Original Article

Fusarium verticillioides INOCULUM POTENTIAL AND ITS RELATION WITH THE PHYSIOLOGICAL STORED CORN SEEDS QUALITY

POTENCIAL DE INÓCULO DE Fusarium verticillioides *E SUA RELAÇÃO COM A QUALIDADE FISIOLÓGICA DE SEMENTES DE MILHO ARMAZENADAS*

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ABSTRACT: The pathogenic fungi may be associated with the seeds during development, harvest and storage, and it can be disseminated through them. This work evaluated the seeds performance of three hybrid corn cultivars, BM 840 PRO, BM 810 and BM 207, submitted to different inoculum potentials of *Fusarium verticillioides*. Seeds were uniformly distributed over the medium fungal colony developed on PDA (potato dextrose agar) containing mannitol in water potential -2.0 MPa. Seeds remained in this way for different periods of contamination (0, 24, 48, 72 and 96 hours). Before and after seven months of seed storage the evaluations were performed. Seeds treated with fungicide Derosal Plus® (carbendazim + thiram, 250 mL p.c. 100 kg^{-1} of seeds) were used as control. The increased of inoculum potential of *F. verticillioides* fungus on corn seeds caused reduction in physiological quality in the potentials 72 and 96 hours of exposure before and after storage. The highest *Fusarium verticillioides* incidence is observed in non-stored seeds. *Fusarium verticillioides* incidence on corn seeds accentuates the deterioration in storage.

KEYWORDS: *Zea mays* L. Germination. Ear rot. Water restriction.

INTRODUCTION

Corn (*Zea mays* L.) is one of the main cereals grown in the world, providing products for food and feed as well as raw material for the industry. Due to the economic importance of this crop and the adoption of technologies by producers is increasing the demand for maize seeds highest quality, causing the seed companies adopt often stricter quality standards than those set by the seed certification system.

Among several factors affecting the corn seed quality, microorganisms are considered one of the most important, as well as being related to sanitary quality can affect germination and vigor, in addition to accelerating the deterioration process during storage. Furthermore, etiological agents of most diseases that affect the corn crop can be spread and transmitted by contaminated-infected seeds.

Fungal species most commonly associated with corn seeds in Brazil are *Fusarium verticillioides*, and species of the genus *Aspergillus*, often conveyed by these seeds (CASA; REIS, 2003). This association, in most cases reduces physiological seed quality, favoring also dispersing pathogens over long distances and seed pathogen transmission to the plant (COUTINHO et al., 2007). Moreover, these pathogens can also accumulate toxic secondary metabolites to humans and animals, called mycotoxins (FREIRE et al., 2007; REIS et al., 2004).

Some studies have associated the *F. verticillioides* presence with seed deterioration (WILLIAMS et al. 2006 and Wilke et al. 2007), germination and seedling emergence reduction, especially in adverse conditions (CASA et al. 2005; MACHADO et al. 2013). On the other hand, Henning et al. (2011), Solorzano and Malvik (2011) did not observe any negative effect of *F. verticillioides* incidence on germination and vigor of corn seeds. However, these results are still divergent and have no information whether the infection levels influence differently on seed quality during storage.

Thus, aimed to evaluate the fungus *Fusarium verticillioides* influence on seeds performance of three hybrid corn cultivars, BM 840 PRO, BM 810 and BM 207, submitted to different inoculum potentials and storage.

MATERIAL AND METHODS

The experiment was conducted at the Seed Center Laboratory and Seed Pathology Laboratory - Agriculture and Phytopathology Departments of the Federal University of Lavras (UFLA), Lavras, MG, Brazil.

Three hybrid corn cultivars were used, BM 840 PRO, BM 810 and BM 207, freshly harvested, provided by the company Biomatrix, being BM 840 PRO classified as moderately resistant and the others as moderately susceptible to ear rot disease, according to the company information. Seeds were initially sterilized with sodium hypochlorite solution 1% for one minute, washed and dried under filter paper in laboratory conditions. As control were used seed treated with fungicide Derosal Plus® (carbendazim + thiram 250 mL p.c. 100 kg^{-1} of seeds), in order to obtain seeds with low fungus incidence.

Obtaining and inoculum multiplication

The fungus *F. verticillioides* was isolated from corn seed by *blotter test*. After identification of *F. verticillioides* colonies discs of 5 cm diameter were transferred to Petri dishes containing PDA medium and maintained in a growth chamber with 25°C temperature for seven days. Obtained pure cultures were maintained in micro-tubes until their use.

Seed inoculation

Corn seeds were inoculated with *F. verticillioides* by the water restriction method on agar substrate (Machado et al., 2001). For the inoculation was used water potential of -2.0 MPa and mannitol addition to PDA 2% in Petri dishes (15 cm diameter) that were inoculated with conidial suspension from pure fungus cultures. Then, the plates were distributed at random in growth chamber with temperature 25°C and photoperiod of 12 hours for five days. After this period, the seeds were equally distributed over the colonies and maintained on substrates for: 0, 24, 48, 72 and 96 hours. Immediately after exposure to different time periods (inoculum potential), the seeds were placed to dry for 24 hours in aseptic laboratory conditions. The evaluations were done just after fungus inoculation and after seven months of storage in uncontrolled environment.

Seed sanity test

The seeds sanity was evaluated by the *blotter test* with freezing. The seeds were previously sterilized with sodium hypochlorite solution 1% for one minute to remove presumable surface contamination by *F. verticillioides*. Eight repetitions of 25 seeds distributed in Petri dishes (15 cm diameter) containing three sheets of filter paper previously sterilized and wetted with sterile distilled water were used. The seeds were incubated for 24 hours at 20 ± 2°C in a chamber with photoperiod of 12 hours and then, transferred to the freezer for more 24 hours and again incubated at 20 ± 2°C for five days. To identify *F. verticillioides* incidence on the seeds, stereoscopic microscope was used and the results expressed in percentage.

Seed germination test

The substrate for seeding was the paper *Germitest*, wetted with distilled water in an amount 2.5 times the dry paper weight. The seeds were placed in germination chambers regulated at temperature 25°C and the counts carried out on the 4^{th} and 7^{th} day after sowing. Four replications of 50 seeds and the results expressed as a percentage of normal seedlings were used.

Seedling emergence test

Four replicates of 50 seeds were sown in plastic trays with dimensions of 60 cm x 40 cm x 10 cm containing as substrate sand + soil in the ratio 2:1 with 60% of field capacity. The trays with seeds were placed in a growth chamber set at 25°C, in the alternate system of light and darkness (12 hours). Irrigation was performed when necessary. The normal seedlings evaluation was performed 10 days after the test assembly.

Cold test

The sown was performed in plastic trays with dimensions 60 cm x 40 cm x 10 cm containing as substrate sand + soil in the ratio 2:1 with 60% of field capacity. The trays with seeds were placed in cold chamber at temperature 10°C for seven days. Then, the trays were transferred for plant growth chamber at temperature 25°C and photoperiod of 12 hours for seven days. Four replicates of 50 seeds were used and percentage of normal seedlings emerged was evaluated.

The experimental design was completely randomized in a 5x2 factorial arrangement with five exposure periods to the fungus (inoculum potentials - 0, 24, 48, 72 and 96 hours) and two storage periods (0 and 7 months), with four replications. Data were submitted to variance analysis using Sisvar® software (Ferreira, 2011), at 5% probability by *F* test (p<0,05). Analyzes were performed separately for each hybrid. Averages were grouping using polynomial regression analyzes were performed.

RESULTS AND DISCUSSION

Fusarium verticillioides incidence in corn seed of the cultivar BM 840 PRO showed lower average 38% in inoculum potential of 24 hours after seed storage (Table 1). But with increasing inoculum potential, these averages did not differ among studied periods, with values close to 100%. For the cultivar BM 810, it was found a significant difference among seeds non-stored with those stored in the potentials of 24 and 48 hours. As for cultivar BM 207, the measure which increases the seed exposure period to the pathogen (24, 48 and 72 hours), observed lower pathogen incidence after storage, except for the seeds that were not inoculated 0 hour and inoculated for 96 hours, which presented no differences. These results are similar to those obtained by Cappelini et al. (2005), that observed *F. verticillioides* incidence reduction after 12 months of storage in uncontrolled environment. It can be inferred that to a certain infection level (72 hours exposure) there is a reduction in the incidence percentage of this pathogen in corn seeds after seven months of storage in uncontrolled environment.

Table 1. Average percentage of *F. verticillioides* incidence and seeds germination (%) of three inoculated corn cultivars in different inoculum potentials before and after storage (0 and 7 months) of seeds of three cultivars.

	Periods	*F. verticillioides* incidence					Germination (%)				
		Inoculum potential					Inoculum potential				
		0	24	48	72	96	0	24	48	72	96
BM 840 PRO	0	0a	100a	100a	100a	100a	99a	96a	98a	92a	85a
	7	0a	38b	99a	100a	100a	100a	99a	84b	66b	54b
CV (%)				5,13					5,37		
BM 810	0	0a	100a	100a	100a	100a	96a	89a	89a	62a	58a
	7	1a	84b	84b	97a	99a	93a	93a	89a	42b	21b
CV (%)				5,30					6,25		
BM 207	0	1a	100a	100a	100a	100a	93a	85a	91a	79a	69a
	7	1a	86b	94b	94b	99a	91a	88a	78b	58b	23b
CV (%)				6,30					8,50		

Lower case letter in the column, in each hybrid corn cultivars, present significant differences according to the variance analysis, $p<0.05$ by the F test.

Based on the results observed for the cultivars BM 840 PRO and BM 207, difference in the germination percentage between two periods analyzed, from 48 hours of the fungus *F. verticillioides* inoculation it was found. For the cultivar BM 810, this difference was observed only after 72 hours (Table 1). As inoculum potential increased, lower germination values were observed in seeds stored after seven months.

Incidence of *F. verticillioides* on corn seeds from the cultivars BM 840 PRO, BM 810 and BM 207, non-stored and inoculated with the pathogen in the inoculum potential (24, 48, 72 and 96 hours) was high, being growing with increased exposure time of seeds to fungus (Figure 1A). For all cultivars, the model fit to the data was the second degree, it is estimated higher level of infection (100%) in 65 hours of the inoculation, *F. verticillioides* is a pathogen that has fast growing and easily penetrates in the seed tissues.

Based on the *F. verticillioides* incidence results (Figure 1B), different behavior was observed for the stored seeds of the cultivar BM 840 PRO in relation to non-stored seeds (Figure 1A). After the storage period, these seeds showed a linear increase of the incidence with maximum value (100%) in 96 hours of inoculation. For the BM 810 and BM 207 cultivars, the highest level of seeds infection (100%) was achieved at 72 and 68 hours, respectively. Based on these results, it is clear that the seeds' permanence time to fungus *F. verticillioides* ranged according to the cultivar studied. It is noteworthy, the hybrid BM 840 PRO has been classified as moderately resistant and the other as moderate susceptibility. Galli et al. (2005) found that the period of 32 hours in contact of corn seeds with the fungus *F. graminearum* was enough for this pathogen infected the seeds. As for Ramos et al. (2014), the period of 16 hours in contact of corn seeds with the fungi *F. graminearum* and *F. verticillioides* was enough to obtain infected seeds.

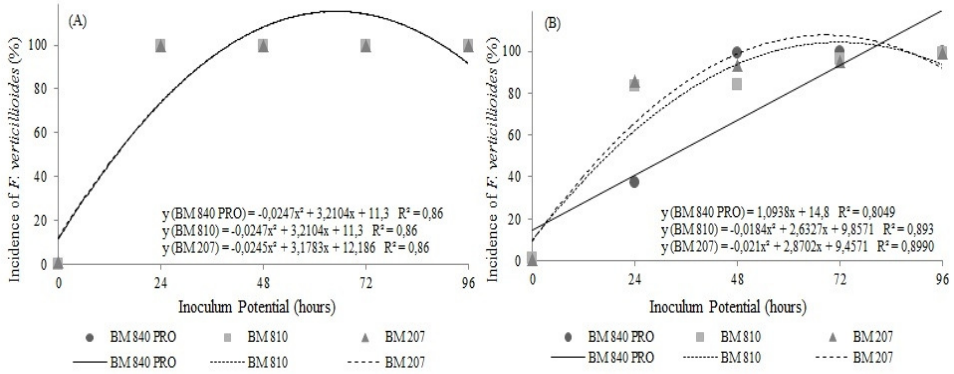

Figure 1. Percentage of *F. verticillioides* incidence on corn seeds from cultivars BM 840 PRO, BM 810 and BM 207 before storage (A) and (B) after seed storage, evaluated by the sanity test and submitted to different inoculum potentials.

The germination data presented showed that for seeds from cultivars BM 840 PRO, BM 810 and BM 207 non-stored and inoculated with the fungus *F. verticillioides*, the increase of inoculum potential provided linear reduction in germination percentage, approximately 13%, 41% and 22%, respectively. Menezes et al. (2011) found that cucumber seeds inoculated with *F. verticillioides* reduces the germination. A similar result was obtained by Yang et al. (2011), that working with barley seeds infected with *F. graminearum* found that the germination was harmed, causing seedlings death and root rot. Probably, this reduction was due to the fungus *F. verticillioides* present a fast and aggressive growth and can cause death of the seeds before germination.

As for the seeds that were inoculated for a period of seven months (Figure 2B), also noticed linear decrease in germination percentage, with severe decrease than non-stored seeds (Figure 2A). Cultivar BM 810 has reduced germination 72%. Since cultivars BM 840 PRO and BM 207 the reduction was 45% and 66%, respectively. It can be said that germination reduction occurred after a stress caused by the high incidence of this microorganism in the seeds, and also by storage conditions, contributing to their rapid deterioration and loss of quality. Barbosa et al. (2013) reported that after the point of physiological maturity or storage, the presence of pathogenic microorganisms reduces physiological potential and sanitary quality of the seeds. In addition, this presence is also associated with decrease in germination and lower seedling development (MUNIZ et al., 2004).

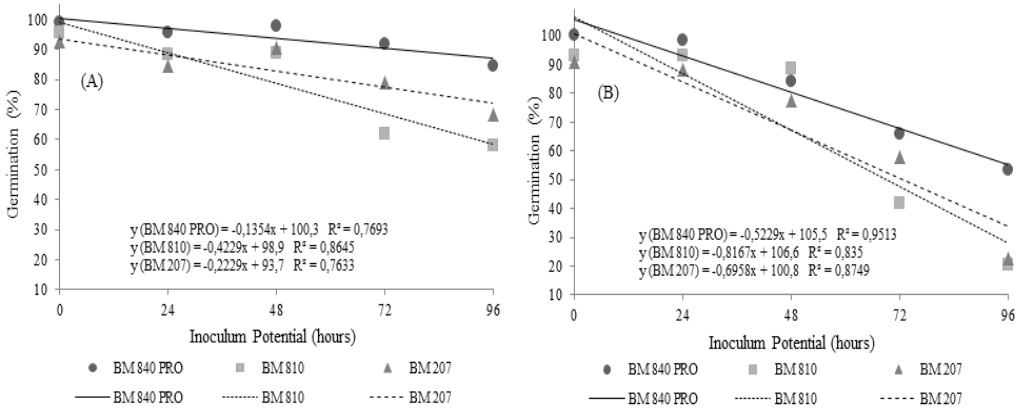

Figure 2. Percentage of normal seedlings of the corn seed from cultivars BM 840 PRO, BM 810 and BM 207 before storage (A) and (B) after storage submitted to different inoculum potentials.

The results observed for the seedlings emergence (Table 2) were similar for the cultivars BM 840 PRO and BM 810, since there was no significant difference between the periods in different inoculum potential (0, 24 and 48 hours). It was observed that there was emergence reduction to the seed inoculated with the fungus only after 72 hours. In relation to the cultivar BM 207 the difference was observed with lower inoculation time (48 hours).

The results observed for the cold test (Table 2), considered a vigor test, were similar in all cultivars among the seeds before and after storage. There was no significant difference between the periods for inoculation potential 0 and 24 hours of the fungus *F. verticillioides* in seeds. In all cultivars, when comparing the average plant emergence in a cold test before and after storage, occurred loss of seed quality from 48 hours of inoculation, with significant reductions of emergence plants which have been stored. Seedlings of the cultivar BM 207 in the inoculum potential 48, 72 and 96 hours when seeds were stored for seven months, had average 50%, 29% and 8%, respectively.

Table 2. Average percentage emergence (%) and seedling emergence in the cold test (%) of three inoculated corn cultivars with *F. verticillioides* in different inoculum potential before and after storage (0 and 7 months) of seeds.

	Periods	Emergence (%)					Cold test (%)				
		Inoculum potential					Inoculum potential				
		0	24	48	72	96	0	24	48	72	96
BM 840 PRO	0	97a	96a	94a	92[a]	65a	100a	98a	91a	94a	80a
	7	100a	97a	94a	51b	41b	98a	96a	55b	34b	18b
CV (%)				5,62					8,12		
BM 810	0	97a	95a	92a	89[a]	78a	98a	82a	92a	84a	61a
	7	97a	95a	94a	66b	49b	95a	89a	49b	42b	19b
CV (%)				5,30					9,26		
BM 207	0	92a	83a	91a	84[a]	78a	89a	85a	63a	71a	62a
	7	94a	89a	70b	46b	31b	93a	78a	50b	29b	8b
CV (%)				8,83					11,0		

Lower case letter in the column, in each hybrid corn cultivars, present significant differences according to the variance analysis, $p<0.05$ by the F test.

By the emergence results and cold test, there was a negative effect of seed storage under uncontrolled conditions, and the pathogen presence also influenced and optimized the loss of physiological quality.

Emergence near to 100% for the cultivar BM 840 PRO with inoculum potential of 26 hours and lower value (68%) provided for 96 hours of the inoculation (Figure 3A). The cultivars BM 810 and BM 207, increasing the exposure time of the seeds to the pathogen causing linear reduction of quality and consequent increase of the number of abnormal seedlings and injuries. The pathogen presence in the seeds damaged emergence, development and seedling establishment.

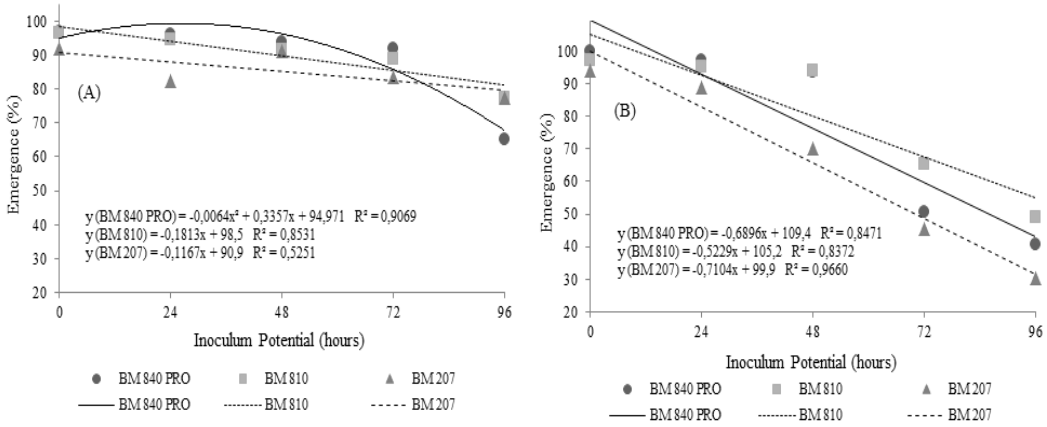

Figure 3. Percentage of emergence corn seedlings from cultivars BM 804 PRO, BM 810 and BM 207 before storage (A) and (B) after storage submitted to different inoculum potentials.

In relation to seedlings emergence after seven months of seed storage, it was observed the same effect for all cultivars BM 840 PRO, BM 810 and BM 207 (Figure 3B). As it increases the period of seed inoculation to the fungus *F. verticillioides* there is a vigor reduction, especially at the end of 96

hours, with severe decrease than non-stored seeds (Figure 3A). Thereby, as in the germination test, this fungus presence on corn seeds can cause irreversible damage and very aggressive depending the exposure time and the achieved infection level, damage these optimized after storage.

For the cold test results, it was found different behavior among the hybrid seeds non-stored and contaminated with *F. verticillioides* (Figure 4A). For the cultivar BM 810 can be observed after 23 hours of the seeds' exposure to the fungus there was a vigor decrease, being maintained until the next 81 hours, after this period it was observed less vigor from these seedlings due to the permanence of seeds with the inoculum for a longer time. As for the cultivars BM 840 PRO and BM 207 the inoculum potential effect in relation to seedling vigor showed a linear behavior, reaching after 96 hours of inoculation, values of 83% and 60%, respectively. Evaluating the vigor of seeds contaminated lots for *F. graminearum*, by the cold test, Cícero and Vieira (1994) concluded that fungus presence in the seeds for a period of seven days in cold storage reduced the performance of the lots according to the level infection. According to Ramos et al. (2014) the fungus presence *F. graminearum* reduced vigor of corn seed exposed to a temperature at 10°C for 8 days.

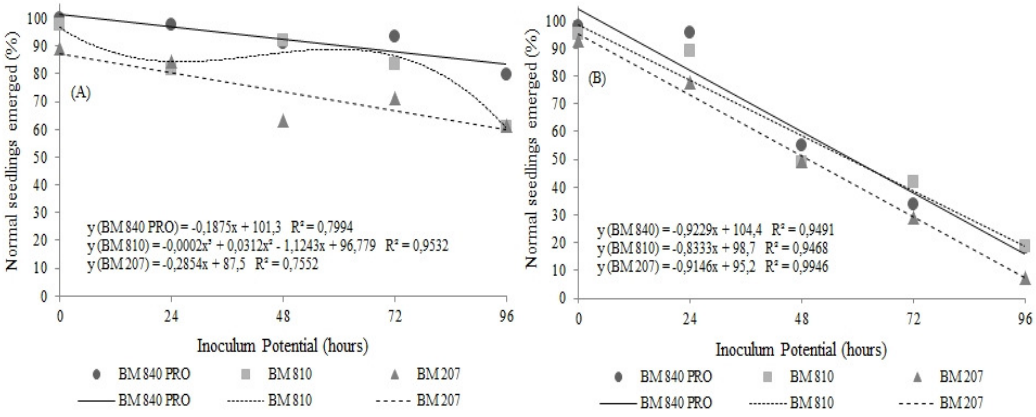

Figure 4. Percentage of emergence of normal seedlings in the cold test for corn seed from cultivars BM 804 PRO, BM 810 and BM 207 before storage (A) and (B) after storage submitted to different inoculum potentials *F. verticillioides*.

As for the estimated vigor by the cold test for the seeds that were stored for seven months in uncontrolled conditions, in all cultivars the trend was linear, with seed vigor reduction as increased the permanence thereof to the inoculum (Figure 4B), with prominent decrease than seen before storage (Figure 4). For the cultivar BM 840 PRO average estimated without fungus inoculation was approximately 100%, whereas with the inoculation 72 hours the value was 38% reaching only 16% of vigorous seedlings with 96 hours. For the cultivar BM 810 the behavior was similar, the seeds which were uninoculated had 99% vigorous seedlings and those inoculated for 72 and 96 hours had 39% and 19%, respectively. Cultivar BM 207 in the inoculation absence presented 95% vigorous seedlings, but after 96 hours of inoculation this value was reduced to 7%. The results found in this study are different from those obtained by Ramos et al. (2014), which was not observed effect of the fungus *F. verticillioides* on the final quality of seeds, but these authors did not work with the storage factor, apart from that, it is possibly to be related to the inactivation of the fungus by the greater exposure period to the low temperatures that authors used.

CONCLUSIONS

There is a reduction of the physiological quality of the seeds inoculated with *F. verticillioides* in the range from 72 to 96 hours of exposure, both before and after storage.

The highest *F. verticillioides* incidence is observed in the non-stored seeds.

The *F. verticillioides* incidence on corn seeds accentuates the deterioration in storage.

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RESUMO: Os fungos fitopatogênicos podem associar-se as sementes durante o desenvolvimento, colheita e armazenamento, e por meio dessas serem disseminados. Este trabalho avaliou o desempenho de sementes de três cultivares de milho híbrido, BM 840 PRO, BM 810 e BM 207, submetidas a diferentes potenciais de inóculo de *Fusarium verticillioides*. As sementes foram distribuídas uniformemente sob colônia fúngica desenvolvida em meio BDA, contendo manitol no potencial hídrico -2,0 MPa. As sementes permaneceram desse modo por diferentes períodos de contaminação (0, 24, 48, 72 e 96 horas). Antes e após sete meses de armazenamento das sementes as avaliações foram realizadas. As sementes tratadas com o fungicida Derosal Plus® (carbendazim + tiram, 250 mL p.c. 100 kg^{-1} de sementes) foram usadas como testemunhas. O aumento do potencial de inóculo do fungo *F. verticillioides* nas sementes de milho causa redução da qualidade fisiológica nos potenciais de 72 e 96 horas de exposição, antes e após o armazenamento. A maior incidência de *Fusarium verticillioides* é observada nas sementes não armazenadas. A incidência de *Fusarium verticillioides* em sementes de milho acentua a deterioração no armazenamento.

PALAVRAS-CHAVE: *Zea mays* L. Germinação. Podridão da espiga. Restrição hídrica.

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