, germination, first count, accelerated aging, seedling length, seedling dry mass. It was concluded that the germination of barley seeds treated with the fungicide Iprodione (Rovral®) and the insecticide Thiamethoxan (Cruizer®), alone and in a mixture applied via IST, maintained the physiological quality of the seeds within the standards for commercialization until 60 days of storage. The use of the insecticide Thiamethoxan (Cruizer®) via IST showed greater damage to the physiology of barley seeds during storage (60 days), applied alone or in a mixture with the fungicide Iprodione (Rovral ®).

Keywords: *Hordeum vulgare* L., IST, insecticide, fungicide, vigor

1. Introduction

Barley (*Hordeum vulgare* L.) is a poaceae widely produced for millennia, being one of the oldest cereals cultivated by man, currently the fourth most harvested cereal in the world (Carpentieri-pipolo, et al., 2021). Barley production in Brazil in 2019 was 428 thousand tons (“Conab” 2019) in which the states of Paraná and Rio Grande do Sul are the largest producers.

Diseases are productivity limiting in barley culture, being the main ones occurring in Brazil: FHB (*Fusarium graminearum*), net blotch (*Drechslera teres*), spot blotch (*Bipolaris sorokiniana*), leaf rust (*Puccinia hordei*), glume blotch (*Phaeosphaeria nodorum*) and powdery mildew (*Blumeria graminis f.sp. hordei*) (Reis & Casa, 2007). As for the pests of the barley crop, the most important are the ground coró, (*Phyllophaga tritico*), grazing coró (*Diloboderus abderus*), cereal aphid (*Schizaphis graminum*), leaf aphid (*Rhopalosiphum padi*), rice root aphid (*Rhopalosiphum rufiabdominale*), lesser grain (*Rhyzopertha dominica*), red flour beetle (*Tribolium castaneum*), borer leaf aphid (*Metopolophium dirhodum*), wheat caterpillar (*Pseudaletia sequax*), fall armyworm (*Spodoptera frugiperda*) and cutworm (*Agrotis ipsilon*) (Dong, et al., 2022).

In this context, it should be noted that the main form of transmission of diseases and pests is via seeds (Menegon, et al., 2005), thus making the application of existing fungicides and insecticides in the market inefficient to eradicate and/or reduce the passage of fungi and pests to the aerial part of the crop, increasing the cost of production due to the need for early application of fungicides on plants. Therefore, industrial seed treatment (IST) is an option, which aims to suppress, control or remove pathogens and initial pests, optimizing farmers' time in the fields, since the seeds are already treated since their processing in the industry (Dan, et al., 2013).

Iprodione is a contact fungicide from the group of dicarboxamides, formula $C_{13}H_{13}Cl_2N_3O_3$, characterized by the mechanism of action called osmotic signal transduction, more specifically it acts at the MAP/Histidine kinase target site. There is a recommendation for its use in the control of net blotch in the treatment of winter cereal seeds like barley (Agostinetto, et al., 2018).

The insecticide Thiamethoxan, belonging to the neonicotinoid group with molecular form $C_8H_{10}ClN_5O_3S$, it has a contact and systemic effect on pests, its active ingredient being transported via acropetal (xylem) through the plant roots and accumulating in the leaves, where it has a translaminar action, in addition to a small amount of the product being transported via the basipetal (phloem) (Scorza & Rigitano, 2012).

In view of the above, the present work aimed to, the present study aimed to evaluate the influence of IST with fungicide (Iprodione) and insecticide (Thiamethoxan), in mixture or not, on the germination performance of barley seeds before (immediate effect) and after storage for 60 days (latent effect).

2. Material and Methods

The seeds used in the test belong to the BRS Brau cultivar and were produced in the 2017/18 harvest. Subsequently, they were brought to the Laboratory of Vegetable Products of the State University of Goiás, Campus Anápolis, to be submitted for analysis. The initial viability of the seed lot was 90%.

A completely randomized design was used, in a 4x2 factorial scheme, with four replications. The treatments consisted of four combinations of applications carried out via IST on barley seeds, of chemical pesticides (fungicide/insecticide), alone or in mixtures, as follows: {T1 – fungicide Iprodione (Rovral); T2 – Thiamethoxan insecticide (Cruizer); T3 – mixture of fungicide/insecticide products (Iprodione + Thiamethoxan); T4 – Control)}, submitted to storage for two months, with evaluations carried out at T0 (treated and analyzed seeds) and T1 (treated seeds stored for 60 days). The doses of Iprodione and Thiamethoxan used in the seed treatment corresponded to 100 and 150 ml p.c./100 kg of seeds, respectively.

The treated seeds were placed in strain bags in a cold chamber, maintained at temperature and relative humidity (± 10ºC and ± 45%, respectively), controlled in order to minimize their metabolic activities and, consequently, the loss of viability and vigor of the seeds. The evaluations were performed according to the intervals mentioned above, using the following tests:

Germination: It was carried out with four subsamples of 50 seeds, arranged in a germitest-type paper substrate, moistened with distilled water in an amount corresponding to 2.5 times the mass of dry paper. Fifty seeds/repetition were placed, rolled and conditioned in a germinator at a temperature of 25 ± 2ºC. Readings were taken on the seventh day after sowing, computing the percentages of normal, abnormal and dead seedlings (Tunes, et al., 2008).

First count: It was conducted together with the germination test, and the evaluation was carried out on the fourth day of setting up the germination test. The percentage of normal seedlings was calculated (Tunes, et al., 2008)

Accelerated aging: For accelerated aging, 250 seeds/repetition were distributed on the surface of a metallic screen fixed and suspended inside a plastic box - gerbox, with 40 mL of water, and kept at 41 ºC and 100% relative humidity, for 72 hours in germinator (Assis & Dalastra, 2021). After this period, the seeds were submitted to the germination test. The percentage of normal seedlings was determined on the 4th (fourth) day after the test was set up.

Seedling length: The procedures described by (Tunes, et al., 2010) were applied, using ten repetitions of 10 barley seeds. A line was drawn on the upper third of the germination paper towel in the longitudinal direction. The papers were previously moistened with distilled water equivalent to 2.5 times the dry mass of the paper. The seeds were positioned so that the micropyle was facing the bottom of the paper. The rolls were positioned vertically in the germinator for seven days at 25 ± 2 ºC. At the end of this period, the parts of the emerged normal seedlings (primary root and hypocotyl) were measured using a ruler. Mean results per seedling were expressed in centimeters.

Seedling dry mass: Normal seedlings obtained in the seedling length test, which had the reserve tissues removed with a scalpel, were evaluated, excluding the cotyledons. The repetitions of each batch were placed in kraft paper bags, identified, and taken to the oven with forced air circulation, keeping at a temperature of 80ºC for a period of 24 hours (Tunes, et al., 2010). After this period, each repetition had its mass evaluated on a scale with a precision of 1.0 mg, and the average results were expressed in milligrams per seedling.

The data were submitted to analysis of variance, and when relevant, the means were compared by Tukey's test at 5% probability. The analyzes were performed using the Sisvar software.

3. Results and Discussion

From the results of the analysis of variance (Table 1), it is verified that there was a significant difference between the treatments in the germination, first count and accelerated aging tests, both for the immediate effect, with treated and analyzed seeds, and for the latent effect, with seeds stored for 60 days. The result of the seedling length test was influenced only by the latent effect, treated and stored seeds, while the seedling dry mass was not significant for any of the factors studied. The interaction of seed treatment and storage did not significantly influence the results of any test applied.

Table 1. Result of analysis of variance of germination (GER), first count (PC), accelerated aging (EA), seedling length (CP) and seedling dry mass (MSP) tests performed on barley seeds treated via IST with insecticide and fungicide, mixed or not, during storage for 60 days. UEG, 2020, Ipameri-GO

Sources of Variation	G.L.	Medium Squares				
		GER	PC	EA	CP	MSP
Treatment(T)	3	78.125*	969.458**	664.833**	1.154^{ns}	0.003^{ns}
Storage (A)	1	276.125*	741.125*	450.000**	14.032**	0.002^{ns}
TxA	3	60.792^{ns}	85.125^{ns}	33.667^{ns}	0.638^{ns}	0.000^{ns}
Residue	25	23.661	79.256	27.362	0.416	0.001
C.V. (%)	-	5.2	11.1	11.1	5.6	29.3

G.L.= Degrees of freedom. *Significant at 5% probability by the F test; ** Significant at 1% by the F test; NS=Not Significant.

In general, good experimental precision was observed in obtaining the data, with the exception of the seedling dry mass test, when compared to the coefficient of variation values established by (Gomes-Pimentel, 2009), who established high values when ≥ 20 and < 30, for experimental precision classification in the field of Agricultural Sciences.

The result of the germination test showed that the treatment of seeds with the fungicide Iprodione (Rovral®) provided a higher percentage of germination of barley seedlings (94%), statistically equating the control, without treatment (95%). On the other hand, the lowest germination rates were obtained in treatments in which the seeds were treated with the insecticide Thiamethoxan (Cruizer®), isolated and mixed with the fungicide Iprodione (Rovral®), whose values were respectively 91 and 92% (Table 2). This reduction observed in the seeds can be attributed to the toxicity effect on the seeds, leading to a reduction in their germination and, therefore, a drop in the percentage of seedling survival (Castellanos, et al., 2017).

Results similar to those obtained in this research were verified by (Avelar, et al., 2011), that evaluated the effect of the application of the fungicide Fludioxonil + Metalaxyl - M(Maxim XL®), Thiamethoxan insecticide (Cruiser®), micronutrient ComoFix® and the fungicide + insecticide + micronutrient mixture, in addition to the application of powder and liquid polymers and a control. They verified that the seeds treated with insecticide showed lower germination after six months of storage, when compared to those of the control and the micronutrient in the treatments without polymer and to all other treatments, except for the micronutrient in the liquid polymer.

Table 2. Mean germination values of barley seeds, treated via IST with insecticide and fungicide, mixed or not. UEG, 2020, Ipameri-GO

Treatment via IST	Normal Seedlings (%)
Iprodione (Rovral®)	94 a
Thiamethoxan (Cruizer®)	91 b
Iprodione (Rovral®) + Thiamethoxan (Cruizer®)	92 b
Control	95 a
General Median	93

Equal means do not differ statistically from each other at 5% probability by the Tukey test.

Seeds treated and stored for 60 days had a lower percentage of normal seedlings than those analyzed before storage. However, these still showed germination of 90%, higher than the minimum required by legislation (equal to or higher than 80%) (Brasil, 2009). This result makes the use of IST in barley seeds acceptable, given the importance of the practice in optimizing farmers' time in the field.

These results are in agreement with those obtained by (Silva, et al., 2019), that evaluating seeds of three soybean cultivars (M7110 IPRO; RR- 8473RSF, M7739 IPRO), subjected to treatments with chemical pesticides (fungicide/insecticide) applied via IST: {T1- control; T2- Carbendazim fungicide + Thiran (Derosal Plus®); T3- Thiamethoxan insecticide (Cruiser®); T4- mixture of fungicide and insecticide products}, concluded that soybean seeds maintained germination percentages within the accepted standards for commercialization (> 80%), with chemical treatment up to 60 days of storage. On the other hand, these authors also observed that the chemical products impaired the quality and vigor of seeds in all cultivars studied after six months of storage, especially treatments containing insecticide.

Table 3. Mean germination values of barley seeds, treated via IST with insecticide and fungicide, mixed or not, as a function of storage periods. UEG, 2020, Ipameri-GO

Storage Periods (days)	Normal Seedlings (%)
0	96 a
60	90 b
General Median	93

Equal means do not differ statistically from each other at 5% probability by the Tukey test.

The effects of chemicals on barley seed vigor verified in the first count test (Table 4) were similar to those observed in the germination test, in which the insecticide Thiamethoxan(Cruizer®), alone and in combination with the fungicide Iprodione (Rovral®) provided the obtaining of seed lots with lower vigor. The systemic and translaminar action of the insecticide thiamethoxan is even more evident in the first count test, with an average of normal seedlings of 79% administered alone and 78% in mixture. The reduction in the percentage of normal seedlings evidences the effect of toxicity on the seeds, leading to a reduction in their germination and, therefore, a drop in the percentage of seedling survival (Castellanos, et al., 2017).

These data corroborate those found by (Silva, et al., 2019), in which soybean seeds treated with the insecticide Thiamethoxan (Cruizer®) alone and in a mixture with Carbendazim fungicide + Thiran (Derosal Plus®) showed lower germination both in the germination test and in the first count. On the other hand, these results contradict the results of some researches (Cataneo, 2008; Acevedo & Porras 2008), which indicate that Thiamethoxan (Cruiser®) can cause physiological effects in plants, promoting an increase in the germination rate of seeds.

Table 4. Mean vigor values of barley seeds in the first count test, treated via IST with insecticide and fungicide, mixed or not

Treatment via IST	Normal Seedlings (%)
Iprodione (Rovral®)	81 a
Thiamethoxan (Cruizer®)	79 b
Iprodione (Rovral®) + Thiamethoxan (Cruizer®)	78 b
Control	83 a
Average	80

Equal means do not differ statistically from each other at 1% probability by the Tukey test.

Regarding the treated and stored seeds (Table 5), the first count test showed lower mean values than the control, due to the seeds treated with the insecticide Thiamethoxan (Cruizer®) mixed or not with Iprodione fungicide (Rovral®), showed a delay in the seed germination process, corroborating the detection of a lower mean in terms of the number of vigorous seedlings in the seed lot in the first count conditioned for 60 days. These results are in agreement with those obtained in the work conducted by Camargo (2018), who noticed a slower response to germination in soybean seeds treated with the insecticides Thiamethoxan (Cruizer®) and/or Fipronil (Amulet®) mixed or not with the fungicide Metalaxil+ Thiabendazole + Fludioxonil (Maxim Advanced®) after eight months of storage.

Table 5. Mean germination values of barley seeds, in the first count test, treated via IST with insecticide and fungicide, mixed or not, depending on the storage periods. UEG, 2020, Ipameri-GO

Storage Periods (days)	Normal Seedlings (%)
0	88 a
60	75 b
Average	82

Equal means do not differ statistically from each other at 5% probability by the Tukey test.

The accelerated aging test at time zero, that is, with seeds treated via IST and without conditioning, showed the greater deterioration of barley seeds exposed to chemical products (Table 6), especially in the treatment with the insecticide Thiamethoxan (Cruizer®), which was significantly smaller than the control, but did not differ from the treatment with Iprodione (Rovral®) fungicide mixed or not with the insecticide Thiamethoxan (Cruizer®). The accelerated aging test results are consistent with those found by Cunha et al. (2015), which verified that chemical insecticides such as Thiamethoxan (Cruizer®), Abamectin + Thiamethoxan + Fludioxonil (Avicta®), Imidacloprid + Thiodicarb (Cropstar®)) among others contributed to a greater deterioration of soybean seeds.

Table 6. Mean vigor values of barley seeds in the accelerated aging test, treated via IST with insecticide and fungicide, mixed or not. UEG, 2020, Ipameri-GO

Treatment via IST	Normal Seedlings (%)
Iprodione (Rovral®)	48 ab
Thiamethoxan (Cruizer®)	43 b
Iprodione (Rovral®) + Thiamethoxan (Cruizer®)	47 ab
Control	51 a
Average	47

Equal means do not differ statistically from each other at 1% probability by the Tukey test.

From the results obtained, the influence of storage time on the vigor of treated barley seeds is evident (Table 7). The accelerated aging test is one of the most studied and recommended for several species and was developed with the purpose of estimating the longevity of stored seeds (Ohlson, et al., 2010). In general, it appears that the seed treatment promoted a reduction in the percentage of normal seedlings (Table 6), evidencing the drop in seed viability after being exposed to the stress situation. These results are inconsistent with those obtained by Flach (2015), who studied the effect of chemical treatment of cotton seeds, with different storage times on seeds treated or not with fungicides and insecticides, including the insecticide used in the present study, and not found significant differences in the accelerated aging test up to 60 days, occurring after 90 days of storage.

Table 7. Mean values of vigor of barley seeds, in the accelerated aging test, treated via IST with insecticide and fungicide, mixed or not, depending on the storage periods. UEG, 2020, Ipameri-GO

Storage Periods (days)	Normal Seedlings (%)
0	51 a
60	44 b
Average	47

Equal means do not differ statistically from each other at 1% probability by the Tukey test.

For the barley seedling length test, it was found that there was a significant difference only in relation to storage time, with the highest value obtained at time zero (Table 8), that is, with seeds analyzed after treatment, without conditioning. The reduction in seedling length may be related to the phytotoxic effect caused by the products used in seed treatment (Castellanos, et al., 2017).

In the work conduced by Dan et al. (2010) it was observed that among the insecticide treatments evaluated, the insecticide Thiamethoxan (Cruizer®) provided the greatest reduction in the length of soybean seedlings, of the order of 0.12 cm per day of storage, this work corroborate the results obtained in this research.

Table 8. Mean values of seedling length from barley seeds, treated via IST with insecticide and fungicide, mixed or not, depending on storage periods. UEG, 2020, Ipameri-GO

Storage Periods (days)	Seedlings length (cm)
0	12.0 a
60	10.7 b
Average	11.3

Equal means do not differ statistically from each other at 1% probability by the Tukey test.

4. Conclusion

The germination of barley seeds treated with the fungicide Iprodione (Rovral®) and the insecticide Thiamethoxan (Cruizer®), alone and in a mixture applied via IST, remains within the standards for commercialization up to 60 days of storage. In addition, it is concluded that the use of the insecticide Thiamethoxan (Cruizer®) via IST presents greater damage to the viability and vigor of barley seeds stored up to 60 days, applied alone or in a mixture with the fungicide Iprodione (Rovral ®).

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Authors contributions

Dr. Itamar R. Teixeira and Dr. Gisele Carneiro da Silva were responsible for study design and revising. Prof. Valter Vaz was responsible for data collection. Prof. Tamires Ester P. Bravo and Prof. Valter Vaz drafted the manuscript and Dr. Itamar R. Teixeira revised it. All authors read and approved the final manuscript.

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Obtained.

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The Publication Ethics Committee of the Canadian Center of Science and Education.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.