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Insecticidal Effect of Central Anatolian Region Diatomaceous Earths Against Confused Flour Beetle (*Tribolium confusum* Du Val.) on Stored Paddy

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Abstract

In this study, insecticidal efficacy of different local diatomaceous earth (DE) deposits obtained Central Anatolian Region in Turkey and commercial DE deposit (German origin), Silicosec® were evaluated against substantial pest on stored grain as *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) at five different concentrations of 100, 300, 500, 900 and 1500 ppm on stored paddy. Mortality of the exposed adults was assessed after 7, 14 and 21 days of exposure. Also progeny productions were assessed after 65 days The tests were carried out at 25±1 oC temperature, 55±5% R.H. under dark conditions. The most effective DE in a short time were assessed AG2N-1 which caused 97% mortality of *T. confusum* adults at 1500 ppm concentration after 7 days of exposure in paddy. Complete mortality of *T. confusum* adults was recorded on AG2N-1 at 900 ppm for 14 days and treatments of AG2N-1, BGN-1, CBN-1 for 21 days at 500, 900 and 1500 ppm respectively whereas 87% mortality rate was determined for 21 days exposure of Silicosec® at the highest concentrations on paddy. In conclusion, this study indicated that Turkish DE deposits, AG2N-1, BGN-1 and CBN-1 had high insecticidal efficacy in comparison with the commercial Silicosec® and would have potential to be used against insects in the pest management of stored paddy.

Keywords: Turkish diatomaceous earths, *Tribolium confusum*, toxicity, paddy, Silicosec

1. Introduction

Currently, the control of insect pests in durable stored food products, such as grains and legumes, is based on the use of chemical methods such as fumigants and residual insecticides. However, the use of these substances is directly related with toxic residues on the final product, as well as serious environmental hazards. These factors, along with the consumers' demand for residue-free food and the development of resistance by several insect pests, have made essential the evaluation of alternative, low-risk and environmentally-friendly control methods. One of the most promising alternatives over the use of traditional pesticides in durable stored products is the use of diatomaceous earths (DEs). DEs are composed by the fossil skeletons of phytoplanktons, also known as diatoms, which occur in fresh and salt water since the Eocene period and produce a soft sedimentary rock, which is composed mainly by amorphous silica ($SiO_2 + H_2O$). The DEs currently mined vary remarkably in their insecticidal activity, depending upon species composition, geological and geographical origin as well as certain chemical characteristics, such as SiO2 content, pH and tapped density (Korunic 1997). DEs are probably the most efficacious natural resource-based dry materials that can be used as insecticides (Korunic 1998). DEs act in the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss. They are non-toxic to mammals (rat oral LD50>5000 mg/kg of body weight), leave no toxic residues on the product and

according to the US EPA they are classified in the category of GRAS (Generally Recognised As Safe) since they are used as food or feed additives (FDA 1995). Regarding their insecticidal use, DEs can be applied with the same application technology with traditional grain protectants, which means that no specialized equipment is required (Athanassiou et al. 2005). Several DEs, based on natural deposits, are now commercially available, and have proved very effective against stored grain pests (Subramanyam and Roesli 2000, Athanassiou et al. 2011). However, the investigation for newer, naturally-occurring DEs that are more effective in insect control is still in progress, especially in areas rich to silicaceous rocks. Based on the first evidence and preliminary samplings, it seems that Turkey is considered rich to natural DE deposits, and there is clear evidence for the existence of large DE deposits at some areas (Özbey and Atamer 1987, Mete 1988, Sıvacı and Dere, 2006, Çetin and Taş 2012). Diatomite reserve of Turkey is 125 million tons. However, there is limited information on the efficiency of local DEs from these areas in Turkey against stored grain insects. In this study, efficiency of three local diatomaceus earth formulations against Confused Flour Beetle (*Tribolium confusum* Du Val.) on paddy, was investigated under laboratory conditions.

2. Materials and Methods

2.1. Test Insects

Confused Flour Beetle adults used in the bioassays were taken from a culture that was kept at the Namık Kemal University, Department of Plant Protection, Toxicology Laboratory on whole wheat at 26 ± 1ºC, 65 ± 5 %. All individuals used in the tests were <2 wk old.

2.2. Diatomaceous earth formulations

The three DE formulations used in biological tests were of three Turkish diatomaceous earth formulations (AG2N-1, BGN-1 and CBN-1) and commercial diatomaceous earth (Silicosec®). Turkish diatomaceous earth formulations were collected from diatomite reserve at middle Anatolia of Turkey.

2.3. Experimental procedure

Exposure studies were carried out at 25 ± 1ºC, 55 ± 3 % RH, and five dose rates of four Turkish diatomaceous earth formulations and commercial diatom earth (Silicosec®) (100, 300, 500, 900 and 1500 ppm) on paddy (*Oryzae sativa* L. variety of Osmancık 97) with %13 moisture content. For each trial (DE formulation-dose combination), five samples of 50 g paddy were taken. Each sample was placed in a small glass jar that was closed, and that was covered with organtine for sufficient ventilation. The samples were treated individually with the respective DE quantity and then shaken manually for 5 min to achieve equal distribution of the dust in the entire product quantity. Five additional tubes, containing untreated paddy, served as control in each case. Subsequently, 20 adults of *T. confusum* were introduced into each tube. The tubes were then placed in incubators, set at above mentioned temperature and relative humidity level. Dead adults of both species were counted after 7, 14, 21 d. After the last count for mortality, all adults (dead and alive) were removed from the DE-treated jars, and the jars were left in the incubators for an additional period of 65 d. Then, the emerged *T. confusum* individuals were counted.

2.4. Data processing and analysis

Generally, the control mortality was very low, but where it was considered necessary the mortality counts were corrected by using the formula of Abbot (1925). The data were analyzed, separately for each species, by using the Anova test of SPSS (SPSS,2009), with insect mortality as the response variable and type of DE formulation and dose rate, as the main effects. For the progeny production counts, with number of progeny as the response variable and type of DE formulation, and dose rate as main effects. Means were separated by using the Anova test at $P<0.05$.

3. Results

Efficacy of Turkish DE's represented different values according to exposure times and concentration at the end of the experiment (Table 1,2 and 3). Complete mortality of *T. confusum* adults was recorded after 14 days of exposure at 900 ppm treated with AG2N-1 (Table 2) and 21 days of exposure at 500, 900, 1500 ppm treated with AG2N-1, BGN-1 and CBN-1 respectively whereas 87% mortality rate were recorded for Silicosec® after 21 day exposure at the highest concentrations on paddy (Table 3). According to the results of the biological tests carried out on the paddy, F1 values of *T.confusum*, including the control group, was not seen in the adult exit. This result wold be caused by confused flor beetle one of the important secondary insect species in the stored product pests and was not able to eat whole kernel of the paddy.

Table 1. Mean percentage mortality (±SE) of *Tribolium confusum* adults exposed to 5 different concentrations of 9 DEs after 7 days

	DE Formulation						
Concentration (ppm)	**Silicosec®**	**BGN-2**	**BHN-1**	**AG2N-1**	**CBN-1**	**F** value	**P** value
1500	3±2 Ac*	25.3±4.3 Ab	0±0 Ac	96.9±2.1 Aa	19.2±4.5 Ab	$F_{4,20}$=105.52	P<0.0001
1000	1±1 Ac	16.2±2.6 Ab	1±1 Ac	82.3±4.5 Ba	1.8±1.8 Bc	$F_{4,20}$=87.38	P<0.0001
500	0±0 Ac	5.3±1.7 Bb	0±0 Ac	54.2±5 Ca	0±0 Bc	$F_{4,20}$=111.33	P<0.0001
300	0±0 Ab	2.4±1 Bb	1±1 Ab	32.3±4.4 Da	1.6±1 Bb	$F_{4,20}$=32.96	P<0.0001
100	0±0 Ab	1.6±1 Bab	1±1 Ab	4.2±2.1 Ea	0±0 Bb	$F_{4,20}$=3.99	P=0.015
Control	0±0	1±1	0±0	4±1.9	1±1		
F value	$F_{4,20}$=1.708	$F_{4,20}$=15.303	$F_{4,20}$=0.5	$F_{4,20}$=74.503	$F_{4,20}$=17.621		
P value	P=0.188	P<0.0001	P=0.736	P<0.0001	P<0.0001		

Table 2. Mean percentage mortality (±SE) of *Tribolium confusum* adults exposed to 5 different concentrations of 9 DEs after 14 days

	DE Formulation						
Concentration (ppm)	**Silicosec®**	**BGN-2**	**BHN-1**	**AG2N-1**	**CBN-1**	**F** value	**P** value
1500	39.4±4.2 Ad	89.8±4.6 Ab	15.2±2.9 Ae	100±0 Aa	80.6±4.1 Ac	$F_{4,20}$=77.96	P<0.0001
1000	5.5±2.7 Bd	66.3±9.2 Bb	4.4±2 Bd	100±0 Aa	25.5±4.4 Bc	$F_{4,20}$=72.54	P<0.0001
500	4.6±3 Bc	21.4±4.7 Cb	0±0 Bc	94.3±1.8 Ba	1.2±0.7 Cc	$F_{4,20}$=97.46	P<0.0001
300	2.6±1.8 Bc	16.3±6.4 Cb	3.6±2.7 Bc	72.7±4.5 Ca	3.9±1.8 Cc	$F_{4,20}$=30.01	P<0.0001
100	1.8±1.8 Bb	2.4±0.6 Dab	1.6±1 Bb	8.4±3 Da	1.2±0.7 Cb	$F_{4,20}$=2.21	P=0.103
Control	1±1	2±1.2	1±1	12±3.7	2±1.2		
F value	$F_{4,20}$=13.74	$F_{4,20}$=33.82	$F_{4,20}$=7.33	$F4_{,20}$=135.58	$F_{4,20}$=75.73		
P value	P<0.0001	P<0.0001	P=0.001	P<0.0001	P<0.0001		

Table 3. Mean percentage mortality (±SE) of *Tribolium confusum* adults exposed to 5 different concentrations of 5 DEs after 21 days

	DE Formulation						
Concentration (ppm)	**Silicosec®**	**BGN-2**	**BHN-1**	**AG2N-1**	**CBN-1**	**F** value	**P** value
1500	86.9±3.4 Ab	100±0 Aa	56.6±3.4 Ac	100±0 Aa	100±0 Aa	$F_{4,20}$=140.39	P<0.0001
1000	29.3±6.6 Bc	100±0 Aa	23.2±8.2 Bc	100±0 Aa	72.6±3.1 Bb	$F_{4,20}$=75.52	P<0.0001
500	11.3±4.6 Ccd	83.3±1.9 Bb	5.7±3.8 Cd	100±0 Aa	14.7±5.9 Cc	$F_{4,20}$=78.53	P<0.0001
300	2.6±1.8 Cc	50±8.2 Cb	5.5±2.7 Cc	94.2±2.6 Ba	7.4±2.7 Cc	$F_{4,20}$=51.93	P<0.0001
100	2.6±1.8 Cb	4.2±1.3 Db	2.4±1 Cb	33.3±7.4 Ca	1.1±1.1 Db	$F_{4,20}$=15.76	P<0.0001
Control	1±1	4±1.9	1±1	13±4.1	5±2.7		
F value	$F_{4,20}$=44.42	$F_{4,20}$=181.57	$F_{4,20}$=15.41	$F_{4,20}$=70.61	$F_{4,20}$=140.14		
P value	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001		

*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.

Discussion

The most effective DE in a short time were assessed AG2N-1 which caused 97% mortality of *T. confusum* adults at 1500 ppm concentration and 82% at 900 ppm concentarition after 7 days of exposure on paddy. Other diatoms showed low toxicity on all concentrations. Complete mortality of *T. confusum* adults was recorded on AG2N-1 at 900 ppm for 14 days and treatments of AG2N-1,

BGN-1, CBN-1 for 21 days at 500, 900 and 1500 ppm respectively whereas 87% mortality rate was determined for 21 days exposure of Silicosec® at the highest concentrations on paddy. Athanassiou et all. (2004), reported that Silicosec was't reached complete mortality at 750,1000 and 1500 ppm concentration on rye, oats and triticale againt *T.confusum* after 21 days application but decrased to number of insect on F_1. In a similar study conducted on rice with *T. confusum* adults, Alagöz (2016) found that Silicosec® commercial diatom was found to be 20% at the end of 7th day, 75% at the end of 14th day and 99% at the end of 21st day, at the end of the day, did not find a new generation of adult outbreaks, including the control group, in all diatoms used in experiments. Ziaee et al. (2012), a study conducted on wheat with *T. confusum* adults, found 51% mortality with Silicosec after 2days at 2000ppm concentration and complete mortality was recorded at 1000,1500 and 2000 ppm after 7 days and more. In conclusion, this study indicated that Turkish DE deposits, AG2N-1, BGN-1 and CBN-1 had high insecticidal efficacy in comparison with the commercial Silicosec® and would have potential to be used against insects in the pest management of stored paddy.

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Twelve years (2005-2017) of scientific and professional work in the field of stored products pests protection in Slovenia

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Abstract

Scientific and professional work in the field of stored products pests protection in Slovenia began in 2005, when we tested the efficacy of entomopathogenic nematodes against the granary weevil (*Sitophilus granarius*) and the sawtoothed grain beetle (*Oryzaephilus surinamensis*) adults under laboratory conditions. In 2007, we participated as partners in the project SEE-ERA.NET "Development of a non-toxic, ecologically compatible, natural-resource based insecticide from diatomaceous earth deposits of South Eastern Europe to control stored-grain insects pests" (coordinated by C. Athanassiou), and we thus became acquainted with the research work in the field of investigation the efficacy of diatomaceous earth in controlling beetles from the *Sitophilus* genus. We have continued the research of different aspects of diatomaceous earth (the influence of geochemical composition and abiotic factors on its efficiency, the effects of individual and combined application, the effects on various harmful insect pests, etc.). In search for comparable substances to diatomaceous earth (regarding the efficacy), we have studied insecticidal effects of quartz sand and entomopathogenic nematodes from Slovenia,