STUDY OF INSECTICIDAL AND BEHAVIORAL EFFECTS AND SOME CHARACTERISTIC OF NATIVE DIATOMACEOUS EARTH AGAINST THE BEAN WEEVIL, *ACANTHOSCELIDES OBTECTUS* (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

In this study, efficacy and some characteristics of the local diatomaceous earth (DE) were investigated against been weevils [*Acanthoscelides obtectus* (Say) (Col.: Bruchidae)] on chickpeas (*Cicer arientum* L.) under laboratory conditions. In the experiments, Turco000, Turco004, and Turco020 derived from native sources (Ankara, Turkey), and Protector®, a commercial product, were used. The bioassays were carried out at 25±1°C temperature, 65±1% r.h. with six concentrations of DEs (100, 200, 400, 600, 800 and 1000 ppm) at the Plant Protection Central Research Institute, Entomology Laboratory in Ankara in 2016. The mortality of adults was evaluated after the 4th, 7th and 14th days of treatments. In order to determine progeny production (F1), the treated chickpeas were further incubated at the same conditions for the 55-days. Turco000 gave 100% mortality to *A. obtectus* adults at 1000 ppm after 4th day as Protector® at 600 ppm concentration did. The lowest mortality rate was observed at Turco020 at a dose of 100 ppm 7 days after treatment. When the effects of diatomaceous earth formulation on the fertility of F1 were investigated, it was determined that all diatomaceous earth formulation had an effect on different rates. There was no progeny production with Turco000 at 200 ppm concentration at the end of 55-day incubation period and this was followed by Turco004 and Turco020, respectively. Results showed that the protective residual effect of Turco000 continued throughout the experiments. In conclusion, Turco000 can be used as a valuable tool for *A. obtectus* in stored product pest management programs.

KEYWORDS:
The bean weevil, Local Diatomaceous earth, Effectiveness, Progeny production

INTRODUCTION

The bean weevil *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) is a major pest of beans and other legumes from the Fabaceae family. The primary host of this weevil is the common bean (*Phaseolus vulgaris*). The weevil also attacks chickpeas (*Cicer arietinum*) and other stored legumes [1]. The consequence of the insect damage to the products causes qualitative and quantitative losses to both nutritional properties and the market value of the stored legumes. The storage period of the legumes, which have an important place in human nutrition, must be carried out under suitable conditions and protected with minimum loss. In stored products, direct or indirect losses from biotic and abiotic factors such as relative humidity, temperature, grain size, and insect species occur during storage. Harmful organisms such as microorganisms, mites, rodents, and insects are among the biotic factors that cause this loss. Stored legumes are particularly damaged by Bruchidae (Coleoptera) family members in the field and/or post-harvest period. These organisms degrade the quality of the product due to the body residues such as exuvia, seta and the substances they release on the product. It has been observed that alternative efforts to the chemical control have recently gained a significant increase in order to reduce this damage rate. Bean weevil, which has a wide spreading area and adapts to environmental conditions, has high tolerances against various insecticides [2]. For this reason, many research and efforts have been made to find effective means of ensuring long-term storage of legumes. The use of natural enemies, plant extracts, volatile oils, and inert dust have been accepted as promising strategies for the control of *A. obtectus* until now [3, 4, 5, 6, 7]. In stored products, various control methods have been applied to insect pests. These methods include; physio-mechanical methods (modified atmosphere, cooling, drying, high temperature, radio waves etc.), biotechnical control methods, cultural control methods and use of synthetic chemicals intensively. Fumigation is the most common chemical control method in the world. Fumigation is preferred because of its rapid penetration, ease of use and low cost in control of storage pests. Methyl bromide, one of the fumigants used intensively, has been banned except in limited applications, under the Montreal Protocol. The residue of some insecticides applied directly to the product to protect stored legumes from insect pests may cause acute or chronic toxicity to the consumer at significant levels. On the other hand, the development of resistance in the pests also causes problems in practice. It has been known that resistance to some important stored product pests against many effective substances such as malathion, chlorpyrifos-methyl, fenitrothion, pirimiphos-methyl, etrimfos and the like which are being used in storage [8]. One of the physical control treatment is diatomaceous earth applications.

Diatoms, either solitary or colonial, microscopic are photosynthesizing algae that have a siliceous skeleton, called frustule and are found in almost every aquatic environment ranging from freshwaters to marine waters. In fact, they are found virtually anywhere moist enough. They have both benthic and planktonic forms that both are restricted to the photic zone, since they are all strictly autotrophic (water depths down to about 200 m depending on the water clarity). Diatoms have a variety of different diameter or length such as 20-200 microns, some even reach up to 2 millimeters in length. They are recorded in geological records since Cretaceous. Diatoms may occur in such large amounts and be well preserved enough to form sediments composed almost entirely of diatom frustules, then called diatomites or partly of frustules, then called diatomaceous earth, in both cases, it becomes economic deposits and may be used in a number of applications including filters, agriculture, paints, toothpaste, and many others.

Elements such as iron, calcium, aluminum, and thorium are present in the form of oxides in the components of the diatomaceous earth, which are basically amorphous silica. In insects, cuticle acts as an exoskeleton and provides protection and support for internal organs. The main barrier to prevent water loss from an insect is the epicuticular lipids. In insect morphology, epicuticular lipids act as a platform for the semiochemicals. Also plays an important role like retention of water in the body, protection from the body external corrosive and toxic substances [9]. Diatomaceous earth absorbs the cuticular lipids and it also abrades the cuticles of insects and causing death by desiccation [10, 11]. Diatomaceous earth can be successfully incorporated into the IPM program as they have proven to be very effective against insect pest species with low mammalian toxicity, long-lasting efficacy and being natural insecticides.

In this study, we intend to determine the effectiveness of native diatomaceous earth obtained from central Anatolia around Ankara region as stored-legume protectants against *Acanthoscelides obtectus*. Also, the present study was designed to assess the effect of particle size and behavioral effect of the tested diatomaceous earth on the insects under laboratory conditions.

MATERIALS AND METHODS

Insect mass-rearing. *Acanthoscelides obtectus* adults were reared in sterile 1-L glass jars on chickpeas in incubators (Nüve ID 501, Ankara, Turkey) maintained at about 25±1°C and 65±1% relative humidity. For the prevention of possible contamination, chickpeas were kept in the deep freezer at -18 ºC at least 7 days. The clean chickpea

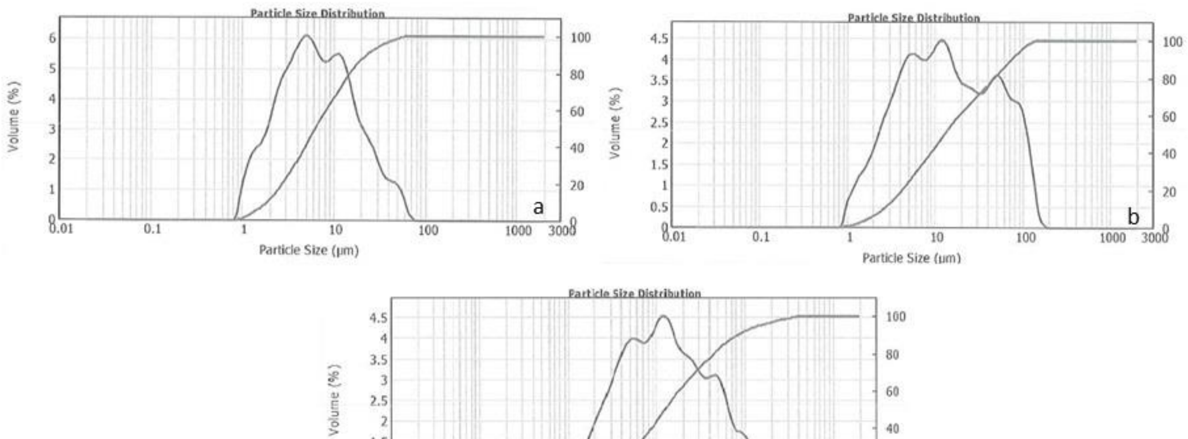

FIGURE 1
Grain size distribution of native DE's (a: Turco000 b: Turco004 c: Turco020).

used as both food source and oviposition area was filled to half of the jar. Approximately, 450-500 mixed-sex adult individuals were transferred in the glass jar. In order to ventilation, the lids of the glass jar were drilled, each lid has eight holes with a diameter of 1mm. The adults on the chickpeas were vacuumed after 24 hours later. Thus, we have obtained 0-24 hours old eggs. The newly emergence adults were observed about 30-33 days after. In the trials, 1-3 days old mixed-sex adults were used.

Diatomaceaus earth characterization. DE's used in this study were acquired from a local commercial company operating in Ankara-Kazan and Beypazarı counties (Beg-tuğ Mineral). Physico-chemical tests and analysis of the DE's (e.g. grain size, chemical content, and morphology) were carried out at General Directorate of Mineral Research and Exploration (MTA) labs in Ankara, Turkey. Grain size distribution and SEM images of the DE's are presented in Figure 1 and 2, respectively. The chemical composition of the DE's is given in Table 1.

Representative splits from each DE were put on glass slides then coated using a gold sputter coater at MTA Labs for the SEM study. Photomicrographs were taken to show diatom varieties in the samples (Figure 2). Based on the SEM observations, each split contained varying amount of diatoms of pinnate type. Centric types were not observed.

About 10 g splits from each sample were pulverized to <450 micron using a Retsch vibratory disk mill (RS 200) and then X-rayed using a Bruker D8 Advance XRD integrated with a dedicated LynEye detector for 0-70º 2Ɵ with 0.019º/sn step speed using 40 KV and 40 mA Cu Kα radiation source at room temperature. Ni filter was used (ITU-JAL labs, Istanbul). Diffraction data were then evaluated using the MDI Jade software to determine crystalline mineralogical contents of the samples and presented in Figure 3).

Mineral contents of the DE are represented by the common soil phases such as feldspars, calcite, quartz, mica and common clay varieties along with some secondary oxides and zeolites. Based on the abundance of the frustules, opal-CT may be detected by XRD.

Insecticidal activity assays. A Turkish chickpea variety, *Cicer arietinum*, Koçbaşı, with 11 % moisture content was used for bioassays. Local diatomaceous earth and Protector® were used at the concentration of 100, 200, 400, 600, 800 and 1000 ppm. Untreated chickpea (control group) was used as the control for the diatomaceous earth samples. For each concentration, 280 g chickpea was used in 1-liter volume PVC bags. Chickpeas and DT were put into plastic bags and then sealed and shaken for 5 minutes to provide a good mixture. To ensure even distribution of DE the bags left for 10 minutes for dust settlement. Each vial (volume of 225ml, 3wx8h cm) was fitted with a plastic lid prepared as for the rearing jars. The vials were then filled with 70 g of DE treated chickpea and with 10 mixed sex adults weevils each. Test vials were placed in an incubator (Nüve ID 501) adjusted at 25±1°C and %65±1 r.h. Assessment of the mortality of the treated live and dead individuals was recorded after 4, 7 and 14 days respectively.

TABLE 1
Chemical contents of three native diatomaceous earth

Treatment	SiO_2 (%)	CaO (%)	Al_2O_3 (%)	Fe_2O_3 (%)
Turco000 (1-10µ)	95.00	0.83	1.42	1.77
Turco004 (10-30µ)	83.26	3.49	6.25	4.91
Turco020 (43-65µ)	83.90	2.74	6.25	3.71
Protector <9.46µ	69.70	0.41	5.80	1.05

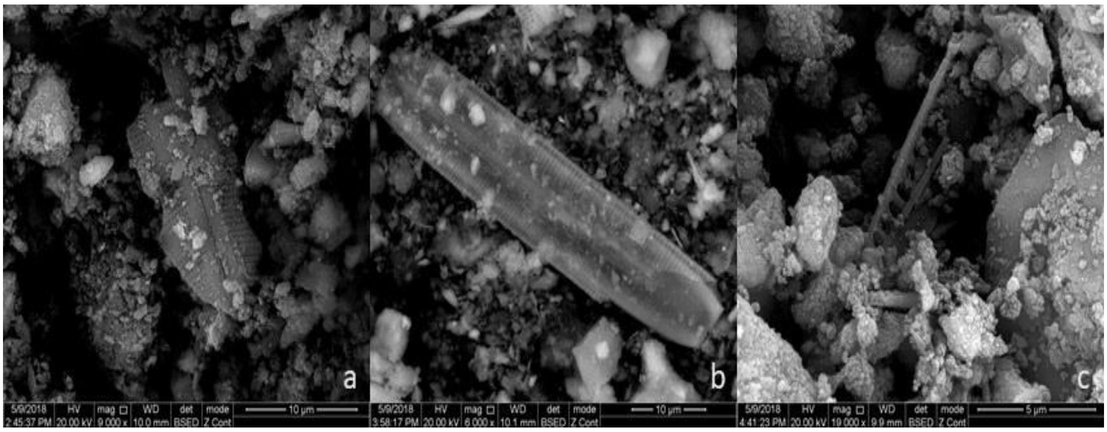

FIGURE 2
SEM images of Turco 000 (a), Turco 004 (b) and Turco 020 (c) showing general size and shape varieties of diatoms.

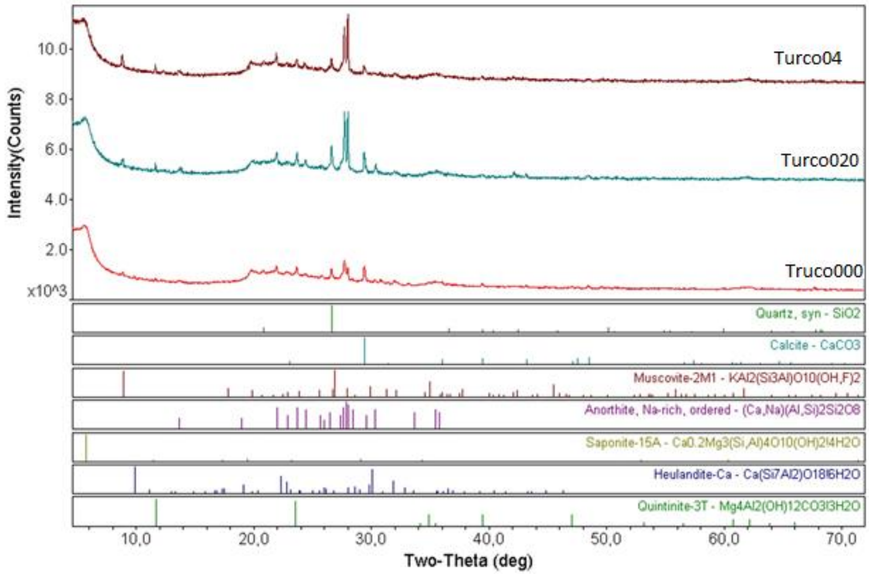

FIGURE 3
Collage of X-ray diffractograms of the subject samples.

TABLE 2
Toxicity of different doses of Turco000 diatomaceous earth against *Acanthoscelides obtectus* adults

Treatment	Mortality (%)±StDev		
	4 DAT	7 DAT	14 DAT
Control	2.57±3.41b*	5.71±2.57c	5.71±2.57c
100ppm	7.47±3.81b	14.64±0.67b	67.82±1.11b
200ppm	90.56±4.71a	100.00±0.00a	100.00±0.00a
400ppm	96.19±5.38a	-	-
600ppm	99.35±2.57a	-	-
800ppm	99.35±2.57a	-	-
1000ppm	100.00±0.00a	-	-
Protector©	100.00±0.00a	-	-

*Different letters in the same column indicate statistically different from each other (Anova P<0,05, Tukey test). DAT: Day After Treatment

Effect of Diatomaceous earth on F1 progeny. After 14 days from the contact toxicity test, dead and live insects were removed from the treated chickpea. The same jars were kept at 25±1°C and 65±1% relative humidity condition until the first adult was seen and then newly emerged adult insects were counted.

Inhibition rate was calculated according to the formula below.

IR = (Cn – Tn) / Cn * 100

IR% : Inhibition rate
*C*n : Number of newly emerged insects in the untreated (control) jar
*T*n : Number of newly emerged insects in the treated jar.

Statistical analysis. Contact toxicity screening test results were firstly converted into percent mortality and then were subjected to arcsine ($n' = \arcsin\sqrt{n}$) transformation and ANOVA. Variance analysis was carried out with transformed data, and additionally, the differences among treatments were analyzed by means of Tukey multiple comparison tests ($p<0.05$). All statistical analyses were conducted with MINITAB® Release 16 package program.

RESULTS

When the insecticidal activity of Turco000, with the lowest particle diameter, on *A. obtectus* was examined, it was determined that all insects applied at the dose of 1000 ppm on the fourth day were died (Table 2). After the four days application, Turco000 was included in the same statistical group with the control group the only dose of 100 ppm. In other doses, Turco000 was in the same statistical group as the positive control group ($F_{(df=7,24)}$=48,03; p<0,05). Insecticidal activity was found to be greater than 90% at application doses of 200 to 800 ppm, but 100% mortality was seen at 1000 ppm. Seven days after treatment, mortality rates were determine as 100% at doses of 200 ppm and above

($F_{(df=7,24)}$=332,34; p<0,05). While all dose applications were in the same statistical group with the positive control group, the only dose of 100 ppm was in the different statistical group.

The effectiveness of Turco 020 was similar to Turco 000 (Table 3). In the evaluation made after four days of treatment, 97% or above mortality rates were determined in all doses except from a dose of 100 ppm ($F_{(df=7,24)}$=147,58; p<0,05). At doses of 400 ppm and above, 100% mortality rate was reached at both the seventh day ($F_{(df=7,24)}$=132,99; p<0,05) and the 14th day ($F_{(df=7,24)}$=187,79; p<0,05) applications. In all three intervals, at doses of 200 ppm and above, all treatments were in the same statistical group as the positive control.

Turco020 has a different effect than other diatomaceous earth used in this study, by terms of mortality rates and particle sizes. At the end of the fourth day, Turco020 did not gave 100% mortality at not any application dose and the highest mortality rate was determined as 97.43% at 1000 ppm dose and only this treatment had taken place in the same statistical group as the positive control ($F_{(df=7,24)}$=76,76; p<0,05) (Table 4). After the seventh day application, mortality rates of all doses were higher than fourth days treatment and it was determined that at the dose of 1000 ppm gave %100 mortality rates ($F_{(df=7,24)}$=116,00; p<0,05). At the dose of 200 ppm gave 90% and above mortality rates after the 14th day after treatment. Application doses of 400 ppm and above were in the same statistical group as a positive control ($F_{(df=7,24)}$=77,17; p<0,05).

It was determined that having different particle sizes and application doses, showed different suppression effects on the progeny production of *A. obtectus*. After the long storage period (55 days), it was seen that Turco000 and Turco004 showed minimum dose and maximum inhibition effect. In the progeny production, made 55 days after the application, the highest inhibitory effect with the lowest dose was found in Turco000 and Turco004. Besides, Turco020 showed lower activity in terms of suppression of F1 production (Table 5).

TABLE 3
Toxicity of different doses of Turco004 diatomaceous earth against *Acanthoscelides obtectus* adults

Treatment	Mortality (%)±StDev		
	4 DAT	**7 DAT**	**14 DAT**
Control	0.65±2.57b*	10.00±0.00b	12.23±0.50b
100ppm	2.57±3.41b	21.83±1.49b	99.35±2.57a
200ppm	97.43±3.41a	98.66±5.28a	100.00±0.00a
400ppm	100.00±0.00a	100.00±0.00a	-
600ppm	100.00±0.00a	-	-
800ppm	100.00±0.00a	-	-
1000ppm	100.00±0.00a	-	-
Protector©	100.00±0.00a	-	-

*Different letters in the same column indicate statistically different from each other (Anova P<0,05, Tukey test). DAT: Day After Treatment

TABLE 4
Toxicity of different doses of Turco020 diatomaceous earth against *Acanthoscelides obtectus* adults

Treatment	Mortality (%)±StDev		
	24 DAT	**48 DAT**	**72 DAT**
Control	0.00±0.00e*	0,65±2.57e	10,00±0.00d
100ppm	0.00±0.00e	12,23±0.50d	70,25±0.81c
200ppm	2.57±3.41de	21,61±2.23cd	90,56±4.71b
400ppm	14.64±0.67d	42,48±0.25c	99,35±2.57ab
600ppm	57.52±0.25c	85,81±1.65b	100,00±0.00a
800ppm	85.08±9.03bc	99,35±2.57a	-
1000ppm	97.43±3.41ab	100,00±0.00a	-
Protector©	100.00±0.00a	100,00±0.00a	-

*Different letters in the same column indicate statistically different from each other (Anova P<0,05, Tukey test). DAT: Day After Treatment.

TABLE 5
The effects of diatomaceous earth on the growth of *Acanthoscelides obtectus*

Treatment	IR (%)					
	100ppm	**200ppm**	**400ppm**	**600ppm**	**800ppm**	**1000ppm**
Turco 000	12,68	100,00	100,00	100,00	100,00	100,00
Turco 004	93,25	98,77	99,39	100,00	100,00	100,00
Turco 020	16,06	59,59	100,00	98,96	100,00	100,00
Protector®	96,96	100,00	100,00	100,00	100,00	100,00

Based on the geochemical and mineralogical analyses, the DE that used in this research contained varying amounts of diatoms. The DE's also contained common soil phases. Pinnate-type diatoms form was prevalent.

DISCUSSION AND CONCLUSIONS

Diatomaceous earth is very important as the only natural bio-rational pesticide of mineral origin in the world. 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 the loss of water from the insect's body through desiccation resulting in death [10]. The aim of this study was to investigate the effect of particle size on the insecticidal and behavioral efficiency of the native diatomaceous earth for controlling of adults of *A. obtectus*. When the results of the study were examined, it was determined that the diatomaceous earth having the lowest particle size had a high mortality effect on the studied insect. Because of the reduction of the particle size, it is thought that the applied DE may be caused by the greater attachment of the insect body due to the widening of the surface area. [12], reported that the particle size distributions of diatomaceous earth particles also play an important role in the insecticidal activity of diatomaceous earth. Similarly, [13] showed that the particle size of the diatomaceous earth on the insecticidal efficacy is not an important parameter but strongly effective on the mortality. In another study conducted with inert powders such as zeolite and diatomaceous earth, contact activity of particle size against pests was investigated. In terms of zeolite, there was no significant correlation between particle size and mortality, while the same was found to be significant for DE. In this bioassay, the differences between particle size and particle size distribution in terms of insect mortality was clearly seen in the same diatomaceous earth. The inert dust, which are the subjects of economic entomology studies, have the potential to be the promising plant protection product especially against the stored product pests. Morphological differences between species are another factor affecting the activity of DE. According to [14], some insects have greater sensitivity to DE because of their anatomy and physiology. Generally, insects with a large surface area in relation to the volume of their body are more sensitive in that they lose relatively greater amounts of water from their body. Insects with a rough or hairy body surface collect more particles per unit of the area causing greater cuticle damage. Therefore, such insects are more sensitive to DE than others. Similarly, the number and location of the setae in the unit area varies in the body of the test insect in this study. Due to these morphological changes, differences were found in the mortality, especially in terms of particle size. Varying DE doses are used for the control of different insect species. These include the volume of the surface area, the state of hair growth in the body, the thickness of the cuticle layer, the biological period of the insect and insect behavior [15, 16, 17]. Turco020 is thought to be retained by bristles before it reaches the cuticle because the particle size in Turco 020 is relatively large compared to the other two formulations. As a result, the turco 020 is not able to fulfill its abrasive effect on the insect cuticle and it is thought to cause slowdown in insect death.

The effect of physiological age on the mortality of insects is important. [18] stated that, when conducting studies on the efficacy of diatomaceous earth on the insects, researchers should standardize the age of the insect in terms of reducing variability. [19] revealed that adult lifespan of the bean weevil adults 7-14 days. For this purpose, age limitation was used to obtain safety data in diatomaceous earth studies so that mixed sex adults were chosen randomly to avoid possible differences between genders.

The results of this study suggest that diatomaceous earth obtained from local sources can be used in the storage of bean weevil in storage conditions.

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