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EFFECTIVENESS OF SOME NATIVE DIATOMACEOUS EARTH AGAINST MAIZE WEEVIL, *SITOPHILUS ZEAMAIS* (COLEOPTERA: CURCULIONIDAE), UNDER CONTROLLED CONDITIONS

SUMMARY

The Maize weevil, *Sitophilus zeamais* Motchulsky (Coleoptera: Curculionidae), is one of important pests of storage maize in Turkey. Laboratory experiments were carried out to investigate the effect of two local diatomaceous earth (Aydın and Ankara) and Silicosec® (Biofa, Germany) against *S. zeamais* adults at 25 and 30°C and 50-65% relative humidity. Maize grains (cultivar Decalp 6664) were treated with three doses of DEs (1000, 1500 and 2000 ppm) and untreated seeds were regarded as control. The dead adult counts were made 2, 3, 5, 7, 14 and 21 days after DEs. The progeny of *S. zeamais* was determined separately for each treatment after 60 days from end of the experiment. The highest mortality (100%) of the maize weevil was observed with 2000 ppm Ankara DE on 14th day after application at 25 °C, which was followed by Aydın and SilicoSec® DEs (99.0 and 90.1%). The insecticidal activity of native DEs increased with increasing application dose, exposure time and temperature. The highest insecticidal activity at 30 °C was determined for Ankara and Aydın DEs with 2000 ppm on 14th day (100%), followed by SilicoSec® (97.9%). All doses of Ankara DE had the highest insecticidal activity than rest of the DEs examined. New adult emergence (F1 offspring) was recorded in all DEs treatments; however, emergence was very low than control treatment. It is concluded that the native DEs (Ankara and Aydın) are very effective and promising against *S. zeamais* adults and might be used for its successful control in storage maize.

Keywords: Diatomaceous earth, *Sitophilus zeamais*, efficacy, storage maize, pest, mortality

INTRODUCTION

Storage grain pests, mainly beetles and moths, such as *Sitophilus* spp. (Coleoptera: Curculionidae), *Tribolium* spp. (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* (Coleoptera: Bostrychidae), *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and *Oryzaephilus surinamensis* Linnaeus

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(Coleoptera: Silvanidae) are especially responsible for weight, germination of stored grains and cause losses up to 100% in Turkey (Özar and Yücel, 1982; Özer et al., 1989; Işıkber et al., 2005).

The maize weevil, *Sitophilus zeamais* Motchulsky (Coleoptera: Curculionidae), is one of important storage pest on maize in Turkey (Yıldırım et al., 2012; Karaağaç and Konuş, 2015) as well as other countries (Trone, 1994; Rees, 2004; Ojo and Omoleye, 2016). The pest also cause problem on small grains for example wheat, barley, sorghum, millet and causes significant quantitative and qualitative damage to storage grains and grains can weight loss up to 90% for not treated storage maize (Giga et al. 1991; Yıldırım, 2012; Ojo and Omoleye, 2016). Insecticides are mainly used for its control in Turkey (Karaağaç and Konuş, 2015) as well as biological control and botanical insecticides among others (Akob and Ewete, 2007). 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 (Foua-Bi, 1993; Korunic, 1998; Owusu, 2001; Doumbia et al., 2014) including *S. zeamais.*

Dusts, such as diatomaceous earth, are known insecticidal or repellant activity products and effectively controls most species of insect pests of storage products (Ebeling, 1971; Sousa et al., 2013). Diatomaceous earth affects larvae and adults, stick to insect's body parts as they move on their surface or inside the treated grain mass (Kavallieratos et al., 2007; Rojht et al., 2010; Shams et al., 2011; Sousa et al., 2013). When dust contacts with insects, it causes the removal of the layer of wax from insect cuticle, bringing about their death by dessication (Rojht et al., 2010). 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 (Eldridge,1964; Ebeling,1971; Korunic,1998). 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 (Çetin and Taş, 2012). The regional deposits of DEs in Turkey, their low cost, easy availability and use warrant their usage as grain protectants. Although abundant and high-quality DEs reserves exist in Turkey, these have not extensively been evaluated for their potential utility in controlling storage products pest.

Many studies have been conducted in Turkey and other countries to determine the insecticidal, biological and repellent activity of native and trade DEs against storage product pests (Korunic, 1998; Masiiwa, 2004; Ferizli and Beris, 2005; Athanassiou et al., 2004; Sousa et al., 2013; Doumbia et al, 2014; Ertürk and Emekçi, 2014; Wakil et al., 2015; Alkan et al., 2019; Şen et al., 2019; Kılıç and Mutlu, 2020). Whereas, biological efficacy and dose studies of various DEs have been conducted such as on wheat, rice and corn related to *S. oryzae* and *S. granarius* species in Turkey, there is no literature available related to efficacy of local DEs for *S. zeamais* on storage corn kernels. In this study, some native diatomaceous earth from Aydın and Ankara provinces were evaluated for efficacy against *S. zeamais* in the laboratory-controlled conditions in 2020.

MATERIAL AND METHODS

Diatomaceous Earth (DE) formulations

Three diatomaceous earth (DEs) (two natives, i.e., Ankara, Aydın and imported, i.e., SilicoSec®) were used in the study. The DE formulations were given in Table 1.

Table 1. Diatomaceous earth formulations used in the study.

DE*	SiO_2 (%)	Al_2O_3	Fe_2O_3	CaO	Na_2O	Particle diameter size (μm)
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.

Test insects

Sitophilus zeamais adults were obtained from cultures maintained in the Entomology Laboratory of Plant Protection Central Research Institute Ankara, Turkey. The adults were reared in sterile 2-L transparent plastic jars on maize grains in incubators maintained at about 25±1°C and 65±5% relative humidity and 16:8 h (L: D) in Insectarium of Plant Protection Department, Agriculture Faculty, Harran University. Maize grains were kept in the deep freezer at -18 °C at least 7 days to prevent contamination. The jars were not filled to the brim for allowing free air circulation and respiration. Approximately, 250-300 mixed-sex adult individuals were introduced in the jars. The adults on the maize grains were vacuumed after 24 hours for obtaining 1-day-old new adults later. The newly emergence adults were observed at ~35-40 days after. Emerged adults were collected with an aspirator and used in experiments when they were 1 to 7 days old.

Laboratory bioassay

Bioassay studies were conducted according to randomized parcel design with four replicates at two temperatures (25 and 30±1ºC), 50-65% RH. Three doses of local (Ankara and Aydın) and SilicoSec® DE formulation (1000, 1500 and 2000 ppm (mg DE/kg maize grain) were used. Untreated maize grains were considered as the control. For each dose, 100 g maize grains were used in 1-liter volume plastic jars. The jars were sealed and shaken manually for 1 min to distribute DE equally. Thirty *S. zeamais* 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.

Effect of diatomaceous earth on F1 progeny

The dead and living adult insects were removed 21 days after the initiation of laboratory bioassay. The same jars (infested maize grains) were kept at 25 and 30±1°C and 65±5% relative humidity condition to allow oviposited eggs to hatch and develop. Then newly emerged adults were counted. Inhibition rate was calculated according to the formula below.

$$IR = (Cn - Tn) / Cn * 100$$

IR% : Inhibition rate
Cn : Number of newly emerged insects in the untreated (control) jar
Tn : Number of newly emerged insects in the treated jar.

Statistical Analysis

All data were converted into percent mortality and subjected to arcsine scale followed by correction of cumulative mortality percentage for the corresponding control mortality (Abbott, 1925). Analysis of variance was conducted to assess the effect of concentration, time of exposure and their interaction with the insect mortalities. The differences among treatments were analyzed by means of Tukey multiple comparison tests ($p<0.01$). All statistical analyses were conducted with Jump (Version 7) package program.

RESULTS

The mortality varied within DEs and their doses. The highest mortality was recorded at higher DEs dose (2000 ppm/100g maize grains) at 25 and 30°C, while minimum mortality was obtained with lower dose. The lowest mortality after 2nd day was 31.12, 36.09 and 13.33% with 2000 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 *Sitophilus zeamais* adults at 25 °C

				Mortality (%)			
DE	**Treatments**	**2. DAT**	**3. DAT**	**5. DAT**	**7. DAT**	**14. DAT**	**21. DAT**
Ankara	Control	0.83 a	0.83 a	0.83 a	0.83 a	5.83 a	13.33 a
Ankara	1000 ppm	3.33 b	10.08 a	29.45 b	38.99 b	48.24 b	75.70 b
Ankara	1500 ppm	26.87 c	62.93 b	84.08 c	89.08 c	95.33 c	100.00 b
Ankara	2000 ppm	31.12 c	65.54 b	91.66 c	96.60 c	100.00 c	100.00 b
				Mortality (%)			
Aydın	Control	0.83 a	0.83 a	0.83 a	0.83 a	4.16 a	10.00 a
Aydın	1000 ppm	5.86 ab	17.58 a	30.20 b	39.51 b	54.89 b	67.40 b
Aydın	1500 ppm	15.91 b	46.89 b	79.02 c	87.47 c	93.98 c	99.16 c
Aydın	2000 ppm	36.09 c	71.35 b	90.69 c	93.25 c	99.03 c	100.00 c
				Mortality (%)			
SlicoSec	Control	0.00 a	0.00 a	0.00 a	0.00 a	6.66 a	10.83 a
SlicoSec	1000 ppm	2.50 a	3.33 a	10.83 b	15.00 b	38.28 b	84.12 b
SlicoSec	1500 ppm	10.00 b	27.50 b	51.66 b	60.00 c	90.13 c	99.10 c
SlicoSec	2000 ppm	13.33 b	28.33 b	60.00 b	77.50 d	91.06 c	100.00 c

*Different letters in the same column indicate statistically different from each other (P<0.05)
DAT: Day after treatment

Increasing of exposure time resulted increased *S. zeamais* adult mortality. The 1000 ppm dose of all DEs was insufficient, mortality increased significantly 5 days after treatment (DAT) with 1500 ppm and reached 84.08% with Ankara DE. The efficacy of Aydın DE was similar to Ankara DE (Table 2). After 7th day, 2000 ppm dose of Ankara and Aydın DEs exhibited high mortality (96.60 and 93.25%), while mortality with the same dose of SilicoSec® (77.50%) was lower. Insecticidal activity was >90% with 1000 ppm; however, 100% mortality was recorded with 2000 ppm dose of Ankara DE 14 DAT. However, SilicoSec® did not reach 100% mortality with all concentrations. The mortality rate at 21 DAT was 100% with 1000 ppm doses of all DEs. The mortality with 1000, 1500 and 2000 ppm doses of all DEs at 30 °C was substantially greater than control at 3 DAT. A considerable increase in mortality was observed with increasing duration in control treatment but remained the lowest (Table 2).

The mortality percentages of *S. zeamais* adults at 2, 3, 5, 7, 14 and 21-DAT at different concentrations of Ankara, Aydın and SilicoSec® DEs at 30 °C are shown in Table 3.

Table 3. Efficacy of different doses of three diatomaceous earth against *Sitophilus zeamais* adults at 30 °C

				Mortality (%)			
DE	**Treatments**	**2. DAT**	**3. DAT**	**5. DAT**	**7. DAT**	**14. DAT**	**21. DAT**
Ankara	Control	1.66 a*	2.50 a	3.33 a	4.16 a	16.66 a	29.99 a
Ankara	1000 ppm	28.79 b	66.77 b	88.81 b	89.44 b	98.81 b	100.00 b
Ankara	1500 ppm	48.94 bc	77.15 b	89.85 b	93.96 b	99.13 b	100.00 b
Ankara	2000 ppm	53.45 c	87.26 b	97.38 b	97.38 b	100.00 b	100.00 b
				Mortality (%)			
Aydın	Control	0.00 a	4.99 a	8.33 a	8.33 a	17.50 a	30.83 a
Aydın	1000 ppm	35.83 b	61.36 b	78.90 b	84.42 b	92.76 b	98.33 b
Aydın	1500 ppm	69.16 c	83.24 c	96.39 bc	97.29 bc	98.10 b	100.00 b
Aydın	2000 ppm	70.83 c	86.75 c	97.32 c	98.21 c	100.00 b	100.00 b
				Mortality (%)			
SlicoSec	Control	0.00 a	3.33 a	6.66 a	6.66 a	23.33 a	40.00 a
SlicoSec	1000 ppm	4.16 ab	9.48 a	33.83 b	51.11 b	80.40 b	83.61 b
SlicoSec	1500 ppm	4.16 ab	23.07 b	42.19 bc	63.26 bc	85.54 b	98.68 b
SlicoSec	2000 ppm	12.50 b	31.13 b	58.67 c	78.44 c	97.95 b	98.75 b

*Different letters in the same column indicate statistically different from each other (P<0.05)
DAT: Day after treatment

Exposure time of insects to different DEs concentrations had significant effects (P<0.01) on mortality of *S. zeamais* adults at 30 °C (Table 3). Increasing DEs doses and exposure time resulted in increased mortality. The mortality after 72 h was 87.26 and 86.75% for Ankara and Aydın DEs, respectively with 2000 ppm dose. SilicoSec® caused very low mortality 31.13% with same exposure time.

The highest mortality (97.3%) was recorded with 2000 ppm concentration of Ankara and Aydın DEs on 5th and 7th DAT, and reached 100% on 14th DAT, while SilicoSec® caused 58.67 and 78.44% mortality on 5 and 7th DAT, respectively. The highest mortality (98.75%) for SilicoSec® was noted 21 DAT and did not reach 100% with any application doses. Mortality in the untreated control remained constant after the insect exposure to untreated maize grains.

Different DEs and application doses showed different suppression effects on the progeny production of *S. zeamais*. After the long storage period (60 days), F1 adult emergence was recorded from all DEs and their all concentrations. The highest number of insects emerged from Aydın, Ankara and SilicoSec® were 30, 26.5 and 15.5 adults respectively in the lower concentration treatment (1000 ppm) at 25 °C. The F1 adult numbers were decreased with increasing applications doses (1500 and 2000 ppm), which were 1, 2.5 and 7.8 adults for Ankara, Aydın and SilicoSec®, respectively at 2000 ppm (Figure 1).

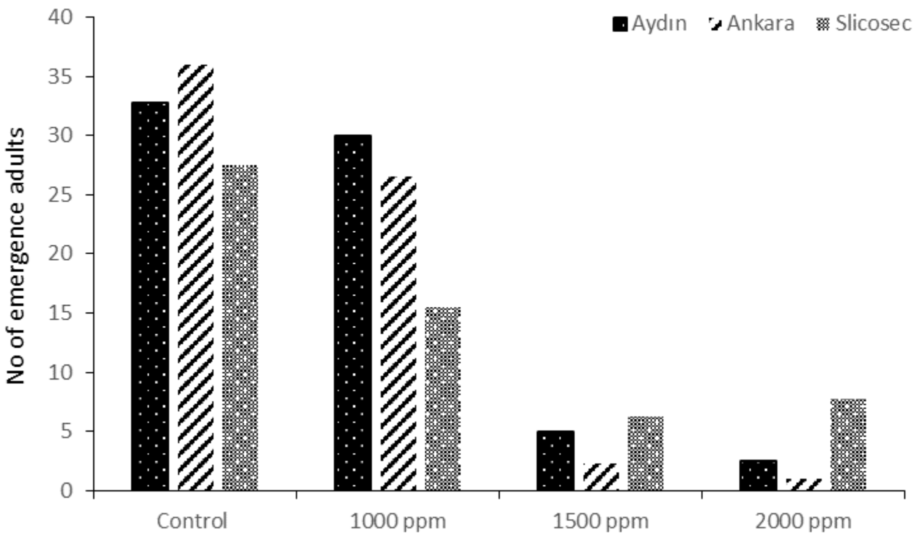

Figure 1. Progeny assessment after 60 days of *Sitophilus zeamais* exposure to the various DE at 25 °C

Unlike the data shown in Figure 1 of 25 °C, F1 adult emergence was very low with all doses of tested DEs with no differences between all DEs at 30 °C. The number of insects emerged, at the end of the exposure period of 60 days, reduced with the increasing DE doses (Figure 2).

The highest dose (2000 ppm) of Ankara and Aydın DEs was very effective in terms of suppression of F1 production (Figure 2). The number of insects emerged from grains treated with DEs at 1500 and 2000 ppm at 30 °C was substantially lesser than the grains in the control

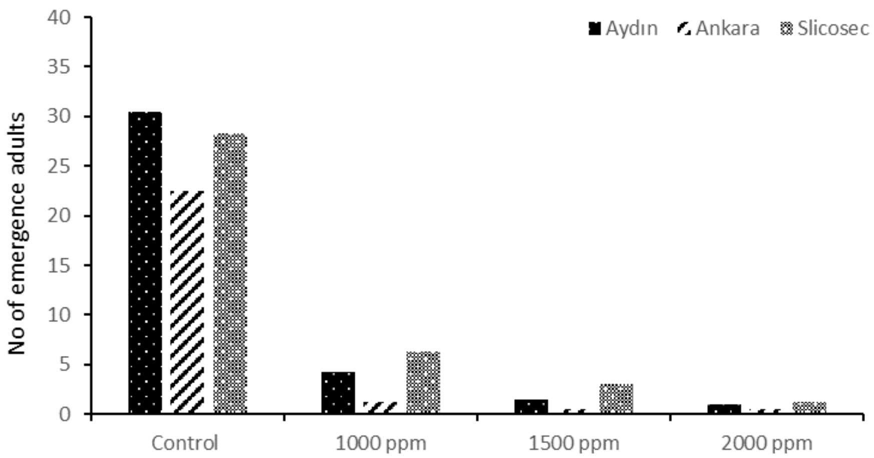

Figure 2. Progeny assessment after 60 days of *Sitophilus zeamais* exposure to the various DE at 30 °C

DISCUSSION

The purpose of this study was to investigate the effect of two native diatomaceous earth (DEs) on mortality of *S. zeamais* adults under controlled conditions. The results of the study indicated that the efficacy of all DEs was considerably influenced by doses, time of exposure, temperature and type of DE formulations. Similar results have been reported by previous researchers (Korunic, 1998; Arthur, 2001; Arnaud et al., 2005; Vayias et al., 2006; Shams et al., 2011; Alkan et al, 2019; Şen et al., 2019; Kılıç and Mutlu, 2020). These studies indicated increased mortality of storage product pests exposed to inert dusts with increasing exposure time. Exposure time of two-day of DEs was not enough to obtain significant mortality with tested DEs in the current study. However, the mortality rate was increased at three DEs, as increased day of exposure after 5^{th} day and reached higher mortality rate on 14^{th} day at 25 and 30°C in the study. Similar result reported by Arthur (2001) who stated that mortality of *Oryzaephilus surinamensis* L. generally increased as exposure interval and temperature increased. Extended exposure time may be needed to increase mortality in adults, because more dust particles would be trapped by their bodies, losing more water and causing death by desiccation (Fields and Korunic, 2000; Arthur, 2001; Rigaux et al., 2001; Shams et al.2011).

The effectiveness 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 (Sousa et al., 2013). The particles and size of DEs have abrasive peculiarities but more importantly the ability to absorb lipid molecules from the cuticle of the insects and subsequently, in water loss, dehydration and death (Quarles and Winn, 1996; Korunic, 1998; Subramanyam and Roelsi, 2000). Altough the particles size of all DEs is very close, SilicoSec® had moderate effect on *S. zeamais* adults. The results indicated that DEs from different locations vary in their efficacy against the maize weevil. This has also been explained by Korunic (1997) and Golob (1997) that DEs from different geological locations have different efficacies and DEs from marine areas are the most common but less efficacious.

The mortality was considerably increased with increasing temperature and was higher at 30ºC than 25ºC. The earlier studies on the influence of temperature on the efficacy of some local and traded DEs against storage pest (*S. oryzae*, *T. confusum* and *R. dominica*) indicated that increasing temperature resulted in increasing insecticidal efficiency against *S. oryzae* adults (Fields and Korunic, 2000; Arthur, 2002; Athanassiou et al., 2005; Vassilakos et al., 2006; Rojht et al., 2010, Şen et al., 2019). The results of the current study agree with Vassilakos et al. (2006) who reported that insecticidal efficacy of SilisoSec® against *S. oryzae* increased with increasing temperature. However, increased temperature would also increase feeding and therefore moisture replacement through the food and production of metabolic water. The synthesis of cuticular waxes may be faster at higher temperatures because of temperature effects on the biochemical pathways (Turnbull and Harris, 1986; Şen et al., 2019). High temperatures stimulate the movement of insects within the grain mass, allowing them greater contact with DEs. In addition, water loss is likely to be increased at higher temperatures (Arthur, 2001; Fields and Korunic, 2000).

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. Smilar results were obtained at 30 °C which clearly indicated that the local DEs were effective than SilicoSec® against the maize weevil. The differences between SilicoSec® and local DEs can be explained by the differences in physical, morphological and chemical characters of DEs. Kılıç and Mutlu (2020) found the highest biological activity with local DE Aydın on *Trogoderma granarium* Everts larvae followed by Ankara DE, while SilicoSec® had the lowest activity larvae at 30°C. However, Alkan et al., (2019) reported that the local DE Turco000 caused 100% mortality to *Acanthoscelides obtectus* Say. adults on chickpea at 1000 ppm 4 DAT and can be used as a valuable tool for bean weevil suppression in storage product pest management programs. Besides, complete mortality of *T. confusum* and *S. oryzae* can be achieved at lower concentrations ranging from 500 to 900 ppm local DE formulation (ACN-1) and this DE has potential to be used for control of storage-grain insects reported by Şen et al. 2019.

The study results showed that at least 1500 ppm dose at 30 °C caused >90% mortality 5 DAT. The results of the present study indicated that complete mortality of *S. zeamais* achieved at higher concentrations, ranging from 1500 to 2000 ppm. These results do not agree with Masiiwa (2004) who reported different results with local African DEs on the maize weevil. Insect mortality caused by DEs is attributed to the dehydration provoked by the abrasiveness of the small particles of this inert dust and by adsorption of oils in the body of the insect, which breaks the layer of wax on the epicuticle, exacerbating the fatal loss of water reported by Subramanyam and Roesli (2000). Therefore, at higher concentrations, the adsorption of wax and abrasiveness caused by the product occurs faster, causing death in a shorter time compared with low concentrations (Shams et al. 2011). In addition, the higher the concentration of DEs can be the more effective, because dust applied to cover containers and grain surface will be greater chances of the insect picking up the dust to cause enough damage (Masiiwa, 2004). Maize grain surface is rough, and adherence is greater than small grain like wheat, millet which are smooth surface have low adherence.

The number of emerged adults in progeny reduced with increasing temperature, reaching ~5% at 1500 and 2000 ppm doses of all DEs at 25 and 30°C. The high mortality was observed for 30°C 5 DAT. These results could be due to the fact that higher temperatures might reduce the rate of oviposition and limit the survival of insects and reduce the number of individuals in the progeny (Vardeman et al., 2006). Alkan et al. (2019) reported that there was no progeny production with local DEs, Turco000, Turco004, Turco020 at 200 ppm concentration at the end of 55-day incubation period on *C. maculatus*. Kılıç and Mutlu (2020) stated that 1000, 1500 and 2000 ppm of local DEs (Aydın and Ankara) applied to wheat resulted low mortalities of *T. granarium* larvae and higher doses did not prevent reproduction.

The results presented in this study suggest that 1500 ppm dose of Aydın DE with high temperatures can be recommended to control the maize weevil with 5 days exposure time.

CONCLUSIONS

The native diatomaceous earths obtained from local sources (Ankara and Aydın) showed high efficacy against the maize weevil under controlled conditions. The study also indicated that temperature had significant effect on the insecticidal efficacy of local DEs against the maize weevil, *S. zeamais*. Based on the results of the bioassays, mortality increased with increasing dose and the exposure time. In conclusion local DEs (Ankara and Aydın) have potential to be used for control of maize weevil.

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