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RESEARCH ARTICLE

Chemical treatment and storage period influence on physiological characteristics of maize seeds

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The seeds physiological quality is a fundamental characteristic to obtain competitive and productive plants, where seeds treatment is an important tool to keep seeds physiological high-quality. The present study aimed to evaluate chemical treatments and storage period influence on seeds physiological quality. It was used a factorial (4 x12) randomized complete block design, with eight replications. In factor A, the chemicals (thiamethoxam, metalaxyl + fludioxonil + thiabendazole and pyraclostrobin + thiophanate-methyl + fipronil) and control. In the factor B, the treated seeds storage periods (0, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 90 days). The variables evaluated were vigor and germination, and the tests were performed at $p \leq 0.05$. The results indicate that the maize seeds vigor is maintained with the fungicide application, regardless of the vigor test used. As the treated seeds storage period increases, their vigor was reduced by the first count test. However, maize seeds germination was not influenced by seed treatment or storage period.

Highlighted Conclusions

1) The maize seeds vigor is maintained applying fungicides, regardless of the vigor test used.
2) The first count test indicates that the longer the untreated or treated maize seeds with thiamethoxam are stored the lower the seed vigor.
3) Maize seeds germination is not influenced by the thiamethoxam, metalaxyl + fludioxonil + thiabendazole and pyraclostrobin + thiophanate-methyl + fipronil chemicals, not even by the storage period up to 90 days of treated seeds.

The highest maize (*Zea mays* L.) yields or any other agricultural crop, occurs applying new technologies and scientifically proven techniques. In the world maize stands out as the most produced cereal, and in the 2016/17 harvest, production was 1.36 billion tons. Brazil produced 99.85 million tons in the same year harvest, the third largest producer (USDA 2017). In this scenario, maize crop presents economic, social and cultural expression, contributing to the farms food security. It is understood that the seed treatment is basically the net application of a small amount material uniformly on the seeds, presenting a potential to improve its performance (Murphy 2017). In addition, other practices are used, such as seed coating with polymers, dyes and fillers, which make it possible to minimize the active ingredients (insecticides) leaching from treated seeds (Avelar et al. 2012, Pedrini et al. 2017).

The application uniformity over a short period of time, low material cost and the fact that no additional energy is required for drying, makes liquid seed treatment widely used for most grown crops (Murphy 2017). The seeds chemical treatment with fungicides favors to keep the vigor, especially for maize seeds, since they are efficient to control the main storage grain fungi, especially when the seeds present low physiological quality, such as germination and vigor (Govender et al. 2008, Pereira et al. 2008, Ramos et al. 2008).

For the insecticides applied in the seeds by chemical treatment, there are studies indicating that they may negatively influence the germination, more reported with the increase of the storage time for sunflower seeds (Mrđa et al. 2010), but without phytotoxicity for rice (Tang et al. 2017). For maize seeds, the insecticides applied by seeds chemical treatment can affect the seeds germination and vigor, with possible increase with in longer storage time (Salgado and Ximenes 2013, Tonin et al. 2014).

The initial maize seedlings growth is directly related to seed vigor (Mondo et al. 2013), consequently, this will reflect on the seedling performance in the field (Dias et al. 2010). In addition, vigor is directly related to the competitive ability and maize grain yield (Mondo et al. 2012). In order to evaluate seed vigor, the main tests used are: Cold tests, Conductivity tests, Seedling performance tests and Tetrazolium test (Yang and Wen 2017).

Seed germination is the main test performed and required for the commercialization of a particular seed lot, but tests that express seed vigor are useful in monitoring seed performance during storage, as it precedes seeds viability loss (Yang and Wen 2017). Deterioration is an inevitable and progressive process, which causes a decrease in seed vigor and viability, mainly due to fungi and insects attack (Deuner et al. 2014).

Seed storage is currently being studied and discussed, as several studies have already shown that storage conditions directly influence crop seed quality. The seeds physiological potential evaluation is the main component in the seeds quality control after the storage period, regardless of whether the seed is chemically treated or not (Govender et al. 2008, Kamizake et al. 2014).

In view of the above, this work aimed to evaluate different maize seeds chemical treatments and storage period influences to the seeds physiological characteristics.

MATERIAL AND METHODS

The experiment was conducted in the Federal University of Fronteira Sul (UFFS) Campus Erechim-RS seed analysis laboratory. The design was a bifactorial completely randomized (4 x 12), with eight replications. In factor A, the 4 seed treatments were allocated: T1 – Control, T2 – Thiamethoxam, T3 – Metalaxyl + Fludioxonil + Thiabendazole and T4 – Pyraclostrobin + Thiophanate-methyl + Fipronil, presented in Table 1. In factor B, the treated seeds storage times: 0, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 90 days. Seeds were stored in room temperature, simulating a conventional seed storage warehouse, packaged in paper bags.

Table 1. Maize seeds treatments used submitted to storage periods.

Treatments	Concentration ($g\ L^{-1}$)	Active ingredient dose (g)*	Product dose (L)*
T1 – Control	---	---	---
T2 – Thiamethoxam	350	42,00	0.12
T3 – Metalaxyl + Fludioxonil + Thiabendazole	20 + 25 +150	0.60 + 0.75 + 4.50	0.03
T4 – Pyraclostrobin + Thiophanate-methyl + Fipronil	25 + 225 + 250	5.00 + 45.00 + 50.00	0.20

* Equivalent dose to apply to 60,000 maize seeds.

It was used the Dow 2B587 Power Core maize hybrid seeds, and the treatment process was carried out in a 10 L plastic buckets, simulating the mixture performed by a seed treating machine. After mixing, it was homogenized by shaking the seeds in the buckets for two minutes. It was followed the technical recommendations for each product in the process (Table 1).

Seed vigor was evaluated by the first germination count tests, carried out along with seed germination test and the first count of the accelerated aging test. The first germination count test criteria is when seeds emit the primary root (greater than 2 mm) in seedlings, which is already validated as a vigor test for maize seeds (Khajeh-Hosseini et al. 2009). This test is based on the principle that high-vigor seeds emit the primary root faster than those less vigorous when comparing different lots (Pereira et al. 2008, Coimbra et al. 2009). The second vigor test was performed through the accelerated aging test, in which 50 seeds with eight replications were arranged on aluminum screen, in a single layer, in gerbox-type boxes (0.11 x 0.11 x 0, 03 m) containing 50 ml of distilled water in the bottom. The gerbox-type boxes were capped and kept in a B.O.D type incubator, set at 42 °C for 72 hours in the dark. After this aging period, the seeds were submitted to the germination test, according to the Regras de Análise de Sementes (Seed Analysis Rules) (Brasil 2009), with a single evaluation at the fourth day after the test installation, where normal seedlings were considered showing at least 1 cm of aerial part and root protrusion.

For the germination test were analyzed 400 seeds of each treatment, dividing into eight replications of 50 seeds each. Germitest paper, moistened with distilled water (2.5 times dry paper mass), was kept in a germination chamber at 25°C with 12 hours of daily light. The evaluations were carried out based on the Regras de Análise de Sementes (Seed Analysis Rules) (Brasil 2009), accounting for the normal seedlings percentage at four days (1^{st} count) and seven days (2^{nd} count) after the test installation.

The data were submitted to analysis of variance by the F test, and when significant Tukey's test was applied for the treatments means in the qualitative factor (seed treatment) and regression was applied for the quantitative factor (storage time). The model's choice for the quantitative factor was based on statistical significance (F test), on the coefficient of determination (R^2) and on the biological model meaning, as proposed by Adati et al. (2006). All tests were performed at $p \leq 0.05$.

RESULTS AND DISCUSSION

Seeds vigor results by the first count and accelerated aging tests were presented in Table 2 and 3, where the seed treatment factor showed a significant effect. In Table 4 we have the germination data, which did not show a significant effect by the F test. For the quantitative factor (treated seeds storage periods), only the control treatments and thiamethoxam application presented significance, represented by a decreasing exponential equation. The other variables evaluated did not present a significant effect (Figures 1, 2 and 3).

It was observed that maize seeds vigor, by the first count test, after being submitted to the seed treatment and 90 days stored periods, presented differences between the evaluated products. Only the 3 days seeds storage did not cause significant difference. In the other periods, treatment with metalaxyl + fludioxonil + thiabendazole showed superiority, except in the 49 days period, in which the treatment with pyraclostrobin + thiophanate-methyl + fipronil was the most effective.

Table 2. Maize seeds vigor (%) by the first count test, due to treatments and storage period.

Storage period (days)	Maize seeds treatment			
	Control	Thiamethoxam	Metalaxyl + Fludioxonil + Thiabendazole	Pyraclostrobin + Thiophanate-methyl + Fipronil
0	89 ± 1.9 B*	94 ± 1.7 A	96 ± 1.6 A	90 ± 1.2 B
3	94 ± 1.9 A	95 ± 3.0 A	97 ± 2.4 A	96 ± 1.6 A
7	95 ± 3.3 B	95 ± 1.9 B	98 ± 0.9 A	97 ± 1.2 AB
14	91 ± 1.9 B	91 ± 1.7 B	96 ± 2.3 A	93 ± 3.3 B
21	88 ±1.7 B	92 ± 1.4 A	92 ± 2.4 A	85 ± 2.4 C
28	91 ± 3.2 BC	90 ± 1.6 C	93 ± 3.1 AB	94 ± 1.4 A
35	90 ± 2.0 B	90 ± 2.1 B	96 ± 0.9 A	97 ± 1.4 A
42	89 ± 1.7 C	90 ± 1.3 BC	96 ± 1.6 A	92 ± 0.9 B
49	91 ± 0.8 B	90 ± 1.6 B	91 ± 2.1 B	97 ± 2.3 A
56	89 ± 1.4 B	90 ± 1.9 B	96 ± 0.4 A	95 ± 1.4 A
63	91 ± 1.5 BC	90 ± 1.3 C	93 ± 0.8 AB	94 ± 1.6 A
90	89 ± 2.0 BC	91 ± 1.7 AB	92 ± 1.9 A	88 ± 3.1 C
Means	91 C	91 C	95 A	93 B
C.V (%)	2.22			

* Means and standard deviation followed by the same letter in the line do not differ from each other by the Tukey test at $p \leq 0.05$.

With the exception of the three-day storage period, in all other periods, the control and treatment with the thiamethoxam insecticide showed a reduction in the vigor rate by the first-count test (Table 2). This fact can be explained by the insecticide effect on the maize seeds vigor and germination stored for a long period (Salgado and Ximenes 2013, Tonin et al. 2014) and possibly because they do not have fungicide active ingredients. Both treatments may have been influenced by the seeds pathogens degradation, as fungicides work to control the main maize seeds fungi (Govender et al. 2008, Ramos et al. 2008).

When comparing the storage periods up to 90 days, the results demonstrate that only the control and thiamethoxam treatment were influenced, so that the storage period increase, decreased the seeds vigor exponentially by first count test (Figure 1A and 1B). Germination is directly linked to seed vigor and has been negatively influenced by the insecticides used in sunflower seeds treatment, which is more noticeable with increasing storage period (Mrđa et al. 2010), corroborating with the results obtained in the present work.

Metalaxyl + fludioxonil + thiabendazole and pyraclostrobin + thiophanate-methyl + fipronil treatments did not show differences as the storage period progressed up to 90 days (Figure 1C and 1D). These treatments obtained average vigor, by the first count test, of 95 and 93%, respectively (Figure 1). These two products demonstrated similar effects when comparisons were made between them (Table 2). Possibly, the fungicides contributed to minimize, at 90 days period, the seeds deterioration. The deterioration is an inevitable and progressive process, which causes a decrease in seeds vigor and viability mainly due to fungi and insects (Deuner et al. 2014).

Table 3. Maize seeds vigor (%) by the accelerated aging test, due to treatments and storage period.

Storage period (days)	Maize seeds treatment			
	Control	Thiamethoxa m	Metalaxyl + Fludioxonil + Thiabendazole	Pyraclostrobin + Thiophanate-methyl + Fipronil
0	87 ± 2.0 C	91 ± 2.3 B	96 ± 2.0 A	96 ± 1.6 A
3	93 ± 2.6 B	93 ± 1.4 B	99 ± 0.9 A	98 ± 1.7 A
7	85 ± 3.0 C	91 ± 1.9 B	95 ± 2.0 A	97 ± 1.9 A
14	81 ± 2.2 C	85 ± 1.6 B	96 ± 1.0 A	98 ± 0.4 A
21	87 ± 1.9 B	86 ± 4.9 B	97 ± 2.0 A	97 ± 0.9 A
28	90 ± 2.1 B	88 ± 1.6 B	96 ± 1.6 A	96 ± 1.2 A
35	85 ± 1.4 B	85 ± 2.9 B	98 ± 1.7 A	97 ± 1.4 A
42	88 ± 1.6 C	90 ± 1.7 C	97 ± 0.9 A	94 ± 2.3 B
49	91 ± 1.7 B	88 ± 1.8 B	97 ± 0.9 A	96 ± 1.6 A
56	83 ± 3.5 D	87 ± 3.0 C	97 ± 0.9 A	91 ± 1.7 B
63	81 ± 1.3 C	86 ± 3.0 B	89 ± 1.9 A	91 ± 1.3 A
90	89 ± 2.6 B	88 ± 2.2 B	93 ± 1.9 A	96 ± 1.4 A
Means	87 C	88 B	96 A	96 A
C.V (%)	2.35			

* Means followed by the same letter in the line do not differ from each other by the Tukey test at p≤0.05.

Table 4. Maize seed germination (%) due to products and storage periods.

Storage period (days)	Maize seeds treatments			
	Control	Thiamethoxam	Metalaxyl + Fludioxonil + Thiabendazole	Pyraclostrobin + Thiophanate-methyl + Fipronil
0	99 ± 0.7^{ns}	99 ± 1.0	100 ± 0.0	99 ± 1.4
3	99 ± 1.3^{ns}	99 ± 1.0	99 ± 1.4	99 ± 0.9
7	99 ± 1.0^{ns}	99 ± 0.7	99 ± 0.9	99 ± 1.4
14	98 ± 1.0^{ns}	98 ± 1.4	98 ± 1.7	98 ± 2.2
21	98 ± 1.3^{ns}	98 ± 1.3	99 ± 0.7	98 ± 1.3
28	98 ± 2.7^{ns}	98 ± 1.3	99 ± 1.4	98 ± 0.9
35	97 ± 2.4^{ns}	98 ± 1.2	99 ± 1.0	98 ± 1.0
42	99 ± 0.9^{ns}	98 ± 1.6	99 ± 1.4	99 ± 0.9
49	99 ± 1.4^{ns}	98 ± 0.9	98 ± 1.9	99 ± 0.7
56	98 ± 1.3^{ns}	98 ± 1.3	98 ± 1.9	99 ± 1.4
63	99 ± 1.0^{ns}	98 ± 1.0	99 ± 0.7	99 ± 2.0
90	99 ± 1.0^{ns}	98 ± 1.9	99 ± 1.0	99 ± 1.3
Means	98^{ns}	98	99	98
C.V (%)	4.82			

^{ns} Not significant by Test F a p≤0.05.

Regarding the maize seeds vigor by the accelerated aging test, it was observed that the control and thiamethoxam were the ones that most affected the vigor, independently of the storage time. As regards the thiamethoxam effect, studies have reported that this product has a harmful effect on maize seeds, affecting vigor and germination as the storage time increases (Corrêa Junior. et al. 2013, Tonin et al. 2014). In addition, it is assumed that fungi seed contamination occurred, which allowed the degradation, as the increase in optimal conditions for microorganisms' development favors the seeds degradation (Stefanello et al. 2015).

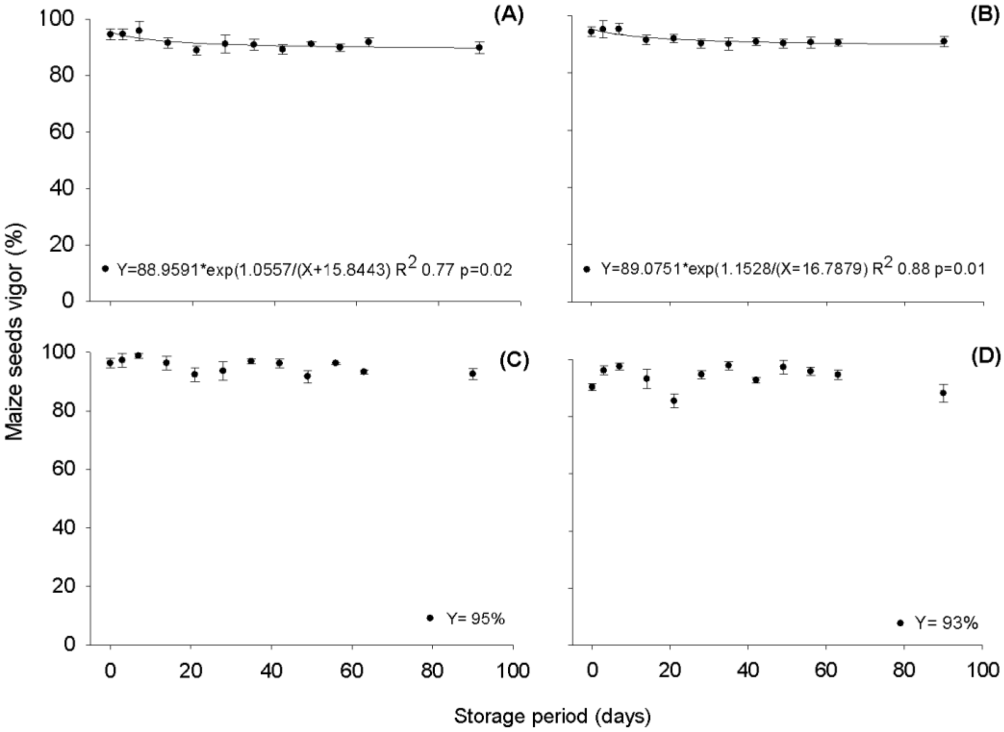

Figure 1. Maize seeds vigor (%) by the first count test as a function of the storage period and seeds treatments: control (A) thiamethoxam (B), metalaxyl + fludioxonil + thiabendazole (C) and pyraclostrobin + thiophanate-methyl + fipronil (D).

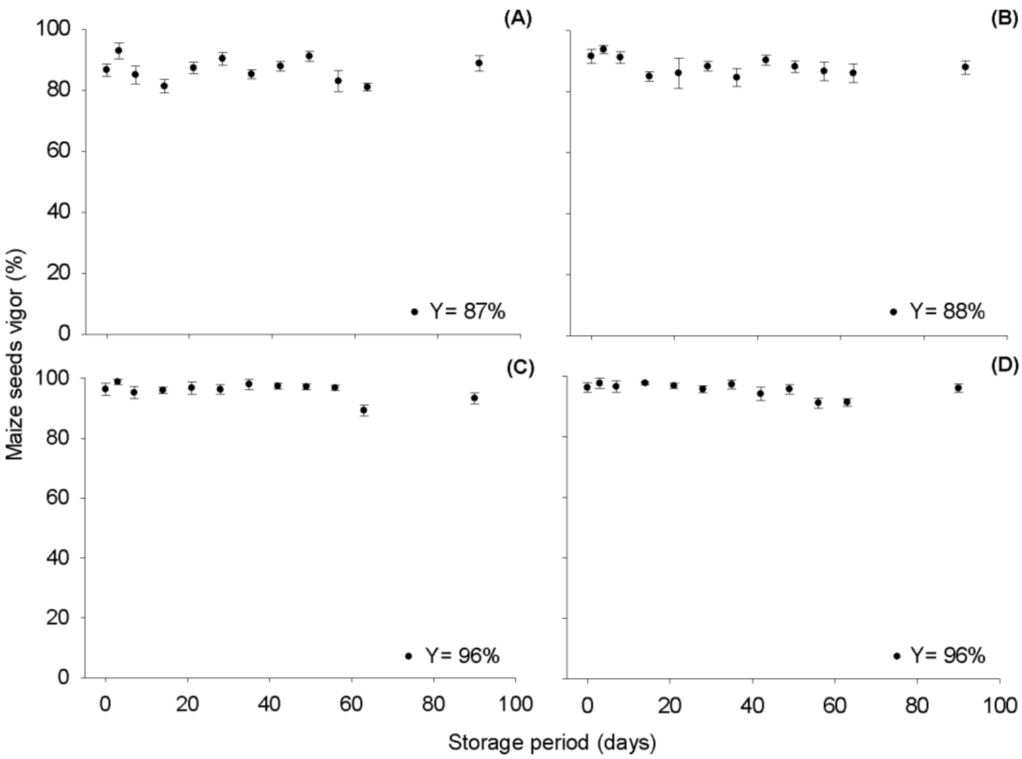

Figure 2. Maize seeds vigor by the accelerated aging test due to storage period and treatments: control (A) thiamethoxam (B), metalaxyl + fludioxonil + thiabendazole (C) and pyraclostrobin + thiophanate-methyl + fipronil (D).

For storage periods general average was observed that the control was the most affected, which resulted in a decrease of 1.70, 9.27 and 8.84%, when compared with treatments involving thiamethoxam, metalaxyl + fludioxonil + thiabendazole and pyraclostrobin + thiophanate-methyl + fipronil, respectively (Table 3). It was observed that the greatest percentage difference occurred when the treatments had some fungicide treatment, a result that corroborates with Kunkur et al. (2007), reporting that cotton seeds physiological characteristics remain with high quality when fungicides were applied along with insecticides.

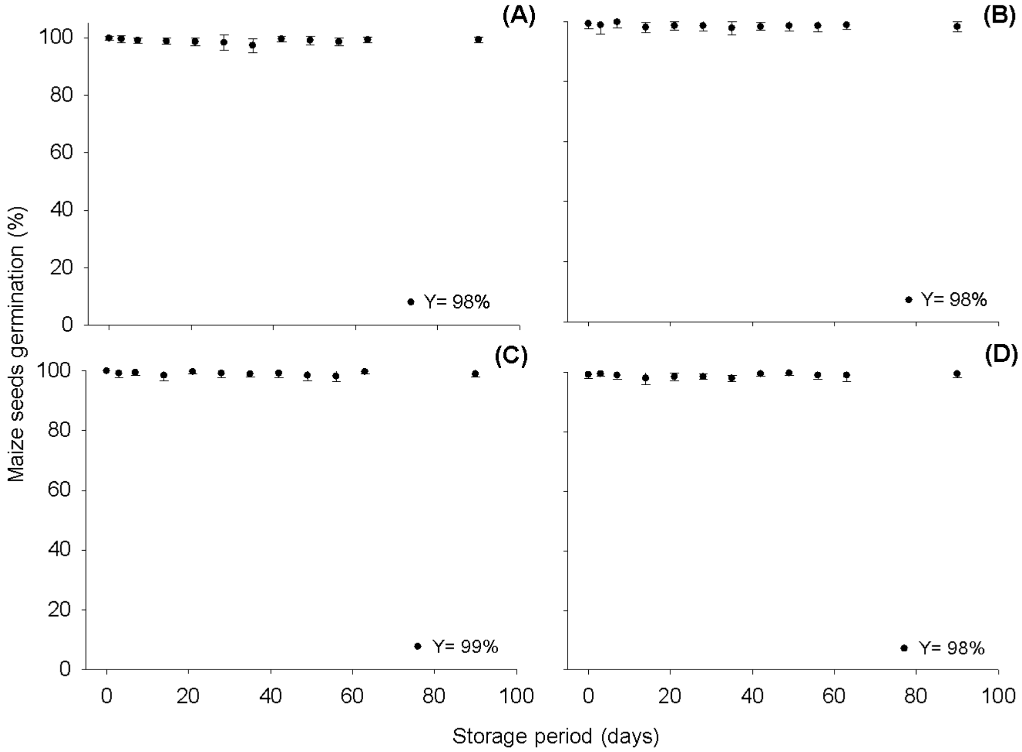

Figure 3. Maize seeds germination (%) due to storage period and seed treatments: control (A) thiamethoxam (B), metalaxyl + fludioxonil + thiabendazole (C) and pyraclostrobin + thiophanate-methyl + fipronil (D).

In Figure 2 it can be observed that there was no significant effect on the maize seeds stored during the 90-day period, treated or not with insecticides and/or fungicides. The seeds vigor was performed by the widely used and reliable accelerated aging test, that is mainly used to monitor seed quality during the storage process (Yang and Wen 2017).

Treatments average vigor were: 87; 88; 96 and 95%, for control, thiamethoxam, metalaxyl + fludioxonil + thiabendazole and pyraclostrobin + thiophanate-methyl + fipronil, respectively (Figure 2). It can be seen in Table 3 that the worst result for vigor was obtained with the control, differing statistically between the treatments. Probably there was a greater seeds degradation by microorganism action, mainly fungi. Seed degradation is an unavoidable process that causes seed vigor and viability to decrease (Deuner et al. 2014). In this way, the fungicides application is necessary to keep the stored maize seeds viability to sown in the next growing season and to avoid fungi attack (Govender et al. 2008).

The treatments with fungicides presented a high standard vigor, both by the first count test and by the accelerated aging test (Tables 2 and 3 and Figures 1C, 1D and Figure 2C and 2D). The seeds vigor is very important to the crop establishment, because seedlings with high vigor have a dominant effect on the maize yield, when compared to plants from low vigor seeds, besides showing a higher competitive ability (Mondo et al. 2012).

Differently from the first count test vigor, the germination did not present significant effect, both comparing seeds treatment and also storage periods. Nowadays, there is a very wide range of products on the market, and these can respond in a differentiated way, mainly insecticides can affect the germination and maize seeds vigor, and still be potentiated as an increase in storage period (Salgado and Ximenes 2013, Tonin et al. 2014).

Even control that showed the lowest vigor in relation to the chemical seed treatments we have not found significant negative effects on germination, it was found 97% average germination, exceeding the minimum required (Brasil 2009) (Table 4 and Figure 3A). It is important to emphasize that the physiological seeds potential is the main component in quality control, and seed germination is the most important test to be performed to obtain this information (Yang and Wen 2017).

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