Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(5): 858-864

Received: 21-07-2017
Accepted: 22-08-2017

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The evaluation of the mortality and repellency effect of diatomaceous Earth (Sayan®) on three coleopteran store pests

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Abstract
Replacing common insecticides with natural compounds can be a suitable method for controlling damage to stored products by pests. In this research, the mortality and the repellency effect of Sayan® formulation of Diatomaceous Earth on adult *Sitophilus oryzae L., Oryzaephilus surinamensi,* and *Tribolium castaneum (Herbst)* have been investigated. A petri dish with an 8 cm diameter and a height of 1.5 cm was used for the bioassay. The mortality assessment was dose and time dependent. The LC_{50} values were 2.5, 1.9 and 12 gr/kg food for *S. oryzae* L., *O. surinamensi* and *T. castaneum,* respectively, at 96 h post-treatments. Consequently, O. *surinamensi* and *T. castaneum* had the highest and the least sensitivity to this compound, respectively. The maximum mean of the repellency effect of Diatomaceous Earth on *S. oryzae* L was 48.95%. Overall, the results of this research have shown that Diatomaceous Earth can be used against stored products pests and it presents an alternative way for the chemical control of these pests.

Keywords: Diatomaceous Earth, stored pests, repellency

Introduction
Rice weevil, saw-toothed beetle and flour beetle are three significant stored product pests, which while feeding on various stored products, leave their larval extinctions and shells, thereby causing immeasurable damage to these products [11, 27, 7]. Mostly, chemical pesticides are used to control these pests. Notwithstanding their benefits pertaining to relatively short and cost-effective control of pests, the unconventional use of these compounds have created many problems for human health and the environment as well. In recent years, many attempts have been made to replace these pesticides with non-chemical compounds. Moreover, public demand for no chemical residue in products and food has also increased. Also, resistance to insecticides among insect populations has increased, and the effect of pesticides has decreased [1].
Diatomaceous earth has been directly used for controlling pests of stored products and in empty warehouses in some countries [13, 37].
Diatomaceous earth has long been known as a potential protector of grains, because it's safe to use and does not affect the final quality of grains. It leads to long-term protection, and compared to the other methods, involves lower cost in grain control [25].
Diatomaceous earth clings to the insect's cuticle wax layer by surface absorption, causing scratches on the surface of the insect's body, and this action causes the body to lose its water and eventually die [12]. This compound also has a repellency effect on some pests [44].
Many diatomaceous earth (DE) formulation are effective against many stored products pests which one of them is Sayan® formulatin. (New Sentences)
Several diatomaceous earth (DE) formulas are now commercially available, and many are effective against a wide range of stored pest species [37].
In Iran, due to the abundance of sources of diatomaceous earth, there have been less perceptive studies of these resources as well as less research on its effects in agriculture [29].
Therefore, this research investigated diatomaceous earth's (Sayan® formulation) ability of causing mortality and repellency on three pest strands under laboratory conditions.

Materials and Methods
Preparation of Trial
Diatomaceous earth with the trade name of Sayan®, with an average particle diameter of 50 microns, was obtained from Kimia Sabzirovar Co., Tehran. The diatomaceous earth was used in combination with food (wheat).

Insect rearing
The tested pests in this research were rice weevil, saw-toothed beetle and flour beetle. The saw-toothed beetle was reared on mixed wheat and yeast (10:1), the flour beetle was reared on mixed white flour and yeast (10:1), and the rice weevil was reared on rice in transparent plastic containers with dimensions of 8 × 15 × 20 cm in a germinator at a temperature of 27 ± 2°C and relative humidity of 65 ± 5%.

Bioassay tests
Mortality effects
First, a preliminary test was conducted to determine the minimum and maximum concentrations on each pest, and then three concentrations between the two concentrations were considered as the logarithmic intervals [34] and the main tests of the five final concentrations with controls were achieved. The concentrations used for the rice weevil were 7, 76, 4, 23/3, 2/2 and 1.5g/kg of food, for the saw teeth beetle, they were 7.7, 73.3, 91.1, 97/0 and 0.5g/kg of food, and for the flour beetle, 32, 38/22, 84/15, 22/11 and 8 g/kg of food. Each of the treatments was prepared in a mixture of 20 grams of wheat and poured into glass pots with a diameter of 8 cm and a height of 1.5 cm. For the control treatment, food was used alone. Then, 12 full insects were placed between 1 and 3 days in each of the Petri dishes. The containers were transferred to the germinator at 27 ± 2°C and 65 ± 5% relative humidity, and then the number of dead and live insects was counted for 24, 48, 72 and 96 hours after the treatment. This experiment was repeated six times.

Repellency effects
According to LC_{50} obtained in the bioassay tests, an experiment was conducted to evaluate the repellency of diatomaceous earth powder in a randomized complete design with four treatments and four replications for each pest. Concentrations of 1, 0.9, 0.8 and 0.7 g/kg of food were considered for this experiment. To do this, petri dishes with a diameter of 12 cm and a height of 1.5 cm were used. After dividing each petri dish into two equal parts by a thin cardboard wall, 0.01, 0.009, 0.008 and 0.007 grams of diatomaceous earth were mixed with 10 grams of wheat and poured into one half of the Petri dish. The other half of the dishes were filled with food alone (control). Then, 12 adult insects (1 to 3 days old) were placed at the center of each container. After 24, 48 and 72 hours of the treatment, the containers were examined and the number of insects in each of the two treatments and controls and the percentages was evaluated. The inclusion was calculated by using the formula PR = [(Nc-Nt) / (Nc + Nt)] × 100. In this formula, Nc is the number of insects on the untreated surface; Nt is the number of insects on the treated surface. Finally, the mean percentage of repellency was in one of the 6 groups (group 0-I-II-III-IV-V). The percentage of repellency in these groups is as follows: 0.1-0.0 (0), 1 / 0-20 (I), 20.1-40 (II), 40.1-60 (III), 60.1-80 (IV), and 80.1-100 (V) (Viglianco *et al*., 2008). This experiment was repeated four times.

Analysis of data
In case of an instance of death in the control, the mortality rate of the other treatments was corrected by the Finney formula [14]. The probit analysis of the data recorded in the bioassay tests was calculated by using SPSS 16.0 software, and the mean of the data was compared with the LSD test. Charts were plotted using Excel software.

Results
Mortality
Except for high concentrations (7, 7.3 and 32 g/kg of foodstuff), the other concentrations did not cause mortality in the tested insects 24 hours after treatment, but with increasing treatment time, the mortality rate in each of the three species increased (Fig. 1). For 72 hours after treatment in the case of the highest applied concentration, 100% mortality was observed in rice weevil, which had significantly higher mortality than that in the other treatments (df = 3.12; F = 147.64; P = 0.0001). The highest concentration also resulted in a mortality rate of 97.5% with Sawtoothed beetle, while the lowest mortality rate was 52.5%. The comparison of the sensitivity of the tested insects to the compositions showed that the flour beetle had higher tolerance to the repellents than the two other insects. The mortality caused by different concentrations of diatomaceous earth in the total number of insects of this pest was 72 hours less than the losses registered in other pests. Also, at the end of the experiment, there was a significant difference between the mortality rate of the total insects of this pest at different concentrations of diatomaceous earth (df = 4.15; F = 31.45; P = 0.0001).
The LC_{50} value of diatomaceous earth was estimated at 21.3, 10.1 and 192.3 g/kg, respectively, for 48 hours after treatment on rice weevils, saw-toothed beetles and flour beetles (Table 1). Accordingly, rice weevils were more susceptible than the two other pests. Over time, the death rate increased and the deadline dropped by 50%. In 96 hours after treatment, the index for these pests was 2.5, 1.9 and 12 g/kg, respectively. Based on the 50% mortality index and the 95% confidence interval, it was found that the total number of saw-toothed beetles was the highest, and most flour beetles had the least sensitivity to the diatomaceous earth.

Repellency effects
The percentage of repellency of different concentrations of diatomaceous earth for the total number of rice weevils, saw-toothed beetles and flour beetles is shown in Table 2. In all the three tested species, the amount of repellency effects increased with increasing treatment time as well as concentration. The highest concentration (1 g/kg), after 72 hours of treatment, was 64.57%, 45.83% and 70.83%, respectively, for the mentioned pests. The time factor for all the three pests was significant. If the highest concentration used, after 48 hours of treatment, the percentage of repellency effects for all the three tested pests increased significantly. However, the highest repellency effects rate was due to the highest concentration of diatomaceous earth and in the whole flour beetle pests, but higher percentage of repellency effects of other concentrations for rice weevils caused the highest average repellency effects percentage for the total number of insects of this pest. The comparison of the mean values showed that the percentage of repellency effects of this concentration of diatomaceous earth, and at the end of the experiment in the total insect count of this pest were significantly lower than in the other two species (df = 11.36; F = 54.68; P = 0.0001). Based on the results, the effect of time

factor on all the three pests was significant. When the highest concentration was used, the percentage of repellency effects for all the three tested pests increased significantly after 48 hours. Although the highest repellency effects rate was due to the highest concentration of diatomaceous earth and in the whole pest population of the tested flour beetles, but the higher percentage of repellency of the other concentrations for rice weevils caused the highest average repellency effects percentage for all the tested insects of this pest. When the average repellency percentage of the four concentrations was used 3 days from the time of treatment of the rice weevils, saw-toothed beetles and flour beetles, 48.95, 32.29 and 43.75 percent, respectively, the difference was significant There was no significant difference between them (df = 2.9; F = 1.06; P = 0.3858) and in groups III, II, and III, when six groups were divided into reversal effects. The mean percentage of repellency was in one of the 6 groups (group 0-I-II-III-IV-V). The percentage of repellency in these groups is as follows: 0.1-0.0 (0), 1 / 0-20 (I), 20.1-40 (II), 40.1-60 (III), 60.1-80 (IV), and 80.1-100 (V) (Viglaianco *et al.*, 2008). This experiment was repeated four times.

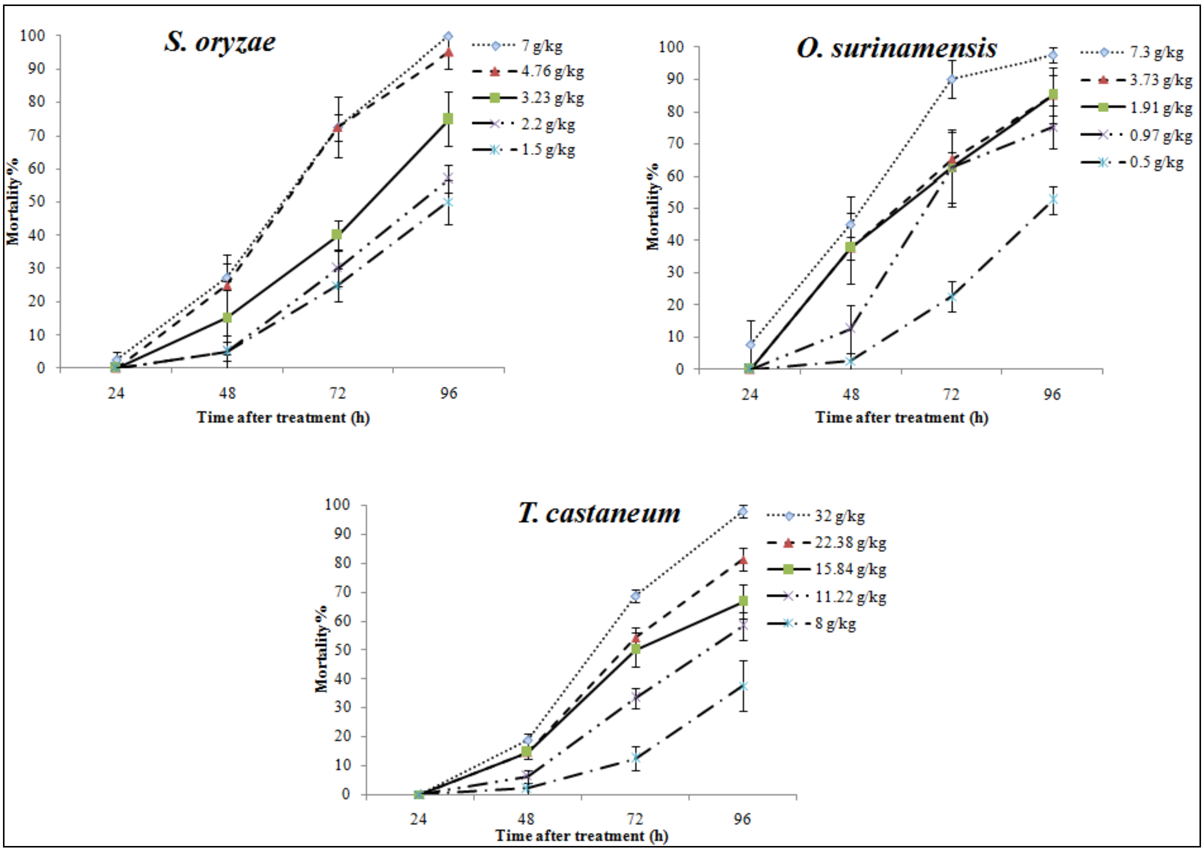

Fig 1:%Mortality of *S. oryzae*, *O. surinamensis*, and *T. castaneum* treated with different concentrations of diatomaceous earth at different treatment times

Table 1: Probit analysis of bioassay data on adult *S. oryzae, O. surinamensis and T. castaneum*

Pest	Time (h)	Total	LC50 (g/kg food) (CI*)	LC90 (g/kg food) (CI)	Slope	χ^2	p-value
S. oryzae	48	432	21.3 (12.9- 125.6)	107.5 (36.9-620.21)	1.82	1.62	0.65
	72	432	5.6 (1.77- 31.5)	24.2 (10.6- 31.9)	2.01	6.8	0.07
	96	432	2.5 (1.4- 8.9)	7.7 (3.8-11.1)	2.59	10.47	0.01
O. surinamensis	48	432	10.1 (8.2- 15)	33.3 (19.7-97.8)	2.56	2.52	0.47
	72	432	2.6 (1.59- 3.44)	12.5 (8.8- 25.9)	1.89	4.93	0.17
	96	432	1.9 (1.02- 2.6)	7.8 (3.55-8.5)	2.08	2.39	0.49
T. castaneum	48	432	192.3 (82.6- 1350.4)	1640.7 (300.4-11185.8)	1.37	1.68	0.64
	72	432	28 (23- 35.9)	125.6 (78.6- 334.02)	1.95	2.69	0.44
	96	432	12 (9.07- 14.6)	38.1 (30.5-55.7)	2.55	2.85	0.41

*CL: denotes confidence limit

Table 2: Repellency% (±SE) caused by different concentrations of diatomaceous earth on three coleopteran stored pests

Pest	Concentration (g/kg food)	Time (h)			Mean repellency% (after 72 h)	Repellency classes
		24	48	72		
Sitophilus oryzae	1	39.57±2.08 c (B)*	49.99±3.4 b (B)	64.57±2.08 a (AB)	48.95±6.83 A	III
	0.9	24.99±3.4 b (C)	50±0 a (B)	56.24±2.08 a(BC)		
	0.8	20.83±2.4 b (C)	33.33±3.4 a (CDE)	37.49±2.4 a (DE)		
	0.7	0±0 b (D)	8.33±3.4 b (G)	37.49±2.4 a (DE)		
Oryzaephius surinamensis	1	24.99±3.4 b (C)	37.49±2.4 a (BCD)	45.83±2.4 a (DC)	32.29±7.86 A	II
	0.9	24.99±3.4 b (C)	33.33±3.4 b (CDE)	45.83±2.4 a(DC)		
	0.8	16.66±0 a (C)	20.83±2.4 a (EFG)	20.83±2.4 a (FG)		
	0.7	0±0 b (D)	12.49±2.4 a (FG)	16.66±0 a (G)		
Tribolium castaneum	1	52.08 ±2.08 b (A)	66.66±3.4 a (A)	70.83±2.4 a (A)	43.75±9.84 A	III
	0.9	45.83±2.4 b (AB)	45.83±2.4 a (BC)	45.83±2.4 a(DC)		
	0.8	25±0 a (C)	25±3.4 a (DEF)	29.16±2.4 a (EF)		
	0.7	16.66±3.4 b (C)	20.83±2.4 ab (EFG)	29.16±2.4 a (EF)		

*Different lowercase letters showed significant differences in each row and different uppercase letters showed significant differences in each column ($P<0.05$).

Discussion

The excessive use of chemical compounds has irreversible effects on beneficial arthropods, drinks, fish and invertebrates [36]. On the other hand, the spread of resistance to insecticides has led to numerous problems in pest control [38, 8]. Delet this sentences: Several reports from different regions of the world have revealed vast resistance of several pest populations to various insecticide groups, including organophosphates, carbonates and fumigant pesticides. In some cases, the replacement of chemical compounds with other control methods is inevitable. Therefore, research on non-toxic methods of pest control are very important [36]. Diatomaceous earth is one of the materials that can be easily used due to its effective properties. Diatomaceous soils have proved to be capable of controlling various groups of insect pests, and can replace the common chemical insecticides [17]. On the other hand, given that diatomaceous earth acts physically, there is no occurrence of genetic resistance due to the use of these compounds [12]. Diatomaceous earth (Sayan formulation) used in this study showed that it had potential against all the three tested pests, and with increasing concentration and time, the mortality rate also increased, which was in accordance with the effect mechanism of diatomaceous soils (scratching the cuticle and the body losing water), and increasing the likelihood of its physical contact with the body of the insect over time is a possible result and is consistent with the findings of other researchers [3, 18]. Based on the results, the susceptibility of the three species tested for diatomaceous earth was different. The sensitivity of insects to diatomaceous earth is very complicated on the basis of their morphological and physiological structures [24]. Some insects are more susceptible to diatomaceous earth on the basis of anatomy and physiology. Delet this sentences: In general, insects with a large area (the size of their bodies) are more sensitive than smaller insects, as the former lose more water from their bodies. Insects with a rough body or hairy body accumulate more diatomaceous earth particles per unit area, resulting in more damage to the cuticle. These insects are more sensitive than others [9]. The variation in the cytokine lipid of cuticle building in various insect species was first reported by Hackman [16]. Neville [30] concluded that any single cuticle combination was linked to environmental factors. On the other hand, the effectiveness of diatomaceous earth depends on its type, target species, treated products, and moisture content [28, 24, 39]. The comparison of the sensitivity of the tested insects to diatomaceous earth based on 50% lethal concentration showed that the sensitivity of flour beetles to diatomaceous earth was lower than that of other coleopteran species.

Previous studies with other types of diatomaceous earth have shown that the *Tribolium spp* pest is resistant against diatomaceous earth [41, 6, 1, 13, 3]. On the other hand, *S. oryzae* and *F. rhyzopertha* dominica have higher sensitivity to this species [2, 21]. One of the reasons that cause more resistance to diatomaceous earth in flour beetles is probably because the insect can restore its body water due to its food intake. Also, the presence of flour can help insects cleanse off diatomaceous earth [1]. On the other hand, since the insect's body does not have much hair, fragments of diatomaceous earth cannot easily stick to the body and maximize its effectiveness [13]. Adult saw-teeth beetles were the most sensitive species against these compounds. The results of the experiments revealed that the mobility of an insect species increases its susceptibility to diatomaceous earth [33]. The saw-teeth beetle's higher sensitivity to diatomaceous earth can be due to the high mobility of this species, and therefore the likelihood of dealing with diatomaceous earth parts is higher. On the other hand, the saw-teeth beetle is small and can easily lose its water due to the properties of diatomaceous earth [24]. According to reports, the death of insects by diatomaceous earth is because of the loss of water due to the crunching of small pieces of pellets and the absorption of oil in the insect's body, thereby breaking the cuticle's wax layer and causing the loss of water and eventual death of the insect [39]. At higher concentrations, the absorption by the waxy layer and the roughness further increases the impact velocity as compared to the lower concentrations. In the present study, the effect velocity was more pronounced in the higher concentrations. The bodies of dead insects were also wrinkled and dried, indicating the effect of diatomaceous earth on the cuticle of the insect body and the loss of water. In other studies, the effectiveness of diatomaceous earth for storing pest control has been proven. Golestan-Hashemi [15] and co-workers estimated the amount of LC_{50} of diatomaceous earth on full insects of *Tribolium confusum* Du Val. Two weeks after the treatment, 183.28 ppm was estimated. Rezaei Torshizi [32] and co-workers studied the effects of diatomaceous earth at different temperatures on adult insects of *Callosobruchus maculatus* (F.), a four-point beetle, and showed that the total insect mortality increased with increasing temperature and duration of heating. The researchers suggested that 60-ppm of diatomaceous earth, with a temperature of 40°C for 30 to 40 minutes, can be used to protect the legumes perfectly against the entire tested population of the four-point beetles. Generally, the effect of diatomaceous earth decreases with increasing humidity and relative humidity, but increases with increasing temperature [39]. In a similar study, the effect of diatomaceous earth of SilicoSec® formulation on the total insects of *T. castaneum* flour beetle was investigated, and it was reported that the LC_{50} of this compound at 48 hours after treatment was 203.135 ppm [35]. In the present study, the LC_{50} of diatomaceous earth of the Sayan® formulation for all the tested insects was the same at 192.3g/kg of foodstuff, which was much higher than the lethal concentration of 50% of the formulations used in the study by the other researchers. The lower toxicity of diatomaceous earth of Sayan® formulation as compared to the SilicoSec® formulation could be due to lower silica content in the Sayan® formulation. Since the amount of silica in the diatomaceous earth is lower, the efficiency of the formulation is also lower [22]. Various reasons such as different formulas of diatomaceous earth, differences in the sensitivity of various biotypes of different pests in different regions, the type of feeding bed, and other reasons have led to a change in the mortality rate of pests [43, 45, 19]. Although the highest susceptibility to diatomaceous earth was reported in the case of the saw-teeth beetles, the least impact was observed in the same pest. Mostly, the effects of repulsion and fecundity were not in the same direction. Similar results were obtained by Kabiri Raeis Abbad *et al*. [20], who showed that, although the pagan cultivar of the citrus had the highest levels of fumigant toxicity on flour beetles, the highest percentage of repellency was attributed to the two orange essential oils and mandarin cultivars. In the present study, the highest repellency rate was observed in rice weevils with an average of 97.79%.

According to the results, to control 50% and 90% of the total population of *T. castaneum* insects, respectively, amounts of 12 g and 38 g of diatomaceous earth in a kilogram of food appeared to be potentially high. In order to reduce the dose, most diatomaceous earth formulations are mixed with other compounds such as silica gel, dry honey, inactivated yeast, and sugar [31, 39]. Considering that *tribolium* genus species are often a major pest, it is important to note that the presence of diatomaceous earth in flour is generally not affected by bread [23]. Also, according to Dowdy and Fields [10], it was found that the use of diatomaceous earth in flour as a treatment increased the heating efficiency to control *T. confusum*. Therefore, the combination of diatomaceous earth with heat can reduce the consumption of this compound by the pest in flour storage depots.

In the management of stored products pests, the treatment time of the products as well as the concentrations of the compounds used are very important. If the concentration used is less than the diatomaceous earth, a long exposure period is required to achieve a satisfactory level of insect control. This is due to the effect of diatomaceous earth on insects [3]. This is because walking for a long time on the treated surface causes more dust particles to be absorbed by the insect's body, thereby resulting in greater wound and loss of water [1]. In the present study, it was also found that if we want to use low-density diatomaceous earth, we need to increase the treatment time so as to maximize the efficacy of this combination. Therefore, the time of exposure to the dust is very important because insects that survive (due to exposure to a small concentration of diatomaceous earth) cause dispersion of the treated surfaces and contamination of other parts of the product mass [39]. This should be taken seriously when treating incomplete cereal grains with diatomaceous earth, such as surface treatment of the product, so that diatomaceous earth serves as an obstacle to product contamination [25]. For all the five concentrations, diatomaceous earth showed maximum efficiency after 96 hours of treatment. Typically, diatomaceous earth samples need more time to handle than fumigant and contact toxins in order to maximize their effectiveness. On the other hand, this compound has high durability. A study by Athanassiou *et al*. [3] showed that diatomaceous earth caused more than 90% mortality in *S. oryzae* population in wheat treated with this product after 270 days of the test.

To reduce the concentration of diatomaceous earth as well as to reduce toxicity toward non-target organisms, diatomaceous soils can be combined with other materials, such as plant or chemical compounds [5, 4, 41]. Diatomaceous soils are, however, generally not toxic to consumers who use the products treated with these compounds. This is because silica does not accumulate in the mammalian body and is excreted as silica. Thus, the daily absorption of silicates from water and plants and from soil by ruminants is high. On the other hand, more than 99% of diatomaceous earths undergo washing, and modern production plants also use special steps to clean grains before milling [24].

Delet This Section: 6. Conclusion

The results indicated that the diatomaceous earth sample of Sayan formulation used for the three coleopteran infestations used in the research, and considering the low risk to the environment and human health, can be considered as one of the most suitable methods in pest control. The control of these pests was undertaken in the laboratory level, although additional testing is required at the warehouse level to prove this hypothesis.

Delet this section: Authors' Contributions

This article is part of the thesis of master student in agricultural entomology, which has done at dep. of plant protection in Sari Agricultural Science and Natural Resources University

Delet this section: Funding/Support

This research is financially supported by Sari Agricultural Science and Natural Resources University.

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