

Efficacy of Phytohormone on Physiological and Biochemical Changes in Aflatoxin B1 Treated Wheat Seed (*Triticum Vulgare* L)

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Abstract – Wheat (*Triticum aestivum* L.) is a cereal of great economic significance and capacity for production. Nonetheless, different diseases that require the use of fungicides during crop development can be affected. The objective of this study was to evaluate the physiological quality of wheat seeds when treated with concentrations of fungicides during crop development. The foliar application of fungicides with the active ingredients strobilurin and triazole plus foliar adjuvant has been cultivated in plants of the Mirante variety. The seed moisture content, germination percentage, accelerated ageing, controlled deterioration, cold test, sand emergence, electrical conductivity and the presence of aflatoxins were evaluated following harvesting. The results obtained showed that when the studied fungicides were used, the physiological quality and aflatoxin presence in wheat seeds were not affected. Even when subjected to stressful conditions, the seeds showed a high index of seedling germination.

Key Words – Foliar application. Germination, *Triticum Aestivum* L. Vigor.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) has a great economic importance among the cold climate cereals. It is grown under the most diverse environmental conditions, with a large capacity for grain yield, nutritional quality and a high degree of adaptability (MARINI et al., 2011). In Brazil, the increase in wheat yield is of economic interest because its cultivation improves soil conditions in addition to meeting demand and provides waste for summer crops, such as soybeans and maize (BARBIERI et al., 2013). High physiological quality of wheat seeds is very important due to high consumption because it increases productivity and results in lower cost of yield. According to Mielezrski et al. (2008), plants with greater physiological potential originated with high seed vigour, which is reflected in higher growth and yield. However, in order to achieve good conditions during the cultivation of wheat, disease control should be carried out from the outset. The management of diseases guarantees the physiological potential for the best seeds. Seeds are transmitted by most diseases that attack wheat, which can cause vigour and germination to decrease. Seeds infected by pathogens may not demonstrate viability or have low vigour, according to Peske et al. (2006). Seeds are a pathogenic vehicle that can sometimes cause disease outbreaks in crops because there may be significant epidemiological significance of small amounts of inoculum.

Wheat is very susceptible to infection with fungi and contamination with mycotoxins. This cereal is highly cultivated in Southern Brazil, which has a subtropical climate in its region. The combination of this type of climate and the non-adoption of no-tillage systems can encourage the onset of serious epidemics, cause severe yield problems and reduce the quality of wheat (ASTOLFI et al., 2011). Fungal pathogens may result in mycotoxin contamination of wheat during flowering, delayed harvest due to wet conditions, and during storage. Several species of *Fusarium*, *Penicillium* and *Alternaria* may infect grain if the harvest is delayed due to wet conditions and if there is sufficient moisture to support fungal growth, isolates of *Aspergillus* and *Penicillium* may infect grain during storage (Jacobsen, 2014). In general, the treatment of seeds consists of the application of processes and substances which preserve or refine their performance. This includes the application, or submission to other physical processes, of pesticides (fungicides, insecticides), biological products, inoculants, etc. It refers to the application of effective chemicals against plant pathogens in a more specific sense (MENTEN; MORAES, 2010).

The presence of chemical contaminants such as pesticides and mycotoxin waste in wheat is not visualised in the final product and is therefore one of the main challenges in food production (EMBRAPA, 2013). To guarantee safe food consumption, quality

control is essential. Contamination of aflatoxin produced by *Aspergillus* spp (*A. flavus*, *A. parasiticus*, *A. niger*, *A. ochraceus*, *A. oryzae*, among other species) has often been identified at high levels in countries with warmer climates for wheat and its by-products (AYDIN; GUNSEN; DEMIREL, 2008; GHALI et al., 2008; JOUBRANE et al., 2011). The Ministry of Health Regulation (RDC No. 7, 18 February 2011) recommends that, for the sum of aflatoxins B1 + B2 + G1 + G2 + G2 + G1, the permissible limits for mycotoxins in Brazil in wheat and its by-products are 5 µg kg⁻¹ (BRASIL, 2011). Aflatoxin B1 (AFB1) is the most potent hepatocarcinogen found in mammals and is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC).

Mycotoxins are poisonous compounds produced by certain species of fungi located in contaminated grain, according to Neme and Mohammed (2017). Aflatoxin, fumonisin, deoxynivalenol (DON), ochratoxin (OT), and zearalenone are five major groups of mycotoxins that may occur in grains: (ZEN). In the field, harvesting, handling, storage, and processing, their occurrence can begin. DON, ZEN, and fumonisins may begin to cause field/or pre-harvest grains, while aflatoxin and OT are mostly present during storage due to improper handling of the post-harvest. The majority of mycotoxin susceptible grains such as maize, peanut/groundnut, sorghum, millet, wheat, and rice have been examined. Mechanical injury, insect infestation, and harvest time, drying method, kinds of storage structure and conditions, handling and processing are the primary post-harvest factors for the cause of grain mycotoxin contamination. The primary factors for mycotoxin growth and development are temperature, humidity, and humidity.

In terms of cultivation, production, and consumption, wheat is the most important cereal crop in Iran. Because of the abundance of production and the primary role that wheat and its meat products play in human and animal diets. In the event of contamination with health-threatening variables, they can play a very important role in endangering human health. Razdari et al. (2014) verified in this context that by reducing wheat storage time and controlling humidity, it is possible to decrease aflatoxin contamination. Results have shown that a lot of mycotoxins with a level of 36.3 to 2.891 mg/g have been found. This fact happens because of the detrimental impact of the milling process. Therefore, it is of great importance to prevent their growth and contamination by decreasing temperature, humidity, and pesticide treatment.

MATERIAL AND METHODS

The samples were wheat seeds produced in the experimental area of the Faculty of Agronomy and Veterinary Sciences in Passo Fundo, RS, Brazil, between July and November 2011. The altitude of 687 m, the humid temperate climate and the annual average temperature are between 12.7 and 22.1 ° C. The minimum is set in June and the maximum is set in

January. The average precipitation is 1,788 mm, with year-round precipitation and temperatures close to or below zero (EMBRAPA, 2013) in soil classified as OXISOL. In the first half of July, seeding was carried out under a no-tillage system with a density of 350 viable m⁻² seeds, with maize as the preceding crop after desiccation with the herbicide glyphosate. Post-emergent herbicide paraquat contact (Gramoxone) at a dose of 1.5 L h⁻¹ was desiccated pre-sowing.

According to soil analysis, the fertiliser was distributed along seeding rows with 250 kg ha⁻¹ fertiliser in the formulation 5-25-0 N-P-K. Application of nitrogen in the coating of 45 kg ha⁻¹ N in the form of granulated urea was carried out between the tillering and elongation stages corresponding to 30 and 45 days following emergence. In areas of average or high fertility with adequate management, the Mirante wheat variety was selected for its productive potential. It is also known to be vulnerable to diseases such as leaf rust, scab and leaf spots, but is resistant to powdery mildew, mosaic and *Pyricularia oryzae* Cav. In Rio Grande do Sul, this variety has an average cycle. Silking occurs after 77 days and after 128 days of maturity. The seeds were treated with triadimenol (Baytan) fungicide at 2.0 mL kg⁻¹ seed and with imidacloprid (Gaucho) insecticide at 1.0 mL kg⁻¹ seed.

In the post-emergence stage, herbicides such as metsulfuromethyl (Ally at 6 g ha⁻¹ dose for dicotyledonous weeds and iodosulfuromethyl (Hussar®) at 100 g ha⁻¹ dose for poaceae weeds, such as oats and ryegrass, have been used to control them. Insecticide imidacloprid + triflurumuron (Connect) at a dose of 500 mL ha⁻¹ was applied to control insects in the shoot during the stalk elongation stage in a single application. In foliar disease prevention applications, fungicides such as Nativo, Opera and Priori Xtra have been used in two applications at a dose of 0.6 L ha⁻¹ (600 mL ha⁻¹). These fungicides have strobilurin and triazole plus leaf adjuvant as their active principles. While a leaf adjuvant, Tensor Plus, has been used as a control. The treatment distribution is illustrated in Table 1.

For the application, a portable pressurised spray with CO₂ and a ha⁻¹ rate of 100 L was used. In order to release fine droplets, the spray nozzles were simple flat jets, the Teejet XR110015 series, and the operating pressure was 2.0 bars. The movement velocity was 5.76 km h⁻¹ (1.6 ms⁻¹). The preparation order for this broth was 1 L of water + Tensor Plus + Nimbus Oil + Priori Xtra Fungicide + the required volume of water to complete the final broth with 2 L of water. In each treatment, the measurement of the pH in the preparation of the broth was also carried out. The water used for this experiment had a pH of 6.64.

Table 1. Distribution of treatments

Treatment		Broth preparation (mL / 2 L)	
Fungicide	Adjuvant	Fungicide	Adjuvant
1. Nativo	—	12 mL	—
2. Nativo	Áureo	12 mL	10 mL
3. Nativo	Break Thru	12 mL	2 mL
4. Nativo	LI 700	12 mL	3 mL
5. Nativo	Glycerine	12 mL	20 mL
6. Opera	—	12 mL	—
7. Opera	Assist	12 mL	10 mL
8. Opera	Break Thru	12 mL	2 mL
9. Opera	LI 700	12 mL	3 mL
10. Opera	Glycerine	12 mL	20 mL
11. Priori Xtra	—	—	—
12. Priori Xtra	Nimbus	—	10 mL
13. Priori Xtra + Tensor Plus	Nimbus	3.0 mL	3.0 mL
14. Priori Xtra + Tensor Plus	Nimbus	5.0 mL	3.0 mL
15. Control	—	—	—

Table 2. Information relating to fungicides application in wheat crop

Applications	First sept, 15th/2011)		Second (Oct., 05th/2011)	
	Beginning	End	Beginning	End
Timetable	15 h 30 min	16 h 40 min	15 h 30 min	16 h 10 min
Air temperature	20.5 °C	23.0 °C	28.1 °C	27.2 °C
Relative humidity	57.5%	48%	50%	48.5%
Wind speed	5 to 6 km ⁻¹	2 to 3 km ⁻¹	2 to 3 km ⁻¹	2 to 2.5 km ⁻¹

In Table 2 the weather conditions, at the beginning and end of applications with fungicides, are indicated.

On November 20, 2011, the harvest took place with a combined harvester parcel, so that eight core rows were collected for each parcel, for a total sample area of 13.6 m². The seeds harvested have been cleaned, weighed and the content of water determined. They were immediately placed in multi-coated paper bags and stored at the Application Technology Laboratory of the University of Passo Fundo at room temperature until the end of January 2012 in a dry environment (50 per cent relative humidity). Then, the samples were placed in four plastic bags and a tablet of the insecticide steaming phosphine (Gastoxin) was placed in each bag to control pests from stored seeds. These bags were transferred to the dry chamber of the University of Passo Fundo's Seed Analysis Laboratory, where they were kept under 20 °C. They were sent to the Seeds and Plant Evaluation Laboratory (LASP) in May 2012, which remained refrigerated until the seed analysis was carried out.

At LASP and Quality of Agricultural Products Control (LACON), both from the Western Paraná State University's Center of Exact Science and Technology (UNIOESTE), Campus Cascavel, PR, Brazil, analyses were carried out. The evaluations were only at the beginning of the experiment to check seeds quality, and the tests were:

a) Germination: For each sample, two repetitions of 100 seeds were placed on a filter paper substrate, in rolls previously moistened with distilled water (2.5 times the weight of dry paper soaked in water) taken at the temperature of the germinator of 25 °C. On the eighth day after sowing, the number of seedlings was carried out and the results were expressed in percentage terms.

b) Cold test: On filter paper, two repetitions of 100 seeds were sown, wrapped in plastic bags and retained for seven days at 10 °C. They were later placed at 25 °C in a germinator and the seedlings were counted on the fourth day (BARROS et al., 1999).

c) Accelerated aging: Four replications of each treatment were previously weighed (± 42.5 g) and spread over the surface of the metal screen gerbox to form a layer of seeds. Therefore, 40 mL of water was added in order to maintain relative humidity inside the box (100 percent RH). For 48 hours after the seeds were submitted to the germination test, plastic boxes remained at 41 °C of the ageing chamber as they were evaluated four days after sowing, and the results were expressed as a percentage of (BRASIL, 2009).

d) Moisture content: four samples of 5 g from each treatment were weighed, placed in aluminum capsules and dried at 105 °C for 24 hours. The seeds were weighed again after dried and then moisture content was calculated according to Brasil (2009).

e) Mass of 1000 seeds: Two samples of 100 seeds were randomly counted and weighted on a precision scale of 0.001 g. The average was expressed in grammes, and thousands of seeds were transformed into a mass. This amount was adjusted according to the moisture content, defined as the adjusted mass, based on the average moisture content, in order to obtain the real weight of the seed mass (BRASIL, 2009).

f) Electrical Conductivity: Four samples of 50 pure seeds were weighed, placed in vials containing 75 mL of deionized water and held for 24 hours at 25 °C in the BOD chamber. The samples were stirred and the electrical conductivity reading was obtained after soaking. The mass and the results expressed as mmhos cm⁻¹g⁻¹ were divided by this value (VIEIRA, 1994).

g) Controlled deterioration: For this test, the water content of the seed samples was adjusted to 15.5 percent, and then the seeds were wrapped in aluminium foil, packed in plastic and kept at 40 °C for 48 hours in a water bath. The seeds were submitted to the germination test and the fourth day of assessment was carried out.

h) Emergency in sand: four replications of 50 seeds from each treatment were used and sown in plastic boxes with washed sand. After seedling emergence, the counting

considered normal seedlings on the eighth day after sowing date. The results were expressed in percentage (NAKAGAWA, 1994).

- i) **Aflatoxins:** By the official 991.31 AOAC method (AOAC, 1982), with immunoaffinity columns, followed by HPLC injection, the determination of B1, B2, G1 and G2 was achieved. The aflatoxin calibration curves were prepared by injecting 0.5 mg L⁻¹ aliquots of standard solutions of aflatoxin (B1, B2, G1 and G2) in order to obtain concentrations of 0.0025; 0.005; 0.01 and 0.025 mg L⁻¹. Area versus mass graphs of the injected aflatoxin B1, B2, G1 and G2 were prepared to record the linearity of the calibration curve, with $r^2 \geq 0.99$. There was a volume of 20 μ L injected into HPLC and a run time of 15 minutes per sample. In duplicate, the samples were obtained and the average was calculated. Under the following conditions, liquid chromatography was carried out: temperature: 40 °C; 4.6 mm x 25 cm column, 5 μ m C18 (Kromasil); mobile phase - methanol:water (40:60); degassed isocratic and fluorescence detector - excitation 365 nm and emission 450 nm. Toxin identification was carried out on the basis of retention time and a quantitative analysis of the toxin area was performed. Aflatoxin G2, G1, and B1 B2 elution occurred at 6.874, 7.813, 10.664, and 12.071 minutes, with quantitative limits of 0.5, 0.5, 0.05 to 0.5 μ g.kg⁻¹ and coefficients of recovery of 68, 71, 93, and 98% respectively.

RESULTS AND DISCUSSION

The germination percentage averages for normal, abnormal seedlings and dead wheat seeds in the initial state and submitted to the accelerated ageing and cold test, treated with fungicide and adjuvant during crop development, are shown in Table 3. In treatments 1 (Nativo without adjuvant), 3 (Nativo + adjuvant Break Thru), 6 (Opera without adjuvant) and 7 (Opera + Assist adjuvant), there was a significant difference, with a lower percentage of germination than the others, with the exception of treatment 1, which showed less than 80% of germination. The percentages of abnormal seedlings and dead seeds were not affected by the treatment, indicating that the products tested did not have any influence.

Table 3. Germination percentage averages for normal, abnormal seedlings and dead seeds of wheat in initial condition and submitted to accelerated aging tests and emergency in sand after treatments with fungicides and adjuvants during crop development

Treatment	% germination			% abnormal			% dead seeds		
	initial	cold test	acc. aging	initial	cold test	acc. aging	initial	cold test	acc. aging
1	79 a	90 a	70 a	7 a	3 a	2 a	14 a	7 a	28 b
2	89 b	94 a	82 b	4 a	1 a	5 c	8 a	6 a	13 a
3	82 a	92 a	80 b	6 a	1 a	5 c	8 a	7 a	15 a
4	86 b	94 a	84 b	4 a	2 a	3 b	10 a	4 a	13 a
5	87 b	91 a	71 b	3 a	2 a	3 b	10 a	7 a	26 b
6	85 a	90 a	68 a	5 a	3 a	9 c	9 a	8 a	23 b
7	85 a	90 a	60 a	3 a	4 a	8 c	12 a	7 a	32 b
8	87 b	93 a	80 b	2 a	2 a	4 b	8 a	5 a	16 a
9	91 b	92 a	77 b	2 a	2 a	8 c	8 a	5 a	15 a
10	91 b	94 a	55 a	3 a	1 a	4 b	7 a	6 a	41 b
11	90 b	93 a	72 b	2 a	1 a	1 a	9 a	6 a	27 b
12	88 b	96 a	53 a	1 a	1 a	5 c	10 a	4 a	42 b
13	90 b	92 a	62 a	2 a	2 a	3 b	9 a	6 a	35 b
14	88 b	95 a	67 a	1 a	1 a	3 b	10 a	4 a	30 b
Control	89 b	93 a	79 b	1 a	2 a	5 a	8 a	5 a	19 a

No significant differences were found between Garcia Júnior, Vechiato and Menten (2008) and the wheat seed germination variety BR 18 Terena with the use of fungicides such as tebuconazole, captan, difeconazole, tolyfluanide, fludioxonil, triflumizole, triadimenol, triticonazole, thiabendazole and methyl thiophanate.

Rampim et al. (2012) studied the physiological quality of seeds of three wheat varieties submitted to bio-stimulant, triadimenol and Azospirillum brasilense therapies, and observed that the interaction between the treatments and varieties used had no influence on germination.

Asghar et al (2016) concluded that, as the responsible pathogens attack and destroy the seedlings, seed borne diseases have been found to influence the growth and productivity of crop plants. Fusarium and spp. of Alternaria It is also responsible for decreasing the rate of germination and for inducing seedling blight. In contrast to healthy wheat samples, the seed germination rate was lower in contaminated wheat. For example, the germination rates of seeds were 84.6 percent and 45.2 percent respectively in healthy and contaminated wheat samples. It has also been concluded, on the basis of the results obtained, that fungal pathogens have adverse effects on wheat seed germination.

The residual effect of fungicides used in soybean seeds was analysed by Gagliardi et al. (2009) through germination, accelerated ageing, sand emergency and sanitation tests. They concluded that the following treatments did not influence the quality of the seeds: Priori + Nimbus, Aproach + Nimbus, Priori Xtra + Nimbus, Sphere + Aureo, Opera, Stratego + Aureo, Nativo + Aureo, Impact Duo + Oppa, Celeiro + Iharol, Battle + Oppa, Aproach Prima + Nimbus and Folicur. However, Rufino et al. (2013) observed that when applied alone or with Zn and polymer combination application, the germination percentage is positively affected by fungicide treatment.

Table 4. Averages of moisture content, mass of a thousand seeds and the mass of a thousand seeds adjusted according to average of seeds moisture content (7.39%) after treatments with fungicides and adjuvants during crop development

Treatments	Moisture content (%)	Mass of thousand seeds (g)	Mass of thousand seeds (g) adjusted to moisture content
1	7.3 a	40.52 b	41.32 a b c
2	6.9 a	43.19 b	46.53 a b d
3	6.7 a	41.21 b	45.80 a b d
4	7.1 a	40.64 b	43.15 a b c
5	6.5 a	40.94 b	46.49 a b d
6	6.8 a	42.23 c	46.07 a b d
7	7.3 a	42.02 c	42.79 a b c
8	6.1 a	42.63 c	52.30 d
9	6.6 a	42.38 c	47.23 b d
10	8.0 a	42.42 c	43.16 a b c
11	8.4 b	41.75 c	36.55 c
12	8.4 b	42.87 c	37.55 c
13	7.9 b	42.98 c	40.14 a b c
14	8.1 b	43.19 c	39.23 a c
Control	8.6 b	33.83 a	28.84 e

Table 4 shows the average moisture content, the mass of one thousand seeds and the mass of one thousand seeds, adjusted according to the average moisture content of the seeds (7.39%). There was a significant difference in the moisture content of wheat seeds in treatments 1 to 10, differentiated from other treatments and controls which showed higher moisture content.

With regard to the mass of one thousand seeds, there is a significant difference between the controls and the other treatments. On the other hand, treatments 1 (Nativo + adjuvant Break Thru), 3 (Nativo + adjuvant Break Thru), 4 (Nativo + adjuvant LI 700) 5 (Nativo + adjuvant Glycerine) and 11 (Nativo + adjuvant Priori Xtra without adjuvant) are less massive than the others. It can be observed that treatments 2 (Nativo + Aureo adjuvant) and 14 (Priori Xtra + Nimbus adjuvant with a dose of 0:25 + 0:15 per cent) showed the highest seed mass and different adjusted mass values, meaning that the increase in humidity was responsible for the increase in mass during treatment 14.

The averages of electrical conductivity, controlled deterioration and emergence in sand are shown in Table 5.

Table 5. Averages of electrical conductivity, controlled deterioration and emergence in sand of wheat seeds after treatments with fungicides during the crop development

Treatments	EC ($\mu\text{S}/\text{cm}^2/\text{g}^{-1}$)	Deterioration (%)	Emergence (%)
1	21.79 b	83 a	73 a
2	17.74 a	84 a	92 a
3	18.84 b	89 a	88 a
4	17.15 a	80 a	95 a
5	17.71 a	91 a	89 a
6	19.98 b	85 a	91 a
7	14.67 a	85 a	93 a
8	17.53 a	77 a	92 a
9	17.53 a	90 a	90 a
10	18.00 a	84 a	92 a
11	23.95 b	83 a	85 a
12	22.86 b	88 a	96 a
13	25.18 b	89 a	93 a
14	21.41 b	89 a	89 a
Control	19.75 b	88 a	89 a

Treatments 1 (Nativo + adjuvant Break Thru), 3 (Nativo + adjuvant Break Thru), 6 (Opera without adjuvant), 11 (Priori Xtra + adjuvant Nimbus), 12 (Priori Xtra +

adjuvant Nimbus), 13 (Priori Xtra (3 mL) Nimbus + adjuvant (3 mL)) and 14 (Priori Xtra + adjuvant Nimbus + 0,25 + 0,15 percent dose) and control showed the highest values, indicating higher deterioration of the membranes of the seeds. These results reflect the lowest seed strength in the treatment, which was significantly different from the other treatments.

The objective of the electrical conductivity test was to evaluate the quantity of ions present in the soaking water and, indirectly, the seed vigour, on the basis that the vigour is related to the integrity of the cell membrane system (MARCOS FILHO; CICERO; SILVA, 1987). Based on the germination of seeds after the controlled deterioration test, there were no significant differences between treatments, including control, in the results. An exception was only treatment 8 (Opera + Break Thru adjuvant), the germination of which was greater than 80% . Although electrical conductivity and accelerated ageing tests have indicated a potential change in physiological quality, vigorous seeds have been shown in the controlled deterioration test. Marcos Filho, Novembre e Chamma (2001) discovered in soybean seeds that the results of the controlled deterioration test were similar to those obtained following accelerated ageing. Both tests were consistent in assessing the physiological quality of the seeds, and therefore differed from the current results, which showed germination in excess of 80% only in two treatments after the seeds were submitted to the accelerated ageing test. Santorum et al (2013) also found that similar results in soybean seeds were shown in the accelerated ageing and controlled deterioration tests, so they are indicated to differentiate the vigour between the batches. There was no significant difference between treatments in the emergency of seedlings in sand, and all of them showed emergence greater than 80% except for treatment 1 (Nativo without adjuvant). These findings are similar to the results of Garcia Junior, Vechiato and Menten (2008), which showed no significant differences in the emergence of fungicide treatment and control in wheat seeds. This indicated an absence of negative or positive effects, without affecting the germination of the seeds of the products tested.

The results for the detection analysis of aflatoxins in wheat seeds treated with fungicides during their development are shown in Table 6.

Table 6. Levels of aflatoxin (B1, B2, G1 and G2) in wheat seeds after fungicide and adjuvant treatments during crop development

Conditions	Sample		Average	Total
	1	2		
1	nd	Nd	nd	Nd
2	nd	Nd	nd	Nd
3	nd	Nd	nd	Nd
4	nd	nd	nd	Nd
5	nd	nd	nd	Nd
6	nd	nd	nd	Nd
7	nd	nd	nd	Nd
8	nd	nd	nd	Nd
9	nd	nd	nd	Nd
10	nd	nd	nd	Nd
11	nd	nd	nd	Nd
12	nd	nd	nd	Nd
13	nd	nd	nd	Nd
14	nd	nd	nd	Nd
Control	nd	nd	nd	Nd

The presence of aflatoxins B1, B2, G1 and G2 in wheat seeds studied did not occur. This indicates that the presence in grains of chemical contaminants and the storage period after harvesting did not favour the presence in wheat seeds of aflatoxins B1, B2, G1, and G2. Perhaps because these treatments were adequate during crop development and the presence of fungi was controlled. In wheat flour, Taheri et al (2012) observed 3.1 percent and 7.4 percent of aflatoxin B1 content, classified as greater than the established limits. According to the authors, samples collected during the summer and winter, respectively, may have interfered with the moisture content during storage. Trombete et al (2014) also assessed wheat grain and trading flour, although the presence of aflatoxin has been detected, the samples did not register with limits greater than those established by Brazilian law (5 µg kg⁻¹), so they are safe as food.

CONCLUSION

The physiological quality of wheat seed is not affected, even under stressful conditions such as cold and controlled deterioration, when fungicides are applied during crop development, with a high germination rate. The products used guarantee the sanitary quality of the seeds with respect to the presence of aflatoxin even during the storage period. The seed moisture content, germination percentage, accelerated ageing, controlled deterioration, cold test, sand emergence, electrical conductivity and the presence of aflatoxins were evaluated following harvesting. The results obtained showed that when the studied fungicides were used, the physiological quality and aflatoxin presence in wheat seeds were not affected.

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