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CONTROL OF MAIZE WEEVIL WITH APPLICATION OF DIATOMACEOUS EARTH IN CORN GRAINS STORED IN DIVERSE TEMPERATURES

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ABSTRACT

The insect known as maize weevil (*Sitophilus zeamais*) is one of the most important pests of stored corn in Brazil. Among the various forms of control, one can highlight the use of inert powders, which has advantages of not providing risk to the environment or to human and animal health, besides not having reports of insect resistance. Thus, this work evaluated the effect of different doses of diatomaceous earth on the development of *Sitophilus zeamais* on two temperature conditions on corn grain storage. A completely randomized design was adopted in a factorial 4x2x4 (diatomaceous earth dose x storage temperature x storage time) dosage, with three replicates. The diatomaceous earth doses were: 0, 250, 500 and 1000 g t^{-1} and subsequently stored at temperatures of 22 and 30°C for 90 days. The bulk density decreased during the storage for all diatomaceous earth doses applied, but in inverse proportion to the dose increment. Diatomaceous earth has proven better efficiency in the control of *S. zeamais*, with increase in dose and storage temperature. Dry weight decrease was observed in all doses and storage temperatures evaluated, but it was more pronounced in those grains that have not received the application of diatomaceous earth.

Palavras-chave:
armazenamento
danos
insetos-praga
pó inerte
Sitophilus zeamais

CONTROLE DO GORGULHO-DO-MILHO COM A APLICAÇÃO DE TERRA DE DIATOMÁCEAS EM GRÃOS DE MILHO ARMAZENADOS EM DISTINTAS TEMPERATURAS

RESUMO

O inseto conhecido como gorgulho-do-milho (*Sitophilus zeamais*) é uma das mais importantes pragas dos grãos de milho armazenados no Brasil. Dentre as diversas formas de controle, pode-se destacar o uso de pós inertes, que possui vantagens de não oferecer riscos ambientais e à saúde humana e animal, além de não apresentar relatos de resistência de insetos. Sendo assim, com o trabalho avaliou-se o efeito de diferentes doses de terra de diatomáceas, sobre o desenvolvimento do *S. zeamais*, sob duas condições de temperatura de armazenagem de grãos de milho. Foi adotado o delineamento experimental inteiramente casualizado, em esquema fatorial 4x2x4 (dose de terra de diatomáceas x temperatura de armazenagem x tempo de armazenamento), com três repetições. As doses de terra de diatomáceas aplicadas foram: 0, 250, 500 e 1000 g t^{-1} e, posteriormente, armazenados em temperaturas de 22 e 30ºC por 90 dias. A massa específica aparente apresentou redução ao longo do armazenamento para todas as doses de terra de diatomáceas aplicadas, porém em proporção inversa ao incremento de dose. A terra de diatomáceas apresenta melhor eficiência no controle do *S. zeamais*, com o incremento da dose e da temperatura de armazenagem. A redução de massa seca foi observada em todas as doses e temperaturas de armazenagem avaliadas, porém, foi mais pronunciada naqueles grãos que não receberam a aplicação de terra de diatomáceas.

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INTRODUCTION

According to FAO (2006), each year in countries that are in the development process, millions of tons of cereals, roots, tubers, fruits and vegetables, do not reach the consumer's table, partly due to problems that occur in processes related to post harvest, as the weakness for procedures, drying and storage equipment, damage from insects, fungi, rodents among other pests, and problems due to logistics, transportation and marketing. According to an estimate by Embrapa (2015), in Brazil, losses related to improper storage of grain totaled around of 15% of total production, mostly attributed to insect pests, fungi and mycotoxins also added to rodent attacks.

Among the main storage insects that attack corn, cite the *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae), popularly known as maize weevil. This is an internal primary pest because adults cause damage to intact and healthy grains, while the larvae feed on the inner part of the grain. The laying is performed on the grains and seeds, the larvae, after they develop, soak and turn into adults still in the grain or seed. Damage results from weight reduction and grain quality (LORINI et al., 2015).

Due to the high losses during storage of corn, one of the ways to reduce the problems caused by insect pests is the use of natural insecticides such as diatomaceous earth (DE), which is an inert powder from fossilized diatoms seaweed shells, which has silicon dioxide as a main component. This insecticide has some advantages over synthetic chemical insecticides, such as not present environmental risk and damage to human and animal health, and have no reports of DE resistance by insects (LORINI et al., 2001; LORINI et al., 2015).

Diatomaceous earth is probably the most efficient natural dust used as an insecticide. Dust particles are trapped by the bodies of the insects as they walk over it. This dust is most effective against insects with setaceous and rough surfaces. Damage occurs to the insects' protective wax coat on the cuticle, mostly by sorption and to a lesser degree by abrasion, or both. The result is loss of water from the insect's body through desiccation resulting in death. The other mode of action of DE is repellence caused by the physical presence of the dust (KORUNIC, 1998).

In recent years several studies evaluating the effect of DE on the control of insect pests of stored grains have been published, highlighting its potential as a protective agent for stored grains (FREDERICK; SUBRAMANYAM, 2016; JAIROCE et al., 2016; WAKIL and SCHMITT, 2015; KAVALLIERATOS et al., 2015; ZIAEE et al., 2014; EISSA et al., 2014; ATHANASSIOU et al., 2014; NWAUBANI et al., 2014; DOUMBIA et al., 2014). However, the studies are largely dedicated to the evaluation of doses and sources, with few studies evaluating the efficacy of DE at different storage temperatures and its residual effect (long-term storage), especially for the protection of stored corn grains against *S. zeamais*, associating its influence on the quantitative parameters of the grains.

According to this, the aim of the study was to evaluate the effect of different diatomaceous earth doses on quantitative parameters and interference in the development of insect pests of the species *Sitophilus zeamais* under two conditions of corn grain storage temperatures.

MATERIAL AND METHODS

The corn grains used were originated from the Agricultural Experimental Station (AES), belonging to the Federal University of Rio Grande do Sul, located at km 47 of the BR 290, in Eldorado do Sul (30º05'52 "S, 51º39'08" W and average altitude of 46 m), cultivated in the crop season 2014/2015.

The insects used for the research were raised in a heated room (25±5 °C, 60±5% RH and photoperiod of 16h) and kept in plastic containers with a perforated lid and covered with a fabric type voile, aiming to allow aeration and curb the flight of insects, according to methodology adapted from Stefanazzi et al. (2011).

The experimental doses of diatomaceous earth (DE) applied to grains, were (0) zero, 250, 500 and 1000 g t^{-1} of the Insecto® commercial product, which has 86.7% of silicon dioxide.

For the experiment 72 plastic containers (experimental units) were used, each with 500 mL capacity containing 140 g of corn, and in 54 of those the respective doses of DE were added to the grains, followed by hand homogenization for 3 min. In the remainder containers, only corn was placed, without the addition of DE. Then each container was infested with 30 adults, unsexed insects of the species *S. zeamais*, aged between 20 and 50 days. These insects were previously identified with tempera gouache paint, aimed at assessing the offspring during the collection of results.

Thereafter, the above steps completed, the containers were closed with "voile" tissue type and then kept in B.O.D type chamber for 90 days under two different temperature storage conditions (22 and 30 °C).

After the period of 30, 60 and 90 days after the application of DE to corn, it was carried out, visually, the counting and removing of the dead, living and emerged insects, as well as evaluation of the dry matter (BOXALL, 1986; ALMEIDA FILHO; FONTES; ARTHUR, 2002), through the weight loss in each experimental unit.

Also, at the time of the experiment and each evaluation period, the determination of moisture content and bulk density was performed as described in the Rules for Seed Analysis (BRASIL, 2009).

To check the residual effect of the DE, after each evaluation period, the insects were removed and then carried further infestation of the corn, using 30 adult insects, aged between 20 and 50 days without the addition of any dose of the product. The evaluations were conducted in the same manner as described above.

For the experiment, a completely randomized design in a factorial 4x2x4 (DE dose x storage temperature x storage time) was adopted, except for the answers related to the development of the insects, which had three storage times, with three replications. The data were subjected to analysis of variance by F test ($p \leq 0.05$), and when accused significant effect, performed regression analysis. The models were selected based on the significance of the equation, by the F test, the significance of the regression coefficients using the "p" value, adopting a maximum of 5% and by the coefficient of determination (r^2).

RESULTS AND DISCUSSION

According to the results of the variance analysis, there was a significant effect among the three evaluated factors (DE dose x storage temperature x storage time) for the variables bulk density, dead insects, live insects, emerged insects and dry matter variation, except for the moisture content, which showed effects of the time x temperature and dose x temperature interactions.

Following shows the results of the bulk density of corn grains (Figure 1), as a function of the application of different DE doses and stored under different temperature conditions.

For the stored grains, at both temperature conditions (22 and 30 °C), significant reductions in the bulk density were observed during the

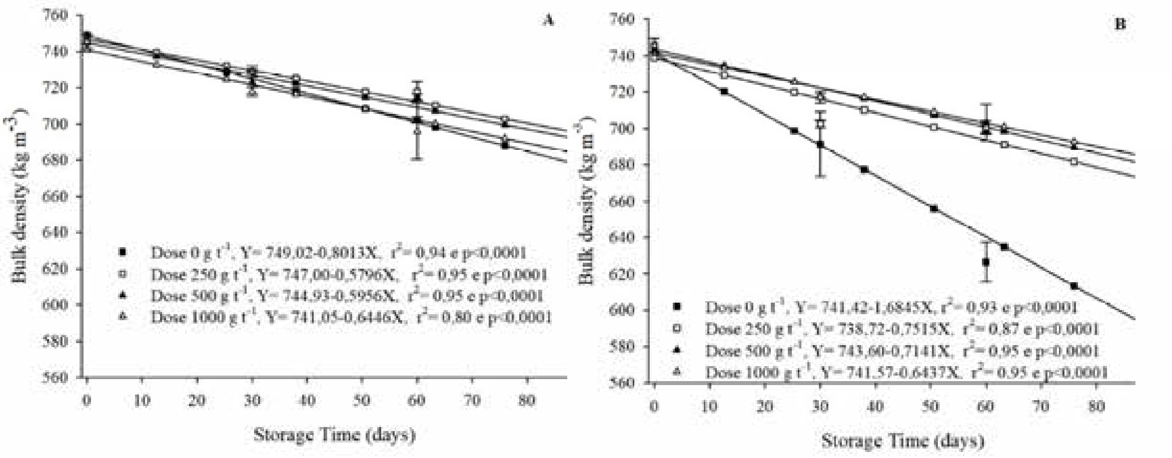

Figure 1. Bulk density of corn grains (13% d.b.) submitted to different diatomaceous earth doses, with subsequent infestation with *S. zeamais* and storage at temperatures of 22 (A) and 30 °C (B).

period, regardless of the DE dose applied (Figures 1A and 1B). For the lowest storage temperature (22 °C), the bulk density reductions were similar, over time, for all tested doses (Figure 1A). At the highest temperature (30 °C), the highest reduction occurred in the grains without the application of DE (control), whereas among the other three doses these reductions during that period were similar but with lower intensity (Figure 1B).

According to the mathematical adjustments, for the temperature of 22 ºC the average reduction of the bulk density was approximately 7.2% at the end of the storage period of the corn grains. For the grains kept at 30 ºC, those that had the addition of DE presented an average reduction of 8.6%, while in those that did not the loss was approximately of 20.6%.

These results are in agreement with Schuh et al. (2011) and Antunes et al. (2014), where they observed a reduction of the bulk density of corn grains during storage. The authors attributed this reduction to the consumption of dry matter caused by an infestation by fungi and/or insects during storage. Also, Ferrari Filho et al. (2012), when evaluating the quality of wheat grains hermetically and not hermetically stored, attributed the reduction to insect attack, the presence of microorganisms and the metabolic activity of the grains.

For the grains without the application of DE and stored at a temperature of 30 ºC, the lowest values of bulk density were obtained at the end of the period, which can be attributed, mainly, to the consumption by the insects, since these grains presented a larger population of live insects. Other authors also observed a reduction of bulk density, when control of the insects in storage was not performed (ALMEIDA FILHO et al., 2002; SANTOS et al., 2002; SILVA et al., 2006; FERRARI FILHO et al., 2012).

However, for the grains without ED application and stored at 22 ° C, no higher reduction of bulk density was observed at the end of the storage period, compared to those where the addition was carried out, but showed a larger reduction over time than the other treatments, demonstrated by the coefficient of the equation. According to Banks and Fields (1995), the optimum temperature range for the development of most stored grain pests is between 25 and 32 °C. Therefore, the temperature of 30 °C is in the ideal range for *S. zeamais* development, allowing full insect development and high dry matter consumption, while the temperature of 22 °C, located in the sub-optimal zone, did not provide promising conditions for population growth and grain consumption.

According to Park et al. (2008), when evaluating the infestation level effect on sorghum grains, concluded that the hectoliter weight presented a reduction proportional to the increase in infestation levels. Then, as the grains stored at 30 °C and without the application of DE presented the largest increment of insects, the largest reduction of bulk density can be attributed, in part, to the consumption of the grains by the insects.

However, for those grains, even when stored at 30 ºC, where the DE doses were applied, it was enough to avoid insect population growth, resulting in lower bulk density reduction, corroborating with the results obtained by Pinto Júnior (2008) and Athanassiou et al. (2014), where found that reduced doses of DE satisfactorily control storage insects.

The results for dead insects, as a function of the application of the DE doses in corn grains, infested with *S. zeamais* and stored in two temperature conditions, are presented in Figure 2.

Grains stored at a temperature of 22 °C showed decreasing mortality over the period, except for the 1000 g t^{-1} dose (Figure 2A), which presented constant mortality during storage, with an average of 29.33 dead insects, which corresponded to approximately 100% of the insects added to each evaluative period. The lower mortality occurred in the absence of DE, but also showed a reduction throughout the period.

For grains stored at 30 °C (Figure 2B), for those without DE and with the dose of 250 g t^{-1}, an increase in mortality was observed over the storage period, at a dose of 500 g t^{-1} this increase was observed only up to 40 days. For the highest dose, the mortality was constant over time, with an average of 30.2 dead insects.

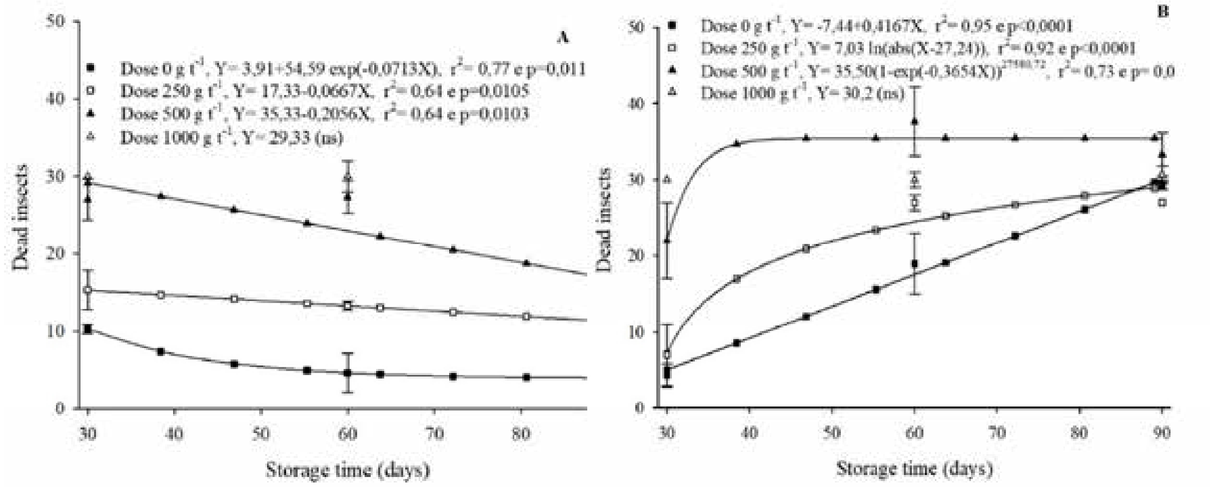

Figure 2. Dead insects due to the application of different doses of diatomaceous earth and infested with *S. zeamais*, with subsequent storage of corn grains at temperatures of 22 (A) and 30 ºC (B).

In general, the increase in mortality was directly influenced by the DE dose increase, being in agreement with the results obtained by other researchers (PINTO JÚNIOR, 2008; CERUTI et al., 2008; CANEPPELE et al., 2010; ATHANASSIOU et al., 2014). This indicates the insecticidal effect of DE at all doses tested and at both temperatures even after 90 days of storage.

The reduction in mortality at the doses of 250 and 500 g t^{-1}, when the grains were kept at 22 °C, may be due to unfavorable temperature conditions for the development of the species. According to Collins et al. (2001), the DE is less efficient when the grains are kept at low temperatures, due to the lower mobility of the insects, thus giving less contact with the product and, consequently, lower mortality.

When evaluating the effect of three corn grain storage temperatures (15, 25 and 30°C) submitted to three doses of DE (500, 750 and 1000 g t^{-1}), Ceruti et al. (2008) observed that the higher the dose and the temperature, higher the mortality, corroborating with the results obtained in the present study. Likewise, Frederick and Subramanyam (2016) found higher mortality rate of *Tribolium castaneum*, Herbst, 1797 (Coleoptera: Tenebrionidae) adults when the grains treated with DE were stored at higher temperatures.

The higher mortality rate, observed when the grains were stored at 30ºC, is probably due to the wide movement of the insects in the grain mass, thus increasing the contact with the DE, as well as the higher emergency rate, compared to the Temperature of 22°C, observed throughout the storage period.

As the DE acts by contact, with action directly related to exposure time and being these ectothermic insects, and therefore more active at high temperatures (FIELDS; KORUNIC, 2000). Added to the effect that these conditions also increase, the evaporation through the blowholes due to increased breathing, affecting the fluid balance of the insect (ZACHARIASSEN, 1991) and, furthermore, there is quicker formation of lethal lesions, where the healing process that counters the lesions become less operative, probably justifying the best effect on insect control when using the 30°C temperature.

As previously discussed, products composed of DE usually require more time to cause insect death when compared to synthetic insecticides, which act on contact. However, the residual effect of DE is generally longer than that synthetic insecticide (MARSARO JÚNIOR et al., 2008b). In this sense, Antunes et al. (2012) state that DE needs 7 to 14 days to present adequate efficiency for the control of storage insects.

The results obtained corroborate with studies carried out by Canepelle (2003), which showed that DE caused up to 50% mortality in the population of *Ephestia* spp (Lepidoptera: Pyralidae) after 210 days of DE application in corn grains.

The results of emerged insects, as a function of the application of different TD doses in corn grains, later infested with *S. zeamais* and stored at two temperatures, are demonstrated in the Figure 3.

At the temperature of 22°C (Figure 3A), emergence increased over the storage period, except for the dose of 1000 g t^{-1} DE, with insignificant values, indicating a negative effect on the emergency. However, even though the other two doses did not prevent the emergency, at the end of the period this rate was, on average, three times lower than that observed in the control treatment.

At the temperature of 30°C (Figure 3B), the highest insect emergence rate occurred in the control treatment (0 g t^{-1}), increasing up to 65 days of storage, reducing until the end of the evaluation period. Although the three doses of DE used showed similar behavior, these rates were much lower, demonstrating a negative effect on the emergence of insects during the period.

Regarding the final number of emerged insects, for both evaluated temperatures, it was observed that the higher the dose of DE, the smaller the number of emerged insects, suggesting that DE inhibits egg laying. However, at 22°C the emergence was lower than that observed at the highest temperature, which according to Athanassiou et al. (2014), is not related to parental mortality, but with the lowest rates of development at this temperature. For *S. zeamais* insects, the highest rates of multiplication and development occur at temperatures close to 28 °C (DOBIE et al., 1984), which may explain the low insect emergence values at 22 °C.

In the work of Kavallieratos et al. (2015), progeny production was not impaired by the doses tested (200 to 1000 ppm), while different source DEs provided different progeny production suppression levels, being considered as a direct consequence of the high parental survival that occurred in this commodity/DE combination. According to Arthur and Trono (2003), the larvae of *Sitophilus* spp. are “invulnerable” by DEs, as their development occurs within the grain. Thus, if they are not killed quickly, the females can lay eggs inside the grains and the larvae will continue to damage.

As adjusted by the mathematical model (Figure 3B), it is suggested that a higher emergence rate, observed between 60 and 70 days of storage, in those grains kept at a temperature of 30 ºC, especially without the addition of DE, occurred due to the posture in the first weeks after infestation of corn grains at the beginning of storage. According to Marsaro Júnior et al. (2008a), the biological cycle of this species varies from 40 to 50 days, depending on the corn hybrid on the insect develops. Considering that the posture occurred within the first 15 days, in the case of an internal primary pest, the highest adult insect emergence rate is expected to occur after 60 to 70 days of infestation of the corn grain mass.

The *S. zeamais* survival results, due to the application of the different DE doses in corn grains and stored in two temperature conditions, are presented in Figure 4.

For the temperature of 22°C (Figure 4A), the

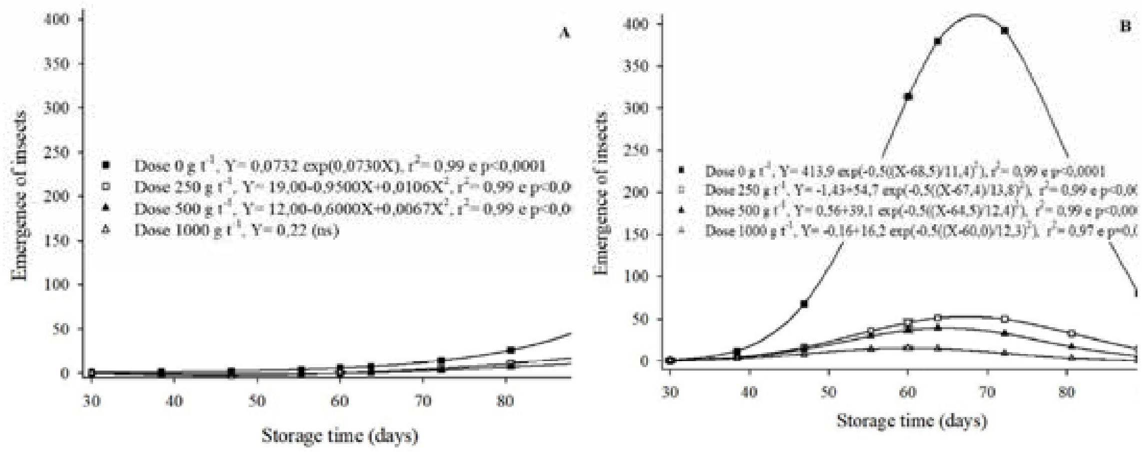

Figure 3. Emergence of insects due to the application of different diatomaceous earth doses in corn grains, with subsequent infestation with *S. zeamais* and storage at temperatures of 22 (A) and 30ºC (B).

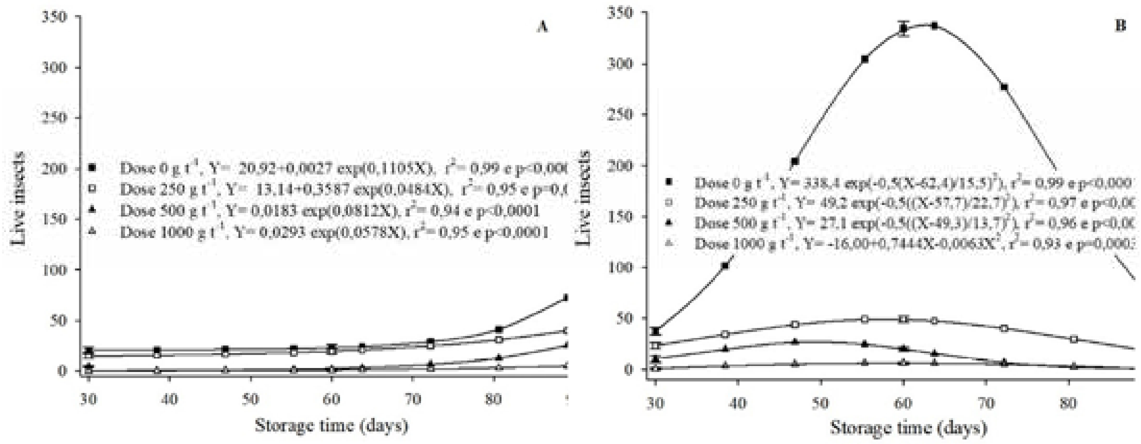

Figure 4. Live insects due to the application of different diatomaceous earth doses in corn grains, later infested with *S. zeamais*, and stored at temperatures of 22 (A) and 30ºC (B).

highest values of live insects occurred at the end of the storage period, being inversely proportional to the applied dose. However, for the 1000 g t^{-1} dose, live insects were observed only from the 62nd day of storage, with an estimate of 5 live insects at the end of the period. These results are in agreement with other studies that affirm that the greater the dose and the contact time of the product with the insect, the shorter is the survival of the pests (MARTINS; OLIVEIRA, 2008; ANTUNES et al., 2012).

According to the results presented in Figure 4B, for the control treatment (0 g t^{-1}), up to 60 days, there was an increase in the number of live insects, with subsequent reduction. With the other doses, similar behavior was observed, but with a lower number of live insects. As each evaluative period all live insects were removed, with the replacement of 30 adults, it is suggested that this reduction is due to the emergency cycle of adult insects, as discussed previously.

However, for both storage temperatures, the grains that received the application of DE had a lower number of live insects, demonstrating the effect of this product, even after 90 days of storage, on the mortality of *S. zeamais*. The results corroborate with those obtained by Ceruti et al. (2008), Pinto Júnior (2008), Antunes et al. (2013), who observed efficient control of *S. zeamais*, in corn grains, with the use of DE, even stored during a certain period.

The results of the dry matter variation of corn grains, at two temperatures during storage, depending on the application of different DE doses, are shown in Figure 5.

At the temperature of 22ºC (Figure 5A) there was no reduction of dry matter during storage for those grains that received the highest DE dose, with an average reduction of 0.17% of dry matter.

The results obtained for the 30ºC temperature (Figure 5B) show a high reduction of dry matter, during the storage, in the grains that did not receive the application of DE (control). In the grains that received the DE application the mass reductions, although increasing over the period, were smaller than that obtained in the control, and inversely proportional to the applied dose.

In a comparison of both storage temperatures, for all three doses of DE, at the lower temperature, the reduction of dry matter was slightly lower than that obtained at higher temperatures, probably due to the lower metabolic activity of the grains and low power consumption by the insects. However, in the grains without the application of DE, the grains kept at the highest temperature showed a reduction of dry matter much higher than the lower temperature, probably due to the greater metabolic activity of the grains and the consumption by the insects.

According to Santos et al. (2012), the elevation of storage temperature increases the respiratory activity of grains and living organisms in the system, resulting in higher CO_2 production and dry matter consumption. In addition, according to Santos (2002), the optimum temperature for *S.*

zeamais development is 27°C, providing greater insect mobility and dry matter consumption. At lower temperatures, according to Collins et al. (2001), insect mobility is reduced, minimizing damage to the grain and, consequently, loss of dry matter throughout the storage period.

The results corroborate with those obtained by Antunes et al. (2014), who evaluated the loss of dry matter as a function of DE application in corn grains with different moisture, observed that the reduction was greater when the product dose was lower. According to Saul and Steele (1966), for open storage systems, the dry matter loss rate increases as the storage time, temperature and moisture of the beans increase.

The moisture content data of the corn grains, during storage, as a function of the DE doses used, are demonstrated in the Figure 6.

The reduction of the moisture content of the corn grains occurred up to 60 days of storage (Figures 6A and 6B), both as a function of the DE dose and storage temperature, followed by a small increase until the end of the period.

Results are in agreement with other studies that evaluated the moisture content of grains under different conditions of temperature and relative humidity, as this variation is attributed to the tendency of the grains to reach the hygroscopic equilibrium, which is dynamic (ALENCAR et al. 2009; ELIAS; OLIVEIRA, 2009; SCHUH et al., 2011).

The stored grains are in continuous and dynamic

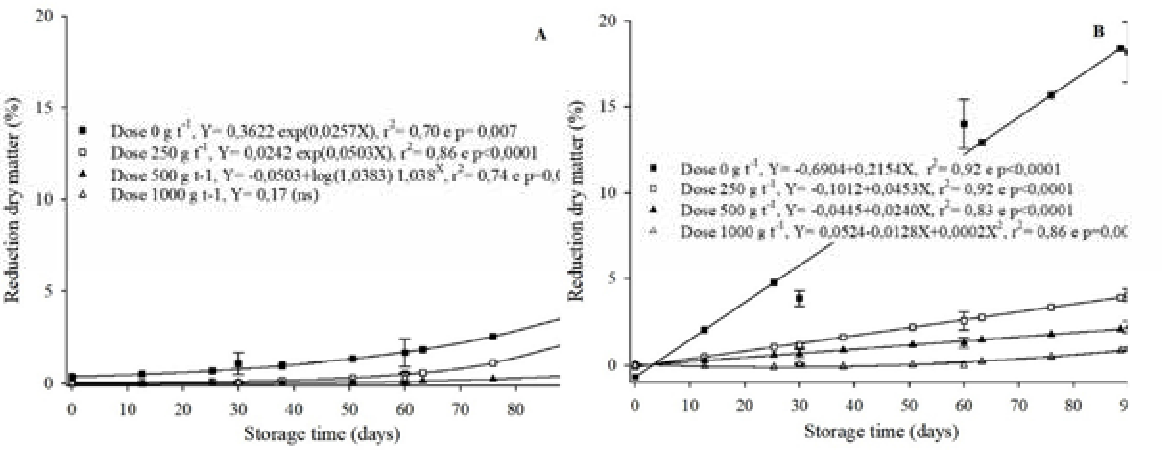

Figure 5. Reduction of dry matter in corn grains, with the application of different doses of diatomaceous earth and stored at a temperature of 22 (A) and 30 ºC (B).

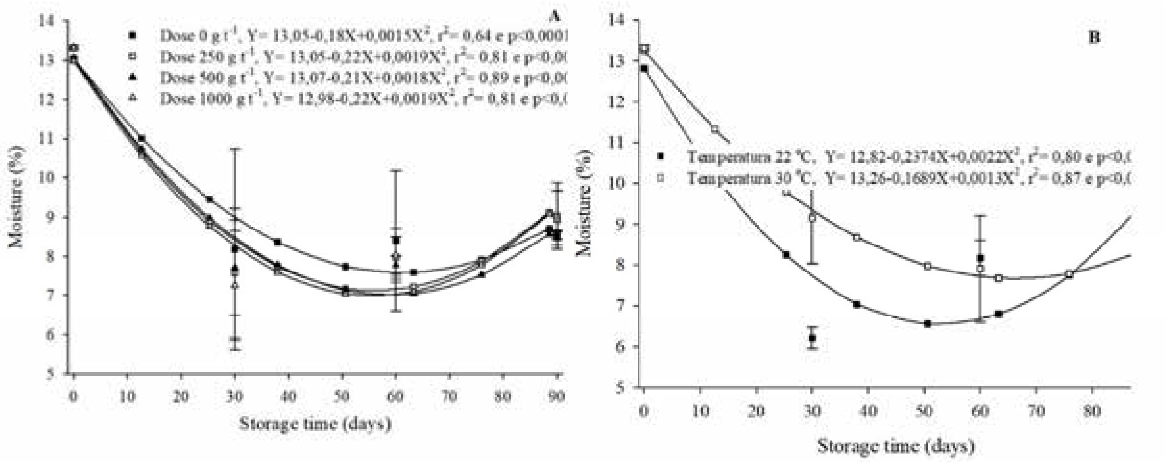

Figure 6. Moisture content in corn grains with different doses of diatomaceous earth and stored at temperatures of 22 (A) and 30ºC (B).

exchanges of heat and water between them and the ambient air until the limit of the hygroscopic equilibrium is obtained, under certain conditions of temperature and relative humidity (ELIAS; OLIVEIRA, 2009).

CONCLUSIONS

- The bulk density, throughout the storage, presented similar reduction for the grains stored at 22°C, regardless of the DE dose applied. The grains stored at 30°C showed a similar reduction of bulk density when treated with DE, except for the control treatment, which showed the greatest reduction in all evaluated conditions.
- For the 22°C temperature, the lowest values of mortality, emergence and live insects occurred, regardless of the doses of DE evaluated throughout the storage. At 30°C, there was an increase in mortality, emergence and live insects at all diatomaceous doses evaluated during storage.
- The use of DE was efficient in the control of *S. zeamais*, presenting better results with increasing dose and storage temperature.
- The reduction of dry matter was observed at all doses and storage temperatures evaluated, but it was more pronounced in those grains that did not receive the DE application and stored at 30°C.

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