

# Sanitary and physiological quality of 'purple' corn (*Zea mays* L.) seeds submitted to thermotherapy

## Qualidade sanitária e fisiológica de sementes de milho 'Roxo' (*Zea mays* L.) submetidas à termoterapia

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**ABSTRACT:** The use of seeds with good sanitary and physiological potential ensures the establishment of the crop, with a uniform, vigorous and disease-free seedling stand, which will result in good productivity levels. Seen that, the objective was to evaluate the sanitary and physiological quality of purple corn seeds (*Zea mays* L.) submitted to thermotherapy. They were subjected to heat treatment with immersion in sterile distilled water (SDW) heated at 50, 60 and 70 °C for 1, 2, and 3 minutes. The control (0 min) correspondens only to immersing the seeds in SDW, which were submitted to sanitary and germination tests in a completely randomized experimental design, in a factorial arrangement 3 × 3 + 1 (exposure time × temperature + additional control). Data were subjected to analysis of variance, and the means were compared with the Scott-Knott test up to 5% of probability, with the aid of the statistical program SISVAR<sup>®</sup>. Immersing *Zea mays* seeds in hot water at 50, 60 and 70 °C for 1, 2, and 3 minutes is efficient to prevent the increased incidence of *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. The heat treatment at 60 °C for 1 and 2 minutes of immersion reduces the incidence of fungal mycoflora without impairing the physiological quality of seeds.

**KEYWORDS:** alternative control; seed pathology; thermal stress.

**RESUMO:** O uso de sementes com bom potencial sanitário e fisiológico garante o estabelecimento da cultura, com estande de plântulas uniformes, vigorosas e livres de doenças, que consequentemente resultará em bons níveis de produtividade. Diante do exposto, objetivou-se avaliar a qualidade sanitária e fisiológica de sementes de milho roxo (*Zea mays* L.) submetidas à termoterapia, por meio de tratamento térmico com imersão em água destilada esterilizada (ADE), aquecida à temperatura de 50, 60 e 70 °C por 1, 2 e 3 minutos. O controle (0 min) correspondeu apenas à imersão das sementes em ADE. As sementes foram submetidas aos testes de sanidade e germinação em delineamento experimental inteiramente randomizado em esquema fatorial 3 × 3 + 1 (tempo de exposição × temperatura + controle adicional). Os dados foram submetidos à análise de variância e as médias foram comparadas pelo teste de Scott-Knott até 5% de probabilidade, com o auxílio do programa estatístico SISVAR<sup>®</sup>. A imersão das sementes de *Zea mays* em água quente a 50, 60 e 70 °C durante 1, 2 e 3 minutos é eficiente para impedir o aumento da incidência de *Aspergillus* sp., *Penicillium* sp. e *Fusarium* sp. O tratamento térmico a 60 °C por 1 e 2 minutos de imersão reduz a incidência da micoflora fúngica, sem prejudicar a qualidade fisiológica das sementes.

**PALAVRAS-CHAVE:** controle alternativo; patologia de sementes; estresse térmico.

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Received on: 11/10/2018. Accepted on: 07/30/2019

## INTRODUCTION

Due to its productive potential, chemical composition, and nutritive value, corn (*Zea mays* L.) is one of the most important cereals grown in the world (MODESTO et al., 2014). However, greater productivity requires not only adequate cultivation methods, but also the use of high quality seeds, which is determined by a set of genetic, physical, physiological and sanitary attributes (ROSSI et al., 2017).

The sanitary quality of seeds can be compromised by fungi in all steps of the production process. These pathogens are responsible for the reduction in physiological quality, can be disseminated all over long distances through infected seeds, and are transmitted from the seed to the seedling (SANTOS et al., 2016). Eliminating or reducing these pathogens is being efficiently achieved through chemical treatments (DOMENE et al., 2016). Nonetheless, there has been growing interest worldwide for alternative seed treatment methods that cause less harm to the environment and human health, such as the use of vegetal extracts, essential oils, biological control and physical treatments (MEDEIROS et al., 2015; PEREIRA et al., 2015).

Physical treatment, such as thermotherapy, is an alternative method with considerable potential, especially to control pathogens adhered to the surface or contained in the interior of seeds (PEREIRA et al., 2015). Although efficient in the control of pathogens, the efficiency of this method is directly related to knowledge of the different degrees of sensitivity to thermal stress between the seed and the pathogen. Thus, the ideal period to expose seeds to heat treatment is when the pathogen can be controlled without causing harm to the physiological quality seeds (FRANÇOSO; BARBEDO, 2014).

Studies have demonstrated the effectiveness of thermotherapy for seeds. SCHNEIDER et al. (2015) found that the immersion of *Jatropha curcas* L seeds in water at 45, 50, and 55 °C for 15 minutes was effective to control *Penicillium* sp., and *Acremonium* sp., without compromising the physiological quality of seeds. By immersing pumpkin (*Cucurbita pepo*) seeds at 60 °C for 6 and 12 minutes, CUNHA et al. (2017) found a reduction in the incidence of *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Penicillium* sp., *Phoma* sp., and *Rhizopus* sp., without affecting the physiological quality of seeds.

Therefore, the aim of this study was to evaluate the sanitary and physiological quality of purple corn (*Zea mays* L.) seeds submitted to thermotherapy.

## MATERIAL AND METHODS

The experiment was conducted in the Phytopathology Laboratory of the Center for Agrarian Sciences of the Universidade Federal da Paraíba, *Campus* II, in the city of Areia, state of Paraíba, Brazil.

## Sanitation test

Two hundred seeds were used per treatment, distributed in 20 replicates of 10 seeds each. The seeds were placed in tulle sacks, which were identified and placed randomly into a water bath at a predetermined temperature, and for a predetermined time.

The heat treatment method consisted of immersing the seeds in sterilized distilled water (SDW), heated to a temperature of 50, 60, and 70 °C for 1, 2, and 3 minutes. For the control (0 min), seeds were immersed in SDW at room temperature, placed into Petri dishes (diameter: 9 cm), containing sterilized double-layer blotter test filter paper moistened with SDW. The dishes were incubated for 7 days at a temperature of 25 ± 2 °C and a 12-hour photoperiod (BRASIL, 2009).

The detection and identification of fungi was performed with the aid of an optical microscope and stereoscope, as well as comparing results to those of specialized literature (SEIFERT et al., 2011). Data were expressed as the percentage of infected seeds.

## Germination test

For the evaluation of physiological quality, 200 seeds were divided into 4 replicates of 50 seeds for each treatment, distributed on 2 sheets of germ-test paper, covered with a third sheet, and organized in a roll form. The paper had been previously sterilized and moistened with SDW at a proportion of 2.5% of its dry weight, placed in transparent plastic bags to avoid losing water through evaporation, and it was finally incubated in a biochemical oxygen demand (BOD) germinator, set at a temperature of 25 °C and a 12-hour photoperiod.

The evaluations were performed four and seven days after sowing, following the criteria established by BRASIL (2009). Germination was evaluated considering the number of normal seedlings on Day Four (first count), and Day Seven. The data were expressed in percentage values. The germination velocity index (GVI) was determined by a daily count of normal seedlings using the formula proposed by MAGUIRE (1962),

$$GVI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \dots + \frac{G_n}{N_n}$$

In which:

$G_1$ ,  $G_2$ , and  $G_n$  correspond to the number of normal seedlings germinated each day;

and  $N_1$ ,  $N_2$  and  $N_n$  to the number of days from the sowing to the first and last counts.

The shoot length (SL), and root length (RL) were determined with the aid of a centimeter ruler, and the result was expressed in centimeters. The shoot dry matter (SDM) and rood dry matter (RDM) content were determined at the end of the evaluation by cutting the parts of the seedlings with the aid of a scalpel. The parts were then placed in Kraft paper sacks

and dried in a thermoelectric oven at 65 °C, until reaching a constant weight (72 h) to determine dry mass. The samples were then weighed on an analytical scale with a precision of 0.001 g and the results were expressed in grams (CARVALHO; NAKAGAWA, 2012).

## Statistical analysis

The experimental design was entirely randomized in a 3 × 3 + 1 factorial scheme (exposure time × temperature + additional control). The data were submitted to analysis of variance and means were compared using the Scott-Knott test with a 5% probability. The analysis was performed with the aid of the SISVAR<sup>®</sup> statistical program.

## RESULTS AND DISCUSSION

### Sanitary quality

In the determination of sanitary quality of purple corn seeds (*Zea mays* L.), the fungi *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Cladosporium* sp., and *Monilia* sp. were found, but the low incidence of the latter two genera was not sufficient to reduce the quality of seeds. The detection of *Fusarium* sp., *Penicillium* sp., and *Aspergillus* sp. is in agreement with data described in previous studies, in which these fungi are described as the main microorganisms associated with corn kernels in Brazil (PINTO, 2001; 2005; RAMOS et al., 2010). A statistically significant difference ( $p < 0.05$ ) was found in the interaction between temperature and immersion time, regarding the percentage of occurrence of *Penicillium* sp., *Fusarium* sp., and *Aspergillus* sp. (Fig. 1), which agrees with data described by NOAL et al. (2014), and DOMENE et al. (2016), who also determined the occurrence of these fungi in corn seeds (*Zea mays* L.).

The maximum occurrence of *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. was 20.5, 63, and 50.5%, respectively, in the 0-immersion time (Fig. 1). These rates are higher than those reported by DOMENE et al. (2016), who investigated the effect of essential oils on corn seeds, and found rates of 3.4% for *Aspergillus* spp., 23.4% for *Penicillium* spp., and 19.8% for *Fusarium* sp. In contrast, NOAL et al. (2014) found higher rates (26% for *Aspergillus* spp., 99% for *Penicillium* spp., and 100% for *Fusarium* sp.) at different harvest points of a corn variety in the state of Rio Grande do Sul (Southern Brazil).

A reduction in the incidence of *Aspergillus* sp. was found in the seeds submitted to thermotherapy, independently of water temperature and immersion time (Fig. 1). However, the seeds treated with a temperature of 60 °C caused the control of this fungus, with a statistically significant difference when compared to the control treatment ( $p < 0.01$ ) (Fig. 1). A similar result is reported by VIEIRA et al. (2011), who found

reductions in the incidence of *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., and *Cladosporium* spp. in coffee seeds (*Coffea arabica* L.), using a heat treatment. The authors also found the elimination of this pathogen (0%) when the seeds were submitted to water at a temperature of 60 °C for 15 minutes.

All treatments were effective at reducing the incidence of *Fusarium* sp. The temperatures of 50 and 60 °C were the most effective, because they considerably reduced the incidence of this pathogen in comparison to the control treatment when seeds were immersed for 3 minutes, and 1 minute, respectively (Fig. 1). These findings are in agreement with data reported by VIEIRA et al. (2011), who describe the effective thermal control of the fungus *Fusarium* spp., with different combinations of temperature and time, but with a considerable reduction when seeds were immersed at 60 °C for 7'30". TANAKA et al. (2003) found that *F. subglutinans* remained viable after thermotherapy, but that many fungi were either controlled or eradicated. COUTINHO et al. (2007) also found a significant reduction in the occurrence of *Fusarium viticillioides* in corn seeds submitted to immersion in water at 60 °C for 5, 10, and 20 min.

According to PINTO (2005) and RAMOS et al. (2010), the high incidence of *Fusarium* sp., and *Stenocarpella* sp. is directly related to the higher incidence of corn kernels. The authors add that other fungi are also found with a high frequency, such as *Penicillium* spp., and *Aspergillus* spp.; however, these pathogens cause more damage to stored grains.

The reduction in *Penicillium* sp. was similar to that found for *Aspergillus* sp., and *Fusarium* sp. The difference between treatments was significant ( $p < 0.01$ ), with a reduction close to 0% under the effect of 60 °C after 2 minutes of immersion (Fig. 1). Unlike the results found, LAZAROTTO et al. (2013) report that the treatment of seeds from the tree *Peltophorum dubium* (Spreng. [Taub.]) with wet heat at 80 °C for over 5 minutes favored the incidence of *Penicillium* sp., and *Aspergillus* spp. BRAGA et al. (2010), and VIEIRA et al. (2011) report similar results to those found in the present study for tomato and coffee seeds, respectively, describing a reduction in the incidence of *Penicillium* sp. when submitted to heat treatment. Therefore, heat treatment should be applied in such a way that it does not affect the seed vigor, which could accelerate its deterioration, and make it more susceptible to the action of seed-rotting microorganisms (LAZAROTTO et al., 2013).

According to MACHINSKI JUNIOR et al. (2001), the high incidence of *Penicillium* sp. in corn kernels is worrisome, since this pathogen produces a mycotoxin (ochratoxin) that is associated with cancer in humans and animals. Despite the lower incidence of *Aspergillus* sp. in purple corn seeds, this pathogen is also considered an important mycotoxin-producing (alphatoxin) agent. The incidence of this fungus immediately after harvest has also been reported in other studies (STEFANELLO et al., 2012), but varies according to the genotype and/or climate conditions (RAMOS et al., 2010).

Although the greater prevalence of *Penicillium* sp. was related to asymptomatic kernels in the present study, PINTO (2001) states that this fungus is normally not associated with corn kernels immediately after harvest, but can be a cob-rotting agent. Moreover, due to the lack of evident signs, asymptomatic kernels can be selected and transported to storage, where the fungus can cause severe damage.

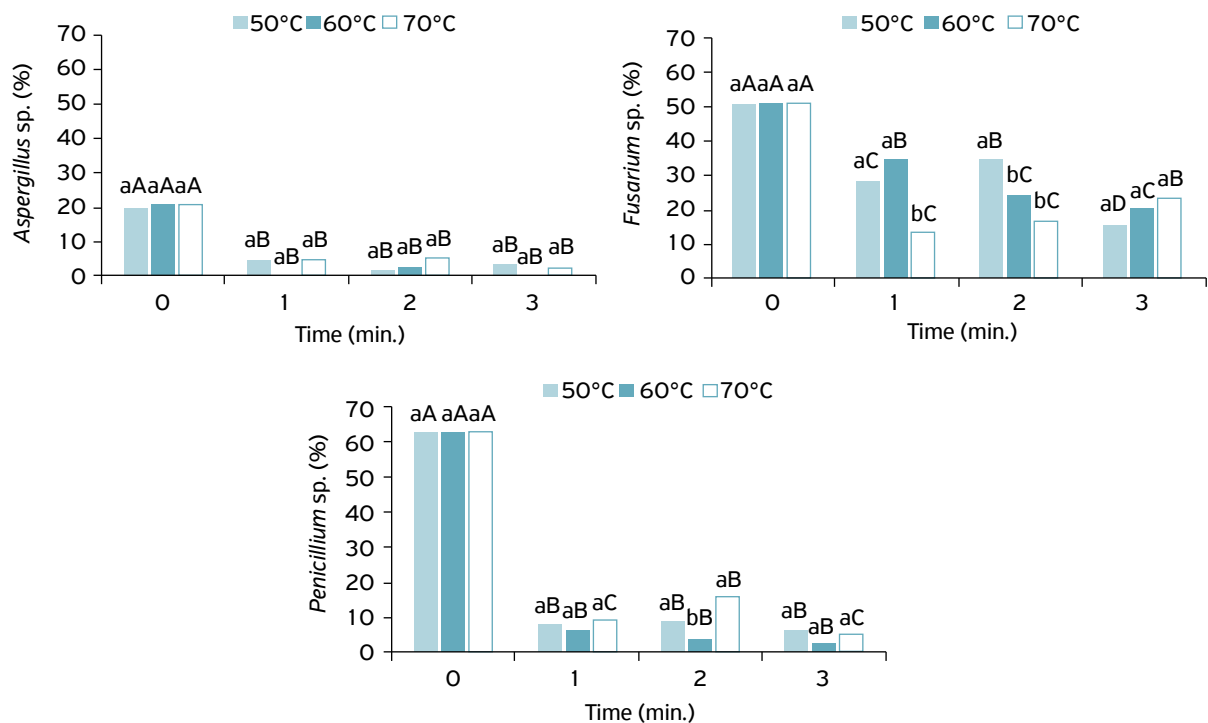
## Physiological quality

Based on the results of the first count and germination, no statistically significant differences ( $p > 0.05$ ) were found between different temperatures, times, and temperature-time interactions, with rates from 47 to 50% for these variables. Therefore, none of the treatments rendered the germination of the seeds unviable. This finding is in agreement with data described by SCHNEIDER et al. (2015), who report no significant effect ( $p > 0.05$ ) of the interaction between thermotherapy temperatures and storage periods on the germination of *Jatropha curcas* L. seeds, describing an increase in the percentage of germination throughout a 90-day period, with a germination rate ranging from 66 to 68%.

For the GVI, SL, RL, seedling length (SdL), SDM, root dry matter (RDM), and total dry weight (TDW), a significant interaction ( $p < 0.01$ ) was found between temperature and immersion time (Fig. 2).

The highest GVI values were obtained with seeds submitted to thermotherapy at 50 and 60 °C, which did not differ significantly from the control treatment (0 min.). In contrast, GVI values decreased significantly ( $p < 0.01$ ), with the increase in immersion time at 70 °C, compared to other treatments (Fig. 2). MATHEUS; LOPES (2007) and SCHNEIDER et al. (2015) also found that the increase in exposure time of *Schyzolobium parahyba*, and *Jatropha curcas* seeds to heated water resulted in a reduction in the germination percentage and GVI, likely due to damage to the embryo, resulting from prolonged exposure to water at a very high temperature. Similar results were found in this study, as a lower GVI was found for seeds submitted to a higher immersion temperature.

A significant temperature-immersion time interaction ( $p < 0.01$ ) was found regarding SL (Fig. 2). Higher values were observed when seeds were submitted to thermotherapy at 50 and 70 °C, with no statistically significant differences in comparison to the control treatment (0 immersion time). However, the immersion at 60 °C for 2 and 3 minutes led to a reduction in SL (Fig. 2). The maintenance of seed viability and vigor above the optimal temperature may be explained by repair mechanisms in membranes, mitochondria, proteins, and RNA, as well as the synthesis of thermal shock proteins and the action of antioxidants (MARCOS FILHO, 2015). According to TAIZ; ZEIGER (2013), the growth and development of seedlings are compromised by a reduction in enzyme activity, and/or



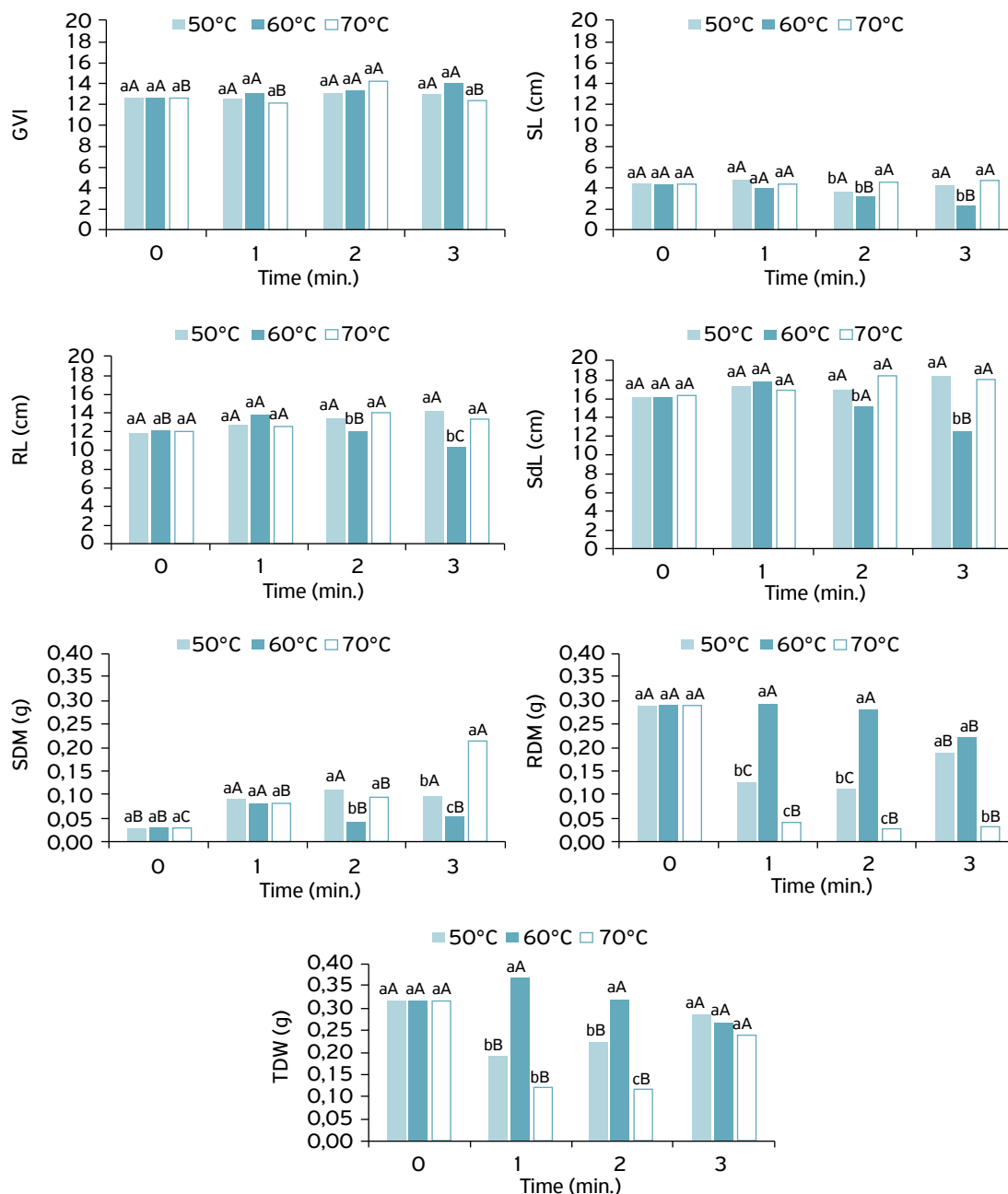
**Figure 1.** Occurrence of fungi associated with purple corn seeds (*Zea mays* L.), submitted to thermotherapy with hot water. Means followed by the same lowercase (comparison of temperatures) and uppercase (comparison of immersion times) letter do not differ significantly ( $p > 0.05$ , Scott-Knott test).

diminished water absorption caused when seeds suffer a moderate degree of stress, such as thermal stress.

Similar behavior was found regarding RL and SdL (Fig. 2), with the highest values found in the thermotherapy treatments at 50 and 70 °C, independently of the immersion time, and with no significant difference in comparison to the control treatment (0 immersion time). SdL results were similar to findings described by DOMENE et al. (2016), who evaluated the

effect of treatment with essential oils applied to corn seeds, and found that most treatments did not have a negative effect on seedling development, like demonstrated by the lack of significant differences compared to the control treatment.

Thermotherapy at 60 °C for 2 and 3 minutes led to a slight reduction in root and SdL, with a significant difference ( $p < 0.01$ ) in comparison to the control treatment (0 immersion time) (Fig. 2). From these results, one may infer that this



GVI: germination velocity index; SL: shoot length; RL: root length; SdL: seedling length; SDM: shoot dry matter; RDM: root dry matter; TDM: total dry matter.

**Figure 2.** Characterization of the physiological quality of purple corn seeds (*Zea mays* L.), submitted to thermotherapy with hot water based on the germination velocity index, shoot length, root length, seedling length, shoot dry matter, root dry matter and total dry matter. Means followed by the same lowercase (comparison of temperatures) and uppercase (comparison of immersion times) letter do not differ significantly ( $p > 0.05$ , Scott-Knott test).

temperature and these immersion times cause more harm to the seeds than shorter immersion times, leading to harm to the seedlings. This is in agreement with data described by SANTOS et al. (2016), who found that thermotherapy led to a reduction in the height of soybean seedlings, especially with exposure times from 30 to 50 minutes to wet heat. However, dry heat can also cause physiological damage, like MENEZES et al. (2012) reported for rice seeds submitted to different dry temperatures.

All treatments led to a statistically significant increase in the SDM content ( $p < 0.01$ ) in comparison to the control (0 immersion time), especially the treatment at 70 °C for 3 minutes, with the accumulation of 0.20 g seedling<sup>-1</sup>, surpassing that of other treatments (Fig. 2). By evaluating the physiological quality of *Peltophorum dubium* seeds submitted to wet heat at 80 °C for 5 minutes, LAZAROTTO et al. (2013) also found an increase in dry matter content, with a maximum of 0.21 g seedling<sup>-1</sup>.

A significant temperature x immersion time interaction ( $p < 0.01$ ) was found for RDM. The best values occurred at 60 °C, independently of immersion time, except when seeds were submitted to this temperature for 3 minutes, which led to a reduction in RDM content (Fig. 2). Thermotherapy at 70 °C considerably affected root development, with the lowest RDM values compared to all other treatments (Fig. 2). Probably, thermal stress altered the balance between the production of reactive oxygen species (ROS) and ROS elimination enzymes, partially reducing the metabolic activity needed to continue and stimulate normal root growth (RASHID et al., 2017).

Regarding TDW content (Fig. 2), thermotherapy at 50, 60, and 70 °C for 3 minutes maintained significant values ( $p < 0.01$ ), with no difference in comparison to the control treatment (0 immersion time). The highest TDW was found with thermotherapy at 60 °C for 1 minute (Fig. 2). Temperature is considered the most important factor to the velocity and percentage of seed germination, and the emergence of seedlings. Moreover, high temperatures can trigger the denaturation of proteins and enzymes responsible for maintaining the tolerance of seeds to heat (DUTRA et al., 2015). It is possible that the maintenance of the dry matter content in the treatments is related to seed vigor, because more vigorous seeds are more tolerant to a broader temperature range without compromising the germination process (SCHNEIDER et al., 2015). When evaluating the effect of temperature on soybean seeds (*Glycine max*), SANTOS et al. (2016) found a reduction in the height and dry weight of seedlings with the increase in exposure time to wet heat at 40 °C.

## CONCLUSIONS

The present findings show that the immersion of *Zea mays* in hot water at 50, 60 and 70 °C for 1, 2, and 3 minutes is effective to prevent an increase in the incidence of *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. Heat treatment at 60 °C for 1 and 2 minutes of immersion reduces the incidence of fungal microflora, without compromising the physiological quality of seeds.

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