COMPARISON OF BIOACTIVITIES OF NATIVE DIATOMACEOUS EARTH AGAINST TURKESTAN COCKROACH [*BLATTA LATERALIS* WALKER (BLATTODEA: BLATTIDAE)] NYMPHS

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Abstract. Cockroaches, *Blatta lateralis* Walker (Orthoptera: Blattidae) have been one of the important groups of insect pests in urban environments. In this study, it was investigated that the effects of different native diatomaceous earths samples against Turkestan cockroach, *Blatta lateralis* Walker (Blattodea: Blattidae) nymph in the laboratory conditions. For this purpose, three different diatomaceous earths (Turco000, Turca004 and Turco020), obtained from domestic sources, were tested in seven different doses (0.50 g/m^2, 0.75 g/m^2, 1 g/m^2, 1.25 g/m^2, 1.50 g/ m^2, 1.75 g/m^2 and 2.00 g/m^2). It was concluded that the increase in the application dose increased the mortality. Turco020 caused 100% mortality rate at the end of the 28th hour at the application dose of 1.25 g/m^2. Similarly, the dose of 1.25 g/m^2 of Turco004 and the dose of 0.75 g/m^2 of Turco000 at the same treatment period produced 100% mortality. Based on the statistical analysis, a positive interaction between particle size and biological activity was detected. These results showed that the diatomite obtained from local sources have an important role in the control of this household pest.
Keywords: *Turkestan cockroach, bioactivity, native, control, particle size, efficiency, household pest*

Introduction

The Turkestan cockroach, *Blatta lateralis* Walker (Blattodea: Blattidae), also known as the rusty red cockroach, red runner cockroach or simply rusty red, red runner is native to a large area of the Middle East extending from Libya eastward to Central Asia including Afghanistan, Pakistan, Uzbekistan, and southern Russia (Alesho, 1997). In addition, Kim and Rust (2013) reported that this species has become an important invasive species throughout the southwestern United States. The adults of Turkestan cockroach are three cm tall and adult females are shorter than male. The Turkestan cockroach females produce between two and 25 ootheca or egg capsules over their life span. Each ootheca contains about 18 eggs. The species becomes adult after five molts, the nymphal developmental period at 26.7ºC is about 224 d. Five generations of Turkestan cockroaches require about 3 years (Kim and Rust, 2013).

Because of the species cause water and food contamination, transfer pathogens that cause diseases in human to induce allergic reactions and diseases (Shahraki et al., 2013). There is also a risk of damage to common household plants and crops that Turkestan cockroach feeds on once the cockroach reaches pest extents (Kim and Rust, 2013). In addition, Cranshaw (2011) reported that this species was known as a nuisance invader of homes in the United States. The use of insecticides is primarily preferred for

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the control of cockroaches. However, alternative control methods have gained importance due to reasons such as the use of insecticides in indoor areas, threatening human and environmental health and developing resistance of cockroaches to insecticides. Some of the strains have shown resistance to up to 8-12 insecticide (Cochran, 2000; Kambayi et al., 2006).

In this context, there is growing interest in diatomaceous earth, which is known to be particularly effective against indoor pests (Faulde et al., 2006a; Alves et al., 2008; Hosseini et al., 2014; Islam and Rahman, 2016; Akhtar and Isman, 2016; Ertürk et al., 2017; Agnew and Romero, 2017; Atay et al., 2018). The mode of action of DE is generally accepted as desiccation affect on the insects (Faulde et al., 2006b). The effect primarily depends on its physical properties. There are different mechanisms of action such as abrasion of the cuticle, absorption of cuticular waxes, damage to the digestive tract, blockage of the spiracles and tracheae, and surface enlargement combined with dehydration (Ebeling, 1971; Faulde et al., 2006b).

The effectiveness of DEs for killing and controlling insect populations can vary depending on geographical origin, formulation process, oil absorption capacity, and chemical/mechanical modification of DEs, test conditions used and the species being tested (Quarles, 1992; Faulde et al., 2006b). This study was carried out to determine the efficacy of insecticidal activity of DE, which is obtained from domestic sources, against *B. lateralis* nymphs under laboratory conditions.

Material and methods

Rearing of Blatta lateralis

Turkestan cockroach nymphs were produced in 30 L plastic containers with 5 cm high peat at the base. A mixture of wheat flour and milk powder was used as a nutrient and water-absorbed paper for water needs (*Figure 1*). The colony was maintained in the climate room at 25 ± 2°C, 65 ± 5% relative humidity and 12:12 (L:D) photoperiod.

Diatomaceous earth

Diatomaceous earth (DE)'s used in this study were acquired from a local commercial company operating in Ankara-Kazan and Beypazarı counties (Beg-tuğ Mineral). The particle sizes of the diatomaceous earth were ranged 1-10 μm, 10-30 μm, 43-65 μm, for Turco000, Turco004 and Turco020, respectively. Local diatomaceous earth mainly composes of SiO_2 (83-95%) and other minerals were found oxidized form of calcium, aluminum, iron in small amounts.

Bioassay tests

Turco000, Turco004 and Turco020 local diatomaceous earth were weighed in seven different doses (0.50 g/m^2, 0.75 g/m^2, 1 g/m^2, 1.25 g/m^2, 1.50 g/ m^2, 1.75 g/m^2 and 2.00 g/m^2) and added to 20 cm^2 glass bottles (Hosseini et al., 2014). Five of Turkestan cockroach nymphs in the same period (60-day-old) were placed into glass bottles. Honey was applied to the inner edges of the bottles to feed of the insects, the mouth of the bottle was covered with insect proof mesh and incubated at 25 ± 2°C and 65 ± 5% r.h. conditions. At the end of the fourth, eighth, 12, 16, 20, 24, 28, 32 and 36 hours live and dead insects were counted and recorded. In the experiment, the control group

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weren't treated with the DE. Trials were set up with randomized block design with 18 replications.

Figure 1. *Rearing of Blatta letaralis in laboratory condiditons*

Statistical analysis

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

Results

It was determined that different doses of Turco020 showed activity in varying proportions against Turkestan cockroach nymphs (*Table 1*).

In all of the studied periods 2.00 g/m^2 showed significant activity on the mortality of the nymphs. After four hours of treatment, the dose of 2.00 g/m^2 had a mortality rate of 72.33%, at the end of the eight hours treatment mortality rate reached 100%. The dose of 1 g/m^2 caused 97.39% mortality rate at the end of the 24th hours and took place in a different statistical group from the control. All doses showing with significant activity were in a different statistical group than the control group. The doses of 0.50 g/m^2, 0.75 g/m^2 and 1 g/m^2 did not provide 100% mortality rate in any time period.

Turco004 showed similar bio-activity together with Turco020 on Turkestan cockroach nymphs (*Table 2*). The lowest dose and the shortest duration time in terms of 100% mortality were 1.25g/m^2 dose and 28 hours of treatment, respectively. However, based on the treatment time, 2.00g/m^2 dose gave 100% mortality rate at the end of the 12th hour. This dose was statistically different from the control group. The doses of 1.25g/m^2, 1.50g/ m^2, 1.75g/m^2 and 2.00g/m^2 gave 100% mortality rate different application time period.

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Table 1. *Mortality rates of Turco020 against Turkestan cockroaches*

HAT	Mortality (%) ± SE*								
	Control	0.50g/m²	0.75g/m²	1g/m²	1.25g/m²	1.50g/m²	1.75g/m²	2.00g/m²	
4	0.00±0.00c[1]	0.07±0.28c	1.06±0.91c	0.80±1.03c	4.05±2.19c	4.50±2.49c	23.85±3.95b	72.33±2.75a	F=27.98, df=7.143, P<0.05
8	0.00±0.00e	4.14±1.70de	11.50±1.61d	38.26±2.28c	78.58±2.02b	90.09±1.73b	99.74±0.53a	100.00±0.00a	F=124.71, df=7.143, P<0.05
12	0.00±0.00e	7.04±1.88d	33.44±1.27c	65.40±2.04b	98.94±0.91a	99.93±0.28a	100.00±0.00a	-	F=211.62, df=7.143, P<0.05
16	0.00±0.00e	18.56±1.73d	41.54±1.90c	79.57±2.21b	99.93±0.28a	100.00±0.00a	-	-	F=204.44, df=7.143, P<0.05
20	0.07±0.28e	20.61±1.45d	50.00±2.61c	89.62±3.10b	99.93±0.28a	-	-	-	F=153.42, df=7.143, P<0.05
24	0.07±0.28d	22.64±1.55c	58.56±1.77b	97.39±2.38a	100.00±0.00a	-	-	-	F=200.31, df=7.143, P<0.05
28	0.07±0.28d	22.64±1.55c	64.55±2.10b	98.09±1.81a	-	-	-	-	F=207.85, df=7.143, P<0.05
32	0.07±0.28d	24.53±1.85c	71.23±2.28b	98.38±1.46a	-	-	-	-	F=199.34, df=7.143, P<0.05
36	0.07±0.28d	27.88±1.50c	74.61±2.64b	98.38±1.46a	-	-	-	-	F=194.04, df=7.143, P<0.05

*Standard Error; HAT: Hour after treatment; [1]Different letters in the same line indicate statistically different from each other (Anova P < 0.05. Tukey test)

Table 2. *Mortality rates of Turco004 against Turkestan cockroaches*

HAT	Mortality (%)±SE*								
	Control	0.50g/m²	0.75g/m²	1g/m²	1.25g/m²	1.50g/m²	1.75g/m²	2.00g/m²	
4	0.00±0.00d[1]	0.00±0.00d	0.07±0.28cd	1.65±1.06cd	3.66±1.48cd	5.74±2.01c	23.30±2.33b	53.91±1.91a	F=30.55, df=7.143, P<0.05
8	0.00±0.00e	5.79±1.91de	15.53±2.38d	46.32±2.70c	81.61±2.24b	94.21±1.91ab	94.16±1.51ab	98.67±1.19a	F=74.51, df=7.143, P<0.05
12	0.00±0.00d	13.95±1.97c	45.10±3.03b	66.99±2.57b	95.31±1.51a	98.02±1.32a	98.94±0.91a	100.00±0.00a	F=95.54, df=7.143, P<0.05
16	0.00±0.00e	21.42±2.02d	61.09±3.33c	86.89±1.86b	99.40±0.74a	99.93±0.28a	100.00±0.00a	-	F=138.81, df=7.143, P<0.05
20	0.00±0.00e	32.28±1.84d	72.33±2.27c	92.31±2.06b	99.74±0.53a	100.00±0.00a	-	-	F=163.61, df=7.143, P<0.05
24	0.00±0.00e	37.82±2.26d	76.42±1.95c	95.31±1.51b	99.93±0.28a	-	-	-	F=180.24, df=7.143, P<0.05
28	0.00±0.00d	40.34±1.72c	77.46±1.91b	98.02±1.32a	100.00±0.00a	-	-	-	F=221.59, df=7.143, P<0.05
32	0.00±0.00d	41.54±1.65c	84.38±1.98b	98.94±0.92a	-	-	-	-	F=240.78, df=7.143, P<0.05
36	0.00±0.00d	42.76±1.82c	86.96±1.96b	99.73±0.53a	-	-	-	-	F=255.58, df=7.143, P<0.05

*Standard Error; HAT: Hour after treatment; [1]Different letters in the same line indicate statistically different from each other (ANOVA P<0.05 Tukey test)

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Turco 000 having the smallest particle size, showed that dose-dependent activity in varying proportions. In terms of the highest activity and the shortest application time, the dose of 0.75 g/m^2 provided 100% mortality at the end of the 28th hours. Besides, at the end of 12th hours, complete mortality was obtained at doses of 1.25 g/m^2 and above (*Table 3*). All doses and times showing significant bioactivity were in a different statistical group with the control group.

Table 3. *Toxicity of Turco 000 against Turkestan cockroaches*

HAT	Mortality (%)±SE*								
	Control	0.50g/m^2	0.75g/m^2	1g/m^2	1.25g/m^2	1.50g/m^2	1.75g/m^2	2.00g/m^2	
4	0.00±0.00c[1]	0.14±0.61c	1.65±1.06c	0.80±1.03c	3.22±1.25bc	3.66±1.48bc	16.53±2.08ab	19.18±2.52ab	F=9.19, df=7.143, P<0.05
8	0.00±0.00e	27.97±1.63d	64.12±1.83c	77.55±2.08c	83.39±1.67bc	94.88±2.21ab	96.34±1.48ab	99.40±0.74a	F=70.41, df=7.143, P<0.05
12	0.00±0.00d	48.77±1.14c	96.83±1.65b	99.73±0.53ab	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	F=329.37, df=7.143, P<0.05
16	0.00±0.00c	60.76±1.67b	99.40±0.74a	100.00±0.00a	-	-	-	-	F=446.07, df=7.143, P<0.05
20	0.00±0.00c	76.53±2.12b	99.93±0.28a	-	-	-	-	-	F=426.91, df=7.143, P<0.05
24	0.00±0.00c	77.47±1.97b	99.93±0.28a	-	-	-	-	-	F=455.25, df=7.143, P<0.05
28	0.00±0.00c	88.58±2.01b	100.00±0.00a	-	-	-	-	-	F=497.8, df=7.143, P<0.05
32	0.00±0.00c	91.64±2.22b	-	-	-	-	-	-	F=447.41, df=7.143, P<0.05
36	0.00±0.00c	95.91±2.10b	-	-	-	-	-	-	F=474.63, df=7.143, P<0.05

*Standart Error; HAT: Hour after treatment; [1]Different letters in the same line indicate statistically different from each other (Anova P<0.05. Tukey test

Statistical analysis showed that there was a significant interaction between diatomaceous earth*dose and diatomaceous earth*time. Interactions of dose, time, and diatoms, both individually and with each other, were found to be statistically significant (*Table 4*).

Table 4. *ANOVA parameters for main effects and interactions for mortality of Turkestan cockroach*

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diatomaceous earth	2	4830	2959	1480	10.45	0.00
Doses	6	178891	49931	8322	58.78	0.00
Time	8	1318661	1034576	129322	913.5	0.00
Diatomaceous earth * Dose	12	39469	6830	569	4.02	0.00
Diatomaceous earth * Time	16	110531	55313	3457	24.42	0.00
Dose * Time	48	496830	443475	9239	65.26	0.00
Diatomaceous earth * Dose * Time	96	536729	536729	5591	39.49	0.00
Error	3213	454856	454856	142		
Total	3401	3140798				

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Discussion

In this study was tested the effectiveness of the native diatomaceous earth, which is a biorational control method, for the Turkestan cockroach nymphs that are important indoor and outdoor pest, in terms of an alternative control method to the chemical compounds. In the trials, the insecticidal properties of local diatomaceous earth were initially tested in different doses and times against Turkestan cockroach nymphs and in this aspect, this study is a first in literature. Results from contact bioassays revealed that nymphs of the Turkestan cockroachs are sensitive to the diatomaceous earth at different rates. The most toxic formulation was Turco000 with the highest mortality rate and minimum exposure time (*Table 3*). It is known that the effect of the particle size on the effectiveness against insects is significant; smaller particles are significantly more effective than larger ones (Korunic et al., 2011). Similarly, Turco004 and Turco020 were less effective because of the having larger particle size than Turco000. The results we found supporting Bhan et al. (2014) findings showed that developed temephos and imidacloprid containing polyethylene glycol encapsulated nanopesticide with the melt-dispersion method and find more active against larvae of *Culex quinquefasciatus* (Diptera: Culicidae).

It is generally known for the diatomaceous earth that high relative humidity content effects the rate of mortality due to the desiccation (Fields, 1998; Subramanyam and Roesli, 2000; Fields and Korunic, 2000; Mewis and Ulrichs, 2001; Athanassiou et al., 2011; Frederick and Subramanyam, 2016). In our study, we used 65 ± 5% r.h. conditions in terms of determining the effect of the r.h. for the toxicity test. However, this relative humidity value needs to be diversified in future studies. Faulde et al. (2006a) revealed that seven kinds of DE formulation have been showing different toxicity at 25 g DE powder per m^2 to the adults of German cockroach, *Blattella germanica* (L.) (Orthoptera: Blattellidae) in a humid climate (85% r.h.), complete populations were eliminated within 10 days with FS 90.0S White, FS 90.0S, and FS 95.0 DE formulations tested. Our findings showed that the doses of 1g/m^2 of Turco000 killed the Turkestan cockroach nymphs at the end of the 16^{th} hour's treatment. It is estimated that morphological differences of different types of cockroaches and the origin of the diatomaceous affects the mortality rates of insects at several rates.

The reactions of biological stages of insects to diatomaceous earth are also different. It is known that larval or nymphal stages of insect more susceptible to the DE formulations. Older larvae or nymph stages are significantly more resistant than young larval periods (Vayias and Athanassiou, 2004). Although the adult cockroach was not used in this study, it can be said that the nymphal periods of Turkestan cockroach are more sensitive to diatomaceous earth. Similarly, Hosseini et al. (2014) reported that the dose of 25 g/m^2 of DE completely kills the nymphal stages of German cockroaches at 72 hours, while LC_{50} and LC_{90} values for adults determined as a 12.9034 g/m^2 and 626.0942 g/m^2, respectively. Vayias and Athanassiou (2004) stated that all larvae of *Tribolium castaneum* Herbst, 1797 (Coleoptera: Tenebrionidae) were killed at the dose of 1.5 g/kg of SilicoSec whereas this rate was 50% in adults after 48 hours exposure period.

As a result, cockroaches emerge as a problem in terms of hygiene in areas where human activity and habitats are intense. Cockroaches are the potential carrier of human diseases, a pathogenic organism such as bacteria, protozoa, fungi, and helminths (Tatfeng et al., 2005; Baumholtz et al., 2008). In addition, increased awareness of side effect of the synthetic pesticides to the society, the resistance statues of insects against

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pesticides, has also led to the need to investigate safe control methods for the cockroaches that can be used in the integrated pest management system.

Conclusions

Diatomaceous earth can be regarded, as have strongly insecticidal properties against Turkestan cockroach. The results show that there is a negative correlation between particle size and activity. In view of these findings, native diatomaceous earth should be investigated in different humidity and temperature conditions against an invasive species Turkestan cockroach adults and nymphs.

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