habitat conditions and the determination of its interaction with other living factors outside cockroaches when applied in natural conditions.

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Efficacy of seven Turkish diatomaceous earths against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchninae) on stored chickpea

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DOI 10.5073/jka.2018.463.163

Abstract

In this study, insecticidal efficacy of seven different local diatomaceous earths (DE) obtained from different deposits in Turkey together with two commercial DEs, Silicosec® (Biofa AG- Germany) and Desect® (Ep Naturals-America) against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchninae) an important pest of stored chickpea at five different concentrations (100, 300, 500, 1000 and 1500 ppm) was evaluated. The local DEs were coded as BGN, BHN, AG2N, AC2N, CB2N, CCN, FB2N. Mortality of the adults was assessed after 1, 3, 5 and 7 days of exposure, and consequently progeny (F1) production on treated chickpeas was recorded 42 days later. The tests were carried out under laboratory conditions of 25±1 °C, 55±5 % R.H. in a dark place. The most effective DEs after 1 day of exposure were CCN, AG2N and BHN causing 75%, 59%, 58% mortalities, respectively at 1500 ppm concentration. Silicosec®, Desect®, BGN, AC2N, applied at 1500 ppm concentration achieved 98-100% mortality of *C.maculatus* after 7 days of exposure, showing similar high insecticidal efficacy. The CCN, BHN, AG2N and CB2N caused 97-99% reduction in progeny (F1) production. Generally, increasing concentration significantly reduced the progeny production. In conclusion, this study has shown that three Turkish DEs, namelyCCN, AG2N and BHN highly toxic to *C. maculatus* after 3 days of exposurein comparison with commercial DEs Silicosec® and Desect®. These local DEs could be used in the management of pests of stored chickpea.

Keywords: Turkish diatomaceous earths, Silicosec®, Desect®, *Callosobruchus maculatus*, chickpea.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the rare plants that have been cultivated since thousands of years and the Turkey's southeast region is known as the homeland of the crop. Globally, it is known that cowpea beetle, *Callosobruchus maculatus* (F.) is the most important pest of stored cowpea and other legumes (Taylor, 1981) and its origin is West Africa (Decelle, 1981). This insect can damage 100% of stored products causing weight losses of up to 60% (Tanzubil, 1991). Fumigants and contact insecticides have several problems such as development of resistance, chemical residues in food, as well as harmful effects on the environment and human health. Researchers are therefore searching for alternative methods of stored product protection. Diatomaceous Earths (DE) have less

resistance problems and leave no residue in the stored product., and less harm to the environment and mammals (Fields 1998). There are a lot of commercial DE formulations which have been applied against a wide range of insect species (Fields 2000, Athanassiou et. al., 2004). The efficacy of DE products depends on several factors such as diatom morphology, DE physical properties, type of grains, temperature and relative humidity (Korunic, 1998).

Turkey is considered to have rich natural DE deposits, and there is clear evidence for the existence of large DE deposits in some regions of Turkey (Özbey and Atamer, 1987; Mete, 1988; Sıvacı and Dere, 2006; Çetin and Taş, 2012). The efficacy of DEs to control stored product insects have been studied by several researchers for almost 20 years. However, there are a few studies using DEs against *C. maculatus* in Turkey. The purpose of this study was to determine the efficacy of two commercially available DEs and seven Turkish local diatomaceous earths formulations against *C. maculatus* on chickpea.

2. Materials and Methods

2.1. Test İnsects

The Test insects, *C. maculatus* were obtained from laboratory cultures maintained on chickpea seeds at the Toxicology Laboratory of the Department of Plant Protection, University of Namik Kemal, Turkey. For this study, new cultures were set up on the chickpea seeds at 25±1°C and 55 % r.h. Adults emerging from the chickpea seeds, aged 48 h old were used in the experiments.

2.2. DE formulations

Seven local diatomaceous earth samples (coded as BGN, BHN, AG2N, AC2N, CB2N, CCN, FB2N) were mostly collected from DE reserves located at middle Anatolia of Turkey, commercial DEs were purchased from the agricultural market.

2.3. Chickpea

The seeds of the chickpea cultivar Koçbaşı (*Cicer arietinum* L.) were obtained from a supermarket. Chickpeas were sterilized at -20°C for 3-5 days before they were used in the experiments

2.4. Experimental procedure

About 500 g of chickpeas used in the experimentsput into 3-liter volume glass jars. Each diatomaceous earth was placed on the products in the glass jar, weighing 0,050, 0,150, 0,250, 0,500 and 0,750 g (100, 300, 500, 1000 and 1500 ppm) on the precision scale. Then the lids of these jars were tightly closed and shaken manually for 3 minutes to homogenize the diatomaceous earth over the chickpea. Each lot was divided into five parts of 100g and in each vial, 20 *C. maculatus* adults of mixed-sex were added with the help of a fine brush .The vials were tightly closed with a tulle which provided air inlet and outlet. One lot was kept as the untreated control. The vials were stored in incubators maintained at 25±1°C temperature and 55±5% relative humidity. Adult mortality was recorded after 1, 3, 5 and 7d. Live and dead adults were counted and recorded. After the 7th day counting, all insects have been taken out from the vials. The chickpeas were seperated according to their doses and placed in plastic containers which were drilled with the help of a pin. They were kept in the dark 80 l volume plastic container for 42 days at a temperature of 26±1°C and 65±5 % relative humidity. Sodium Bromide (94.32 g NaBr / 100 ml water) solution was used to keep the ambient air humidity constant. After 42 d, the jars were opened and the total number of F1 adults were recorded.

2.5. Statistical analysis

Mortality rates obtained from control counts were corrected using Abbot's correction formula (Abbott 1925). After applying the Arcsin transformation to the corrected mortality rates, two-way

variance analysis (ANOVA) was performed in the SPSS 15.0 Evaluation Version statistical program. F1 emergence adults were subjected to direct variance analysis (ANOVA) without any corrections. Using the Duncan test at the 5% significance level, the differences between the mean of mortality rates and the number of new generation adults were determined.

Table 1 Mean percentage mortality (±SE) of *Callosobruchus maculatus* adults exposed to 5 different concentrations of 9 DEs after 72h

Table 2 Mean (±SE) number of *Callosobruchus maculatus* F1 progeny produced after 42 d on chickpea treated with eight DE formulations at five dose rates.
*Two-way variance analysis (ANOVA) was applied to the data and the differences between the averages were based on the 5% significance level. The different uppercase letters in the same column and the different lowercase letters in the same line are statistically different.

3. Results

Toxicity of DEs against *C. maculatus*

DE dose rate (ppm)	DE Formulation / Silicosec®	Desect®	BGN	BHN	AG2N	AC2N	CB2N	CCN	FB2N		
1500	82.9±2.9Acd*	71.7±2.9Ade	82.3±4.8Acd	96.3±1.6Aab	100±0Aa	61.1±8.9Ae	90.6±1.1Abc	98±1.1Aa	78.7±6.2A	15.200	0.0001
1000	62.2±5.2Bb	70.4±3.7Ab	65.3±6.3Bb	92.1±2.6Aa	94.1±1.7Ba	40.3±7.8Bc	66.1±4Bb	96±1.8Aa	62.7±4.8Bb	17.017	0.0001
500	48.3±3.2Cb	25.2±5.8Bd	9.5±1.2Ccd	31.4±2.3Bcd	36±2.8Cc	2.9±2.2Cf	39.7±2.6Cbc	72.9±3Ba	23.9±2Cd	46.989	0.0001
300	20.4±2.6Db	8.7±2.9Cb	5.5±0.9Cb	6.8±1.1Cb	21.6±2.6Da	1±1Cc	22.5±0.9Da	23.8±4.3Ba	20.6±4.1Ca	51.558	0.0001
100	0±0Ed	0.7±1.06Dcd	4.2±2.6Ccd	4.9±1.6Cc	11.3±1.8Eab	3.5±2.4Cd	10.6±1.1Eab	6.4±3.4Cc	17.7±2.8Ca	7.441	0.0001
Control	0±0	0.5±0.5	2±1.2	0±0	0±0	2±1.2	0±0	0±0	1±1	-	-
F	150.222	67.512	68.741	131.617	247.357	28.780	204.950	76.216	35.671	-	-
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	-	-

Percent mortality of *C. maculatus* at the end of the third day of treatment of chick peas with 5 different concentrations of each diatomaceous earth are given in Table 1.

DE dose rate (ppm)	DE Formulation / Silicosec®	Desect®	BGN	BHN	AG2N	AC2N	CB2N	CCN	FB2N	F	P
1500	27.8±3.7Cb*	18.4±3.4Dc	27.8±5.8Cb	1.4±0.5Cd	2.4±0.5Dd	37.8±2.9Ca	2.8±0.8Dd	0.4±0.2Cd	20.4±3.1Cbc	22.343	0.0001
1000	29±12.1Ca	27.8±4.8Da	35.8±6.7Ca	1.2±0.5Cb	6.2±1.1Db	41.6±7.1Ca	22±5.1Dab	5.2±2Cb	36±10.2Ca	5.058	0.0001
500	39.8±5Cd	60.8±10.4Ccd	84.4±6.2Bab	3.6±0.6Ce	57.8±4.5Ccd	97.6±7.3Ba	47.6±7.6Cd	56.6±9Bcd	71.2±6.8Bbc	15.103	0,0001
300	67.6±12.1BCc	97.4±5.9Bab	97±4.9Bab	18.6±1.2Cd	61±8Cc	102.4±5.7ABa	62.4±6.8Cc	109.6±6.7Aa	78.6±6.1ABbc	15.749	0.0001
100	103.4±16.6ABa	107.6±10.5Ba	105±7ABa	77.4±18.9Ba	96.8±12.3Ba	105.2±6ABa	99.2±10.9Ba	111.6±4.9Aa	80.4±12.3ABa	1.002	0.451
Control	138.2±20Aa	93.3±17.1Aa	129.8±17.7Aa	98.8±13.3Aa	98.8±13.3Aa	129.8±17.7Aa	98.8±13.3Aa	138.2±20.2Aa	102.4±9.2Aa	0.497	0.850
F	11.863	28.117	19.149	33.924	38.762	16.841	31.905	36.477	12.919	-	-
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	-	-

Mortality of *C. maculatus* adults after 3 d of exposure to DE treated chickpea increased with increase in DE dose rate.Two-way ANOVAs showed statistically significant effects on the mortality rates of the DEs ($F_{8,180}$ = 73.866, P <0.0001) and concentrations ($F_{4,180}$ = 659.275, P <0.0001) and the interaction between these two factors $F_{32,180}$ = 9.179, P <0.0001) were found to be statistically significant. The CCN and AG2N coded DEswere found to be similar in efficacy at a concentration of 1500 ppm. Within this exposure interval, the highest (100%) mortality were recorded on AG2N coded DE at 1500 ppm. CCN-coded DE showed 96 and 98% mortality rates at 1000 and 1500 ppm

concentrations, respectively. Silicosec® and Desect® commercial DEs showed 82.9% and 71.7% mortality of the beetles, respectively. At the lowest dose rate of each of the DE formulations the efficacy was very low.

F1 progeny production

The main effects of both DE formulation and dose rate as well as their interaction were significant ($P \leq 0.0001$) for number of progeny produced by *C. maculatus*. The mean number of progeny in the untreated control was significantly higher than the numbers that developed on treated chickpeas. Progeny production was reduced by increasing DE dose rate. On treated chickpea seeds, the lowest number of progeny was 0.4 and 1.4, respectively. At 1500 ppm concentration of CCN and BHN coded Des, very few new generation adults emerged. İn all DE treatments, the number of new generation adults that emerged from the control treatments ranged from 93 to 138 and was statistically similar. Generally, as the concentration Des was increased, there was a decrease in the emergence of new generation adults.

Discussion

A few researchers have studied the toxicity of various diatomaceous earth formulations on *C. maculatus.* Most researchers have applied diatomaceous earths of different origins against adult insects at different temperature, humidity and time (Prasantha et al., 2002, Stathers et al., 2004, Islam et al., 2010, Wakil et al., 2010, Shams et al., 2011, Parsaeyan et al., 2012, Tofel et al., 2012, Badii et al., 2013, Chelav et al., 2013, Ofuya et al., 2015, Doğanay et al., 2017). The results presented in this study indicate that local Turkish DE's are effective in controlling *C. maculatus* on chickpea. However, among them, CCN, AG2N, BHN and CB2N coded DEs were found to be more effective after one day of exposure. Our results and those of previous studies suggest that as the DE dose and exposure time increased, the adult mortality rates also increased (Vayias and Athanassiou 2004, Athanassiou and Kavallieratos 2005, Korunic and Fields 2006, Vayias and Stephan 2009, Baytekin and Sağlam 2017). İn the present study at the end of the third day, only AG2N coded DE showed a 100% mortality at 1500 ppm concentration. Stathers et al., (2004) found that 0.1 g of Dryacid® commercial DE killed 100% of adults of *C. maculatus* after 3 days' exposure. Similar results were obtained with the AG2N encoded DE in this current study. When we applied at 1500 ppm concentration of Siicosec® for 3 days 83% mortality was recorded. Shams et al. (2011) conducted studies on Silicosec® on *C. maculatus* at 500 ppm concentration for 2 days and obtained a 95% mortality rate. Tofel et al. (2012) achieved a 100% mortality rate when they applied Silicosec® 2000 ppm concentration for 4 days. Islam et. al (2010) reported that after 3 days of exposure Silicosec® at 1000 ppm concentration the mortality of *C. maculatus* was 90%. İn the present study Silicosec® had a 99% mortality rate on the 5th day, but a 100% mortality rate was reached at the 7th day at 1500 ppm concentration. It is thought that the differences between the studies may be due to differences between the experimental conditions, product type and the insect populations. İn this study, it was concluded that local Des were effective in controlling *C. maculatus* adults on chickpea, but among them, CCN, AG2N, BHN and CB2N coded Des were found to be most effective. Diatomaceous earth is a potential alternative method that can be used in an integrated pest management of stored product pests. Progeny production was also significantly reduced by the application of the local DEs.Field studies on the practicial application of local DEs for the protection of grains against insect pest infestation n Turkey are necessary.

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