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Efficacy of diatomaceous earth and temperature to control the granary weevil, *Sitophilus granarius* (Coleoptera: Curculionidae) in stored wheat

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Abstract

This study was carried out to investigate the suitability of the diatomaceous earth (DE) material in biological control of the granary weevil, *Sitophilus granarius* depending on the temperature. Treatment with DE is an efficient insect control technique in Integrated Pest Management (IPM) programs of stored grain products. Its main advantages are low toxicity to environment, non-target organisms and long lasting efficacy. The objective of this application was to evaluate the efficacy doses and temperatures to control the granary weevil, *S. granarius* in stored wheat. In plastic cups with 100 g of clean and dry wheat grains, 40 non-sexed 7-14 day-old adults of *S. granarius* were submitted to the following applications in three replicates; DE (Detia® Diatom-DDE 90) at 300, 600 and 900 mg kg^{-1}, at 15, 25 and 30 ºC. The mortality was evaluated from the 2nd to 32th day. After this period, the adults were removed and the progeny was kept until the 60th day when the insects were counted and the grain moisture content evaluated. The effect of temperature on *S. granarius* mortality was significant for the three DE doses. The 600 and 900 mg kg^{-1} doses caused the highest mortality at 25 ºC and 30 ºC but with no significant difference between them. The progeny development was significantly higher in the control group compared to the treatments with DE. There was no significant difference in the number of progeny among the three DE doses at any of the temperatures studied. The results support the use of DE as an effective grain protectant against the granary weevil, *S. granarius* in stored wheat.

Keywords: *Sitophilus granarius*, diatomaceous earth, stored grain protectant

Introduction

Stored grain insects may cause a serious threat to grain and seed stored for several months. Significant loss in both grain and seed quality and quantity may occur. It is critical to know what type of insect pests may potentially infest the grain and seed (Khare, 1994) [1].

The stored grain insect pests can be categorized as major or minor pests based on the severity of damage. On the basis of their feeding behaviour, these can be grouped as external and internal feeders of grains or seeds (Srivastava and Subramanian, 2016) [2].

External feeders feed on germ and endosperm from outside. These may attack whole seed and damage the germinal portion or feed on the seeds, which have already been damaged/ infested by other insects or broken mechanically. These insect pests or their stages are generally visible among the seeds (Srivastava and Subramanian, 2016) [2].

Internal feeders that feed on whole grains where the larvae live inside the kernels and seeds causing damage. The damage created by these insects may lead the producer to realize a discount fee at the time of sale if there are 32 or more insect damaged kernels in a 100 g sample of grain (Srivastava and Subramanian, 2016) [2].

Internal feeding insect pests of stored grain and seed feed upon intact, whole kernels. After the eggs are laid in, on or loosely among the kernels and seeds, the larvae burrow into them causing damage within the kernels and seeds where it is not observable. An infestation is known only when adult insects are observed or holes in the kernels are detected. In Oklahoma, there are seven main insect pests that are internal feeders and these occur in three insect groups: I-grain weevils (rice weevil-*Sitophilus oryzae*, granary weevil-*Sitophilus granarius*, maize weevil-*Sitophilus zeamais*, cowpea weevil-*Callosobruchus maculatus*, bean weevil-*Acanthoscelides obtectus*), II-grain borers (lesser grain borer-*Rhyzopertha dominica*), III-grain moths (Angoumois grain moth-*Sitotroga cerealella*) (Abd El-Aziz, 2011) [3].

Shortly, internal feeders mostly lay eggs inside or on the surface of grains, spend a part or entire larval and pupal life inside the grains and only emerge as adults. These contribute significant loss of germination which is not detectable outside (Mondal and Parween, 2000) [4].

The granary weevil, *Sitophilus granarius* Linnaeus, 1758 is a small dark-coloured beetle with a snout. The size of this insect is about $^{3}/_{16}$ of an inch or smaller. It does not have functional wings and can only walk from place to place. Its thorax is marked with oval or elongated punctures (Karakas, 2016) [5].

Granary weevil adults live an average of 7 to 13 months, and each female lays 50 to 250 eggs during this period. The female lays its eggs similar to the rice weevil. The larva hatches from the egg and then consumes the interior of the kernel, pupates and emerges as an adult. The hole is jagged in shape. The life cycle from egg to adult is about 28 to 35 days (Longstam, 1981a; Karakas, 2017) [6, 7].

The grains that the grain weevil generally attacks are rye, wheat, barley, corn, oats and rice. To a lesser extent also buckwheat, pasta, macaroni, spaghetti and flour are affected. The damage caused by the weevil consists mainly of quality and weight loss. In addition, the beetles cause nutrition reduction, flavour decay and germination reduction. This is especially true for parties from the developing world. Parties that are particularly vulnerable to damage are those stored for years in moderate to poor storage (Davis and Bry, 1985) [8].

Control of this insect population around the world is primarily dependent on insecticides and fumigants, and ultimately undesirable effects on non-target organisms, feeding, environmental and human health concerns. Growing concern about the level of pesticide residues in foods has prompted research to look for alternatives to synthetic pesticides (Hamza *et al*., 2016) [9].

Chemical control of insects in storage has been used with severe shrinkage breaks. Synthetic insecticides are expensive and in most cases did great ecological damage with only moderate results (Hamza *et al*., 2016) [9].

In contrast, the low toxicity of botanical insecticides makes the product cheap to handle and apply. In most cases, materials are available locally and are affordable. These have generated tremendous interest in recent years as potential sources of natural insect control agents. There is an urgent need for safe, effective and biodegradable pesticides that have no toxic effects on non-target organisms (Karakas, 2020) [10].

Diatomaceous earth (DE), which is a fine powder composed by diatomaceous algae carapaces, represents one of the most efficient types of inert dusts and has been used for insect control around the world. Interest in this insect control technique has increased enormously in recent years because the number of active ingredients for insect control in grains is restricted to four or five product, mainly due to insect resistance problems (Arthur, 2002; Athanassiou *et al*., 2004) [11, 12].

Diatomaceous earth is not toxic to humans, domestic animals, and environment and does not leave toxic residues in the grain and by products. However, its direct action on insects is slower than that of synthetic insecticides, permitting oviposition and offspring production, although in love numbers. Also, DE effects some physical properties of grains, particularly bulk density (Korunic, 1998) [13].

The action of DE is attributed to the desiccation caused by adsorption and the abrasive properties that breaks the epicuticular wax layer, causing loss of body water and death within hours or days. Aldryhim (1990, 1993) [14, 15] observed that *S. granarius* and *R. dominica* are more susceptible to DE at 30 ºC than at 20 ºC because of increasing water loss at higher temperature. However, *Tribolium confusum* was more susceptible to DE at low temperatures.

The purpose of this research was to evaluate the effectiveness of DE under different temperatures to control *S. granarius* in stored wheat. This information is important for integrated pest management programs when combining the DE technique with artificial grain cooling or heat treatment.

Materials and Methods

Insect rearing

The granary weevil, *S. granarius* was reared in a 1 L wide-mounted glass jars containing (250 g) soft wheat grains (11% moisture). Mouth of the jars covered with a fine mesh cloth for ventilation and to prevent escape of the weevils. Cultures were maintained in an incubator at 27 ± 1 °C temperature and 60 ± 5% relative humidity (RH). Insects used in all experiments were 1 to 7 day old adults. All experimental procedures were carried out under the same environmental conditions as the cultures. The granary weevil adults were obtained from the stock culture of the laboratory of the Plant Protection Department, Faculty of Agriculture, Ankara. The life cycle can be completed in as little 30 to 40 days during the culture conditions but takes considerable longer in cooler conditions. Adult granary weevils can live up to eight months and can produce up to four generations per year.

Insect control material

The DE used was the commercial product Detia® Diatom-DDE 90, which is composed of 90% of silicon dioxide (SiO_2), particles of 10-15 mm; apparent density of 200-230 g L^{-1}, inert ingredients 10% and soluble in water.

Bioassay

The wheat grains were previously disinfected by freezing at -20 ºC for five days, then placed in plastic cups and mixed with different doses of DE: 300, 600 and 900 mg kg^{-1}, and homogenized by vigorous hand agitation for three minutes. From each application were taken three replicates of 100 g placed in plastic cups of 500 ml capacity. Forty non-sexed, 7 to 14 day-old adults of *S. granarius* were placed in each flask, and covered with a screen flap. Three replications of each dosage plus a control group without DE were kept in an incubator cabins at 15, 25 and 30 ºC with 60 ± 5% relative humidity and 18 h dark / 6 h light photoperiod.

For all applications, the mortality was recorded at the 2nd, 4th, 7th, 11th, 18th and 32th days, and all the adults removed by the 32th day. Insects that did not move after being touched with a brush, after two or three minutes, were considered dead. On the 60th day after infestation the wheat grain was sieved and the granary weevil adults of the second generation were counted.

The moisture content of the wheat grain was determined by the oven method (Lazzari, 1997) [16], using three replicates of 15 g of wheat grains placed in an oven during 72 h at 100 ± 5 ºC and weighed out on precision scale.

Statistical data

The mean mortality and the standard error were calculated for each date of sampling for each application. The data were analysed by variance analysis and means were compared by Tukey’s multiple range test at 5% probability, using the IBM SPSS Statistics 22.0.

Results

The average mortality of *S. granarius* at 300 mg kg^{-1} was significantly higher at 25 ºC and 30 ºC than at 15 ºC (Table 1). At 600 and 900 mg kg^{-1} there was no significant differences in mortality at the temperatures investigated.

The average mortality at 25 and 30 ºC for the three DE doses was significantly higher than that for the control group (Table 1). At 15 ºC, the doses of 600 and 900 mg kg^{-1} caused higher mortality than 300 mg kg^{-1}, although at this last dosage the mortality was significantly higher than the control group.

At 15 ºC, the mortality at 600 and 900 mg kg^{-1} DE started by the 7th day of exposure (Table 2), and by the 18th day, the mortality was already 94.0% and 96.8% respectively, for two doses. The difference was not significant between the applications with 600 and 900 mg kg^{-1} of DE in the 18th and in the 32th day after exposure. Application with 300 mg kg^{-1} the mortality was lower than in the other two by the 18th and 32th days after exposure, with cumulative mortality of 78.6% and 82.8%, respectively. In the control group, the cumulative mortality was only 6.4% by the 32th day, when the insects were removed to evaluate the number of the second generation progeny.

At 25 ºC, mortality started by the 4th day in all applications with DE (Table 3) and there was no significant difference between 300, 600 and 900 mg kg^{-1} after the 11th day of application. The cumulative mortality by the 18th day was 96.4% for 300 mg kg^{-1}; 98.3% for 600 mg kg^{-1} and 100.0% for 900 mg kg^{-1}. The mortality in the control group was 76.8% after 32 days after application, due to a non-explained cause.

At 30 ºC, mortality started by the 4th day in all applications with DE (Table 4). There was no significant difference between 300, 600 and 900 mg kg^{-1} after the 18th day of application. The cumulative mortality after the 32th day of exposure was 96.3% for 300 mg kg^{-1} and 100.0% for both 600 and 900 mg kg^{-1}. There was no mortality of *S. granarius* until the 32th day in the control group at 30 ºC.

The average number of offspring produced by the 60th day is shown on Table 5. In the plastic cups with wheat grains treated with 300, 600 and 900 mg kg^{-1} DE and kept either at 30 or 25 ºC, the number of *S. granarius* from the second generation was significantly lower than in the control group. However, at 15 ºC, there was no significant difference between the 300 mg kg^{-1} and the control group. At 30 ºC after 60 days of exposure, the number of descendants was significantly higher (114 insect) than at the two other temperatures for the control group, and the wheat grains were severed damaged at the end. There was no significant differences in progeny production for the three DE doses in the three temperatures; however, there was a tendency of increasing of progeny with increasing temperatures and DE doses, especially at 30 ºC.

Table 1: Average mortality (%) ± Standard Error (SE) of *Sitophilus granarius* in wheat grain after 32 days of exposure to diatomaceous earth (DE) at different doses and temperatures; 60 ± 5 relative humidity and 18 h dark/6 h light photoperiod.

Doses (mg kg^{-1} DE)	**Temperatures (ºC)**		
	15	25	30
300	82.8 ± 1.5 B b	96.4 ± 1.7 A a	98.2 ± 2.7 A a
600	94.4 ± 2.4 A a	99.8 ± 2.7 A a	100.0 ± 0.3 A a
900	98.7 ± 2.1 A a	100.0 ± 0.8 A a	100.0 ± 0.7 A a
Control group	5.98 ± 0.5 B c	87.0 ± 8.3 A b	0 B b
CV (%)	7.04		

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability.
CV: Coefficient of variation at 95%.

Table 2: Cumulative mortality (%) ± Standard Error (SE) of *Sitophilus granarius* in wheat grain after different exposure time and doses of diatomaceous earth (DE) at 15 ºC, 60 ± 5 relative humidity and 18 h dark/6 h light photoperiod.

Exposure time (Days)	**Mortality / DE doses (mg kg^{-1})**			
	0	300	600	900
2	0 A a	0 A b	0 A c	0 A c
4	0 A a	0 A b	0 A c	0 A c
7	0 A a	0 A b	3.2 ± 0.2 A bc	7.2 ± 0.5 A c
11	0 C a	6.4 ± 0.3 BC b	12.0 ± 1.3 B b	34.0 ± 1.7 A b
18	0 C a	78.6 ± 2.3 B a	94.0 ± 1.9 A a	96.8 ± 1.5 A a
32	6.4 ± 0.3 C a	82.8 ± 1.5 B a	94.0 ± 2.1 A a	98.1 ± 2.1 A a
CV (%)	16.68			

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability.
CV: Coefficient of variation at 95%.

Table 3: Cumulative mortality (%) ± Standard Error (SE) of *Sitophilus granarius* in wheat grain after different exposure time and doses of diatomaceous earth (DE) at 25 ºC, 60 ± 5 relative humidity and 18 h dark/6 h light photoperiod.

Exposure time (Days)	**Mortality / DE doses (mg kg^{-1})**			
	0	300	600	900
2	0 A b	0 A c	0 A c	0 A d
4	0 B b	3.4 ± 0.6 AB c	6.7 ± 1.8 AB c	15.1 ± 2.1 A c
7	0 C b	24.1 ± 1.8 B b	28.0 ± 2.2 B b	45.0 ± 2.8 A b
11	0 B b	84.6 ± 1.1 A a	95.1 ± 2.8 A a	95.6 ± 2.8 A a
18	0 B b	96.4 ± 1.8 A a	98.3 ± 2.2 A a	100.0 ± 0.8 A a
32	76.8 ± 7.0 B a	96.5 ± 2.1 A a	98.4 ± 2.6 A a	100.0 ± 0.8 A a
CV (%)	14.43			

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability.
CV: Coefficient of variation at 95%.

Table 4: Cumulative mortality (%) ± Standard Error (SE) of *Sitophilus granarius* in wheat grain after different exposure time and doses of diatomaceous earth (DE) at 30 ºC, 60 ± 5 relative humidity and 18 h dark/6 h light photoperiod.

Exposure time (Days)	**Mortality / DE doses (mg kg^{-1})**			
	0	300	600	900
2	0 A a	0 A c	0 A c	0 A c
4	0 A a	3.5 ± 0.2 A c	15.4 ± 0.4 A c	15.1 ± 0.2 A c
7	0 B a	15.4 ± 0.4 B c	46.6 ± 1.6 A b	48.8 ± 1.5 A b
11	0 C a	46.2 ± 1.2 B b	89.1 ± 0.4 A a	93.4 ± 2.6 A a
18	0 B a	86.8 ± 2.1 A a	95.2 ± 1.6 A a	98.4 ± 1.7 A a
32	0 B a	96.3 ± 0.2 A a	100.0 ± 0.5 A a	100.0 ± 0.8 A a
CV (%)	20.01			

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability.
CV: Coefficient of variation at 95%.

Table 5: Average number (%) ± Standard Error (SE) of second generation progeny of *Sitophilus granarius* in wheat grain after 60 days of exposure to diatomaceous earth (DE) at different doses and temperatures; 60 ± 5 relative humidity and 18 h dark/6 h light photoperiod.

Doses (mg kg^{-1} DE)	**Temperatures (ºC)**		
	15	25	30
300	8.42 ± 1.7 A a	12.27 ± 1.5 A ab	32.53 ± 2.0 A b
600	2.86 ± 0.6 A b	4.01 ± 0.4 A b	13.86 ± 1.3 A b
900	0 A b	2.04 ± 0.4 A b	9.26 ± 0.6 A b
Control group	20.56 ± 3.1 B a	34.02 ± 3.3 B a	114.26 ± 3.7 A a
CV (%)	68.73		

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability.

CV: Coefficient of variation at 95%.

Discussion

Different studies have been conducted on the use of DE in the management against insect pests of stored seed products until today. This study showed that temperature has a significant effect on the insecticidal efficacy of native DE when tested in stored grain beetles.

The insecticidal efficacy of DE is highly influenced by various factors such as temperature and DE formulation and concentration (Kavallieratos *et al*., 2007) [17]. The deaths of *S. oryzae* and *T. confusum* adults generally increased with increasing temperature and the mortality rate was significantly higher than 20 ° C and 25 ° C at 30 ° C. Studies on the effect of temperature on the efficacy of some commercial DEs against *S. oryzae* and *T.confusum* adults have shown that increasing temperature generally increases insecticidal activity against *S. oryzae* adults (Athanassiou *et al*., 2005; Vassilakos *et al*., 2006; Rojht *et al*., 2010) [18-20]. Vassilakos *et al*. (2006) [19] reported that insecticidal efficacy of the commercial DE formulation, Silisosec®, against *S. oryzae* adults increased with increasing temperature. Pinto (1994) [21] observed a correlation between the doses of DE and the time of exposure of *Sitophilus* spp. in corn, having recorded 100% mortality after 19 days of exposure to 500 and 750 mg kg^{-1}. Lorini and Schneider (1994) [22], testing DE at 500, 750 and 1000 mg kg^{-1} to control rice weevil, *S. oryzae* obtained after 7 days of treatment mortality of 19, 87 and 100% respectively. The previous studies supported that DE is less effective against insects at low temperatures (Aldryhim, 1990, 1993; Collins *et al*., 2001) [14, 15, 23], which was probably due to reduced exposure to DE particles when insect mobility decreases. High temperatures would increase insect movement and increase contact with DE, resulting in greater cuticle damage increase temperature would also lead to a higher metabolic rate, which would result in increased water consumption.

But Athanassiou *et al*. (2007) [24] showed that two different DE formulations, were more effective at 20 ºC than at 30 ºC. Paula (2001) [25] observed that the number of second generation progeny of *Sitophilus* spp. in paddy rice was inversely proportional to the dose of DE.

Mewis and Reichmuth (1998) [26] observed similar results in a laboratory experiment with *S. granarius* exposed to treated wheat grains with DE at 25 ºC and 14.5% RH. They reported that the adults died within a few days, which was enough time to produce progeny and result in a considerable population increase after 42 days.

Arthur and Throne (2003) [27] demonstrated that, although adult weevils are killed by exposure to DE, some oviposition could still occur and progeny suppression may not be complete.

Other studies have shown that the other weevil, *S. oryzae* is the most DE-susceptible, followed by *R. dominica* and *T. confusum* (Kavallieratos *et al*., 2005; Athanassiou *et al*., 2014) [28, 29].

Conclusion

These results support the use of DE as an effective grain protectant against *Sitophilus* spp. in stored grains and as an important Integrated Pest Management (IPM) component to attend to the market and consumer pressures. The interactions of DE with several abiotic factors such as temperature, relative humidity, grain moisture content and biotic factors such as insect species, grain kind and all the overall storage conditions such as grain cleaning, fumigants, insecticides should be continuously investigated especially under large scale storage.

References

1. Khare BP. Pests of stored grain and their management. Kalyani Publishers, New Delhi, 1994, 304.
2. Srivastava C, Subramanian S. Storage insect pests and their damage symptoms: An overview. Journal of Grain Storage Research, 2016, 53-58.
3. Abd El-Aziz. Control strategies of stored product pests. Journal of Entomology,2011:8(2):101-122.
4. Mondal KAMSH, Parween S. Insect growth regulators and their potential in the management of stored-product insect pests. Integrated Pest Management Review,2000:5:255-295.
5. Karakas M. Toxic, repellent and antifeedant effects of two aromatic plant extracts on the wheat granary weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae). International Journal of Entomology Research,2016:1(6):24-28.
6. Longstam BC. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): a critical review. Protection of. Ecology,1981a:2:83-130.
7. Karakas M. Use of aromatic plant extracts as bio-insecticides for the control of stored-product insect, *Sitophilus granarius*. International Journal of Entomology Research,2017:2(1):27-29.
8. Davis R, Bry RE. *Sitophilus granarius*, *Sitophilus oryzae* and *Sitophilus zeamais*, *Tribolium confusum* and *Tribolium castaneum*. In: Handbook of Insect Rearing. Vol. I. (Singh P. and Moore R.F), Elsevier, Amsterdam-Oxford-New York-Tokyo, 1985, 287-293.
9. Hamza AF, El-Orabi MN, Gharieb OH, El-Saeady AHA, Hussein AER. Response of *Sitophilus granarius* L. to fumigant toxicity of some plant volatile oils. Journal of Radiation Research and Applied Sciences,2016:9:8-14.

10. Karakas M. Repellency potential of certain herbal root samples against the granary weevil, *Sitophilus Granarius* L. (Coleoptera: Curculionidae) adults in stored wheat grains. Fresenius Environmental Bulletin,2020:29(10):9232-9240.
11. Arthur FH. Survival of *Sitophilus oryzae* (L.) on wheat treated with diatomaceous earth: impact of biological and environmental parameters on product efficacy. Journal of Stored Products Research,2002:38(3):305-313.
12. Athanassiou CG, Kavallieratos NG, Andris NS. Insecticidal effect of three diatomaceous earth formulations against adults of *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae) on oat, rye and triticale. Journal of Economic Entomology,2004:97:2160-2167.
13. Korunic Z. Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research,1998:34:87-97.
14. Aldryhim YM. Efficacy of the amorphous silica dust, Dryacide, against *Tribolium confusum* Jacquelin du Val and *Sitophilus oryzae* (L.) (Coleoptera: Tenebrionidae and Curculionidae). Journal of Stored Products Research,1990:26:207-210.
15. Aldryhim YM. Combination of classes of wheat and environmental factors affecting the efficacy of amorphous silica dust, Dryacide®, against *Rhyzopertha dominica* (F.). Journal of Stored Products Research,1993:29(3):271-275.
16. Lazzari FA. Umidade, fungos a micotoxinas na qualidade de sementes grãos e rações. Curitiba, 1997 134.
17. Kavallieratos NG, Athanassiou CG, Vayias BJ, Maistrou N. Influence of temperature onsusceptibility of *Tribolium confusum* (Coleoptera: Tenebrionidae) populations to three modifieddiatomaceous earth formulations. Florida Entomologist,2007:90:616-625.
18. Athanassiou CG, Vayias BJ, Dimizas CB, Kavallieratos NG, Papagregoriou AS, Buchelos CT. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. Journal of Stored Products Research,2005:41:47-55.
19. Vassilakos TN, Athanassiou CG, Kavallieratos NG, Vayias BJ. Influence of temperature on the insecticidal effect of *Beauveria bassiana* in combination with diatomaceous earth against *Rhyzopertha dominica* and *Sitophilus oryzae* on stored wheat. Biological Control,2006:38(2):270-281.
20. Rojht H, Horvat A, Athanassiou CG, Vayias BJ, Tomanović Ž, Trdan S. Impact of geochemical composition of diatomaceous earth on its insecticidal activity against adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Journal of Pest Science,2010:83(4):429-436.
21. Pinto JR. Uso de pós-inertes no controle de insetos de grãos armazenados. Curitiba, Dissertoção (Mesterado em Entomologia). Universidade Federal do Paraná, 1994, 80 f.
22. Lorini I, Schneider S. Paragas de grãos armazenados: resuldatos de pesquisa. Passo Fundo: Embrapa-Cnpt, 1994; 48.
23. Collins DA, Armitage DM, Cook DA, Buckland A, Bell J. The efficacy of alternative compounds to organophosphorus pesticides for the control of storage mite pests. London: Home-Grown Cereals Authority, 2001, 163.
24. Athanassiou CG, Kavallieratos NG, Peteinatos GG, Petrou SE, Boukouvala MC, Tomanoviæ Z. Influence of temperature and humidity on insecticidal effect of three diatomaceous earth formulations against larger grain borer (Coleoptera: Bostrychidae). Journal of Economic Entomology,2007:100(2):599-603.
25. Paula MCZ. Manutençãu da qualidade do arroz armazenado: monitoramento a controle de insetos. Curitiba. Tase (Doutorado em Entomologia). Universidade Federal do Paraná, 2001, 74 f.
26. Mewis I, Reichmuth C. Diatomaceous earth against the Coleoptera granary weevil *Sitophilus granarius* (Curculionidae) the confused flour beetle *Tribolium castaneum* (Tenebrionidae), mealworm *Tenebrio monitor* (Tenebrionidae). In: Zuxun J *et al*., (Eds.). International Working Conference on Stored-Products Protection 7. Beijing. Chengdu: Sichuan Publishing House of Science and Technology, 1998, 966-973.
27. Arthur FH, Throne JE. Efficacy of diatomaceous earth to control internal infestations of rice weevil and maize weevil (Coleoptera: Curculionidae). Journal of Economic Entomology,2003:96(2):510-518.
28. Kavallieratos NG, Athanassiou CG, Pashalidou FG, Andris NS, Tomanovic Z. Influence of grain type on the insecticidal efficacy of two diatomaceous earth formulations against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Management Science,2005:61:660-666.
29. Athanassiou CG, Kavallieratos NG, Lazzari FA. Insecticidal effect of Keepdry® for the control of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) on wheat under laboratory conditions. Journal of Stored Products Research,2014:59:133-139.