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Efficacy of three diatomaceous earth formulations against cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), on bean

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Abstract

Laboratory experiments were carried out to evaluate the effect of three diatomaceous earth (DE) formulations (two local Aydın and Ankara) and SilicoSec® (Biofa, Germany) against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) adults on bean at 25 and 30°C. DEs were treated with three doses (200, 400 and 600 mg/kg) and control. The adult mortality was assessed after 2, 3, 5, 7, 14 and 21. day of application (DAT). The highest mortality rate (100%) on the cowpea beetle was recorded for Ankara DE on 5. DAT at 25 °C with 600 ppm, followed by Aydın and SilicoSec® DE (84.97% and 63.47%). The biological activity of local DE increased with increasing application dose, exposure time and temperature. The highest mortality at 30 °C was determined for SilicoSec® (100%) with 600 ppm on 2. DAT followed by Ankara and Aydın (94.95 and 94.59% respectively). After 3 DAT, 600 ppm dose showed the highest efficacy all three DEs. There was no new generation emergence of all DEs treatment. It was concluded that the local DEs (Ankara and Aydın) have a high potential to be used for control of *C. maculatus* adults damage for storage bean.

Keywords: Diatomaceous earth, *Callosobruchus maculatus*, efficacy, mortality, stored pulse

1. Introduction

The cowpea beetle, [*Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)], is bean bruchid, widely distributed throughout the world as well as Turkey [36, 3, 42, 22]. The pest is very destructive stored pest of legumes such as cowpea, chickpea, lentil, soybean, bean [3, 31, 22]. The adult beetle infestation may start in the pods before harvest and carry over into storage where substantial losses may occur [10]. The larvae bore into the seed and feed within the same seeds and damage in terms of holes produced by adult emergence from stored seeds can increase to 100% after 3-5 months of ordinary storage conditions [23]. Thus, they cause huge quantitative and qualitative losses in legume seeds [11, 36]. Some control measures need to be application in order to minimize the losses caused by this pest. Most attempts to control this pest relied on synthetic insecticides. The ubiquitous issues of pesticide residue and pest resistance to pesticides are becoming increasingly challenging for society to solve [25, 32]. Insecticides are mainly used for its control and recently some plants essential oil were also used in Turkey [12, 40, 22]. Therefore, there is an increased need for alternative plant protection materials against the cowpea beetle [25]. Besides, several alternatives control methods (natural products such as, palm oil, tobacco extract, leaf essential oils, seed extracts and inert dusts) have been studied against stored pests including *C. maculatus*. [20, 29, 34, 42, 14].

Dusts, such as diatomaceous earth (DE), are known insecticidal or repellent activity products and effectively controls most species of insect pests of stored products [15, 43]. Diatomaceous earth is a naturally occurring, soft, siliceous sedimentary rock and consists of fossilized skeletons of freshwater or marine of diatoms [29]. Diatomaceous earth acts larvae and on adults, stick to insect's body parts as they move on their surface or inside the treated grain mass [26, 39, 42, 43]. When it contacts with insects, it causes the removal of the layer of wax from insect cuticle, bringing about their death by desiccation [39]. Besides, this product is extremely stable and has low toxic effect on mammalian, little potential for insect resistance as well as a good residual effect [16, 15, 29]. Turkey has a huge and good quality diatomaceous earth reserve (125 million tons) and most of them geological deposits occur in some regions (especially Center Anatolia, Aegean region) of Turkey [13]. With the diatomaceous earths from regional deposits as grain protectant in farm stores of Turkey, their potential low costs, readily availability and

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easiness to use, many farmers and grain dealers can use them as grain protectant. Although there are very abundant and high-quality diatomite reserves in Turkey, they cannot be evaluated adequately of these reserves for controlling stored products pest. DE formulations with dry grains are an excellent method of protecting the commodity against stored product pest [36].

Although there are many studies evaluating insecticidal, biological and repellent activity of local and registered DEs against some stored product pests [29, 30, 18, 7, 43, 14, 17, 50, 2, 41, 27], but there is no information on the efficacy of local DEs formulation against *C. maculatus* on bean in Turkey. The main objective of the study was to determine efficacy of two local diatomaceous earth (Aydın and Ankara) and SilicoSec on mortality of DE-treated the cowpea beetle on bean at the laboratory-controlled conditions in 2020.

2. Materials and Methods

2.1. Diatomaceous Earth (DE) formulations

Three diatomaceous earth (two native diatomaceous earth, Ankara, Aydın provinces and SilicoSec®) were used in the study. The DE formulations are given in Table 1.

Table 1: The formulations of diatomaceous earths used in the study

DE*	SiO ₂ (%)	Al ₂ O ₃	Fe ₂ O ₃	CaO	Na ₂ O	Particle diameter size
Ankara	92.8	4.2	1.5	0.3	-	8-12μ
Aydın	94.2	4.6	1.6	0.3	-	8-12μ
SilicoSec®**	92.0	3	1	-	1	8-12μ

*DE: Diatomaceous earth

**A sample of dry formulation of SilicoSec® was obtained from Biofa GmbH, Germany

2.2. Test insects

Callosobruchus maculatus adults were obtained from the cultures maintained at Entomology Laboratory, Plant Protection Central Research Institute Ankara, Turkey. The adults were reared in sterile 1-L transparent plastic jars on chickpea in incubators at 30±1°C temperature, 50-65±5% relative humidity and 16:8 h (L:D) in the insectarium of Department of Plant Protection, Faculty of Agriculture, Harran University, Turkey. Chickpea grains were kept in deep freezer at -18 °C at least 7 days to prevent contamination. Approximately, 200-250 mixed-sex adults were released in the jars. The adults were vacuumed after 24 hours to obtain 1-day-old new adults later. The adult emergence was observed at ~35-40 days. Emerged adults were collected with an aspirator and used in experiments when they were 1 to 3 days old.

2.3. Laboratory bioassay

Bioassay studies were conducted according to randomized parcel design with four replicates at two temperatures (25 and 30±1°C), 50-65% RH and 16:8 h (L:D). Three doses of local (Ankara and Aydın) and SilicoSec® DEs formulation (200, 400 and 600 ppm (mg DE/kg bean) were used. Untreated bean grains (control group) was considered as the control. For each dose, 200 g bean grains were used in 1-liter volume plastic jars. The jars were sealed and shaken manually for 1 min to distribute DEs on-bean grains. Thirty *C. maculatus* adults were introduced in each jar and the insects were kept in controlled condition insectarium rooms at 25 and 30±1°C. The numbers of living and dead adults were recorded after 2, 3, 5, 7, 14 and 21 days respectively.

2.4. Effect of Diatomaceous earth on F1 progeny

The dead and living adults were removed 21 days after the initiation of laboratory bioassay. Infested bean grains were kept at 25 and 30±1°C and 65±5% relative humidity condition to allow oviposited eggs for hatching and development. Then newly emerged adults were counted. Inhibition rate was calculated according to the formula given below.

$$IR\% = (C_n - T_n) / C_n \times 100$$

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.

2.5. Statistical Analysis

The collected data were converted into percent mortality and subjected to arcsine transformation followed by correction of cumulative mortality percentage (Abbott, 1925). Analysis of variance was conducted to assess the effect of concentration, exposure time and their interaction. The differences among treatment means were analyzed by means of Tukey's multiple comparison tests ($p < 0.01$). All statistical analyses were conducted on Jump (Version 7) package program.

3. Results

The mortality varied within DEs and their doses. The highest mortality was recorded at higher DEs dose (600 ppm/100g bean grains) at 25 and 30°C, while minimum mortality was obtained with lower dose. The lowest mortality after 2nd day was 11.72, 3.39 and 7.50% with 200 ppm dose of Ankara, Aydın and SilicoSec® DEs, respectively at 25 °C (Table 2).

Table 2: Efficacy of different doses of three diatomaceous earth against *Callosobruchus maculatus* adults at 25 °C

		Mortality (%)					
DE	Doses	2. DAT	3. DAT	5. DAT	7. DAT	14. DAT	21. DAT
Ankara	Control	1.66a*	1.66a	12.50a	28.33a	61.66a	85.00a
Ankara	200 ppm	11.72a	22.61ab	39.27b	56.70b	61.29a	93.75a
Ankara	400 ppm	11.72a	42.61b	68.64c	83.56c	92.89b	100.00a
Ankara	600 ppm	83.03b	93.27c	100.00d	100.00c	100.00b	100.00a
		Mortality (%)					
Aydın	Control	0.83a	2.50a	10.83a	19.16a	42.50a	60.00a
Aydın	200 ppm	3.39a	12.94ab	26.20a	64.80b	92.24b	100.00b
Aydın	400 ppm	12.58a	29.81b	63.12b	91.44c	98.80b	100.00b
Aydın	600 ppm	30.11b	70.04c	84.97b	99.00c	100.00b	100.00b
		Mortality (%)					
SilicoSec	Control	2.50a	9.16a	24.16a	32.66a	52.50a	95.83a
SilicoSec	200 ppm	7.50a	16.90b	28.27ab	72.84b	94.48b	100.00b
SilicoSec	400 ppm	14.16a	20.89b	53.19bc	89.90bc	100.00b	100.00b
SilicoSec	600 ppm	15.83a	38.39b	63.49c	98.86c	100.00b	100.00b

*Different letters in the same column indicate statistically different from each other ($P < 0.05$)

DAT: Day after treatment

The mortality increased with increased exposure time (3, 5, 7, 14, 21 days). The 200ppm dose of all DEs was insufficient, mortality increased significantly 5 days after treatment (DAT) with 600 ppm and reached 100% with Ankara DE. The efficacy of Aydın and SilicoSec® DEs were similar to Ankara DE and, 600 ppm dose of Aydın and SilicoSec® DEs exhibited high mortality (99.00 and 98.86% respectively) at 7th day. Insecticidal activity was >90% with 600 ppm; however, 100% mortality was recorded with 600 ppm dose of all three DEs at 14 DAT. The mortality rate at 21 DAT was 100% with 400 and 600 ppm doses of all DEs. The mortality

with 400 and 600 ppm doses of all DEs at 25 °C was substantially greater than control at 5 DAT. A considerable

increase in mortality was observed with increasing duration in control treatment but remained the lowest.

Fig. 1: The mortality rate of three DE on *Callosobruchus maculatus* at 25 °C

The mortality at 2, 3, 5, 7, 14 and 21 DAT with different concentrations of Ankara, Aydın and SilicoSec® DEs at 30 °C has been shown in Table 3.

Table 3: Efficacy of different doses of three diatomaceous earth against *Callosobruchus maculatus* adults at 30 °C

DE	Doses	Mortality (%)					
		2. DAT	3. DAT	5. DAT	7. DAT	14. DAT	21. DAT
Ankara	Control	0.83a*	15.00a	42.49a	50.83a	69.42a	91.66a
Ankara	200 ppm	62.71b	83.92b	100.00b	100.00b	100.00b	100.00b
Ankara	400 ppm	85.89c	97.72bc	100.00b	100.00b	100.00b	100.00b
Ankara	600 ppm	94.95c	100.00c	100.00b	100.00b	100.00b	100.00b
Aydın	Control	0.00a	17.50a	30.83a	56.75a	68.33a	91.66a
Aydın	200 ppm	62.71b	83.92b	100.00b	100.00b	100.00b	100.00b
Aydın	400 ppm	85.89c	97.72c	100.00b	100.00b	100.00b	100.00b
Aydın	600 ppm	94.59c	100.00c	100.00b	100.00b	100.00b	100.00b
SilicoSec	Control	0.00a	10.83a	17.50a	36.66a	51.66a	92.50a
SilicoSec	200 ppm	71.16b	75.68b	100.00b	100.00b	100.00b	100.00b
SilicoSec	400 ppm	92.07c	100.00b	100.00b	100.00b	100.00b	100.00b
SilicoSec	600 ppm	100.00c	100.00b	100.00b	100.00b	100.00b	100.00b

*Different letters in the same column indicate statistically different from each other ($P < 0.05$)

DAT: Day after treatment

Exposure time to different DEs had significant effect ($P < 0.01$) on mortality of the cowpea beetle at 30 °C (Table 3). Increasing DEs doses and exposure time resulted in increased high mortality. The mortality after 48 h was 94.95 and 94.59% for Ankara and Aydın DEs respectively with 600 ppm dose. SilicoSec® caused the highest mortality (100%) with

same exposure time. The highest mortality (100%) was recorded with 600 ppm concentration of Ankara and Aydın DEs on 3rd DAT. After 5 DAT, 100% mortality was recorded at three DEs with 200 ppm. Mortality rate in the untreated control group increased as exposure time was extended.

Fig. 2: The mortality rate of three DE on *Callosobruchus maculatus* at 30 °C

There was no F1 adult emergence all DEs, doses and DAT at two temperatures, after the long storage period (60 days).

4. Discussion

This study investigated the effects of three diatomaceous earths (DEs) (two local-Aydın and Ankara) on mortality of cowpea beetle adults under controlled conditions. The results indicated that the efficacy of all DEs was considerably influenced by application doses, exposure time, temperature and DEs formulations. Several factors, including dose, temperature and exposure time influence the efficacy of DEs. Similar results have been reported by previous researchers [29, 5, 4, 33, 35, 9, 2, 41, 27]. These studies indicated increased mortality of stored product pests exposed to inert dusts with increasing exposure time. Exposure time of 2 days of DEs was enough to obtain significant mortality with tested DEs in the current study. However, mortality increased with increased exposure time from 5 DAT and reached to the highest on 14 DAT at 25 °C and the highest mortality reached on 3 DAT at 30 °C. Similar results have been reported by Parasanthi *et al.*, (2002); Parsaeyan *et al.*, (2012); Badii *et al.*, (2014), Kabir and Wulgo (2014) [35, 9, 25] who stated that mortality of *C. maculatus* increased with increasing exposure interval and temperature. Prolonged exposure time may be needed to increase mortality in adults because more dust particles are trapped by insect bodies with increasing exposure time and in turn insects lose more water and died of desiccation [19, 5, 38, 42]. The efficacy of DEs increased with higher doses and temperature. This effect shows that the mortality of insects is dependent on the conditions in which the grains are exposed to DEs [43]. The particles of DEs have abrasive peculiarities and have ability to absorb lipid molecules from the cuticle of the insects leading to water loss, dehydration and mortality [37, 29, 45]. Although particle size of all DEs was similar, SilicoSec® had moderate effect on cowpea beetle adults and the mortality increased after 5 DAT for SilicoSec®. These results indicate that DEs from different origins vary in their efficacy against maize weevil. This has also been explained by Korunic (1997) and (Golob, 1997) [28, 21].

The mortality was considerably increased with increasing temperature and was higher at 30°C than 25°C. The earlier studies on the influence of temperature on the efficacy of some local and traded DEs against stored pest (*S. oryzae*, *T. confusum* and *R. dominica*) showed that increasing temperature resulted in increased insecticidal efficiency against *S. oryzae* [19, 6, 8, 48, 39, 41]. The results of the current study agree with Vassilakos *et al.*, (2006) who reported that insecticidal efficacy of SilicoSec® against *S. oryzae* increased with increasing temperature. However, increased temperature would also increase feeding and moisture replacement through production of metabolic water. The synthesis of cuticular waxes may be faster at higher temperatures because of temperature effects on the biochemical pathways [46, 41]. High temperatures stimulate the movement of insects within the grain mass, allowing them greater contact with the DEs. In addition, water loss is likely to be increased at higher temperatures [19, 5].

The total mortality was influenced by DE types under both temperatures. The local DEs, Ankara and Aydın, showed >90% mortality 5 DAT, while SilicoSec® caused ~60% mortality at 25°C. Similar results were obtained at 30°C, which clearly indicated that local DEs were more effective than SilicoSec® against maize weevil. The differences between imported and native DEs can be explained by the

differences in physical, morphological and chemical characters of DEs. Kılıç and Mutlu (2020) [27] found the highest biological activity with local DE Aydın on Khapra larvae followed by Ankara DE, while SilicoSec® had the lowest activity at 30 °C. However, Alkan *et al.*, (2019) [2] reported that local DE Turco000 caused 100% mortality of *Acanthoscelides obtectus* Say. adults on chickpea at 1000 ppm 4 DAT and can be used as a valuable tool in stored product pest management. Besides, complete mortality of *T. confusum* and *S. oryzae* can be achieved at lower concentrations ranging from 500 to 900 ppm of local DE formulation (ACN-1) and this DE has potential to be used for control of stored-grain insects reported by Şen *et al.*, (2019) [41].

The study showed that at least 600 ppm dose all DEs at 30 °C caused >94% mortality on 2 DAT. The results of the present study indicated that complete mortality of cowpea beetle can only be achieved at higher concentrations, ranging from 400 to 600 ppm. These agree with Nabei *et al.*, (2012) who reported mortality rates for *C. maculatus* were increased as the concentration of DE enhanced. Therefore, at higher concentrations, the adsorption of wax and abrasiveness occurs faster, causing death in a shorter time compared with low concentrations [42]. In addition, higher concentration of DEs can be the more effective, because dust applied to cover containers and grain surface will have greater chances of picking up by the insects and cause enough damage [30] (Masivii, 2004). Contrary to what is believed that bean grain surface is smooth, and adherence is lower than rough surface grain like wheat and maize, the result indicated that three DEs tested were very effective after 2 DAT on *C. maculatus* adults at 30 °C.

There was no F1 adult emergence tested all DEs. The high mortality was observed for 25 °C on 5 DAT and for 30°C on 2 DAT. These results could be due to the fact that high mortality rate of cowpea beetle might reduce the rate of oviposition and limit the survival of the insects and reduce the number of individuals in the progeny [47]. Our results agree with [2] who reported that there was no progeny production with local DEs, Turco000, Turco004, Turco020 at 200 ppm concentration at the end of 55-days incubation period on *C. maculatus*.

The results presented in this study suggest that 600 ppm dose of Ankara and Aydın DEs 30 °C temperatures can be recommended to control cowpea beetle with 3 days exposure time.

5. Conclusions

The two native diatomaceous earths obtained from local sources (Ankara and Aydın) and imported brand SilicoSec® showed high efficacy against cowpea beetle under controlled conditions. The study also indicated that temperature had significant effect on the efficacy of a local DEs against the cowpea beetle. Based on the results of the bioassays, mortality increased with increasing dose and the exposure time. In conclusion local DEs (Ankara and Aydın) have high potential to be used for control of *C. maculatus* adults damage for storage bean.

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