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Original article (Orijinal araştırma)

Evaluation of diatomaceous earth formulations for the control of rice weevil, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) in stored rice[1]

Depolanmış çeltikte pirinç biti, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae)'nin mücadelesinde diyatom toprağı formülasyonlarının değerlendirilmesi

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Summary

A study was conducted between 2012 and 2014 on the protective efficacy of diatomaceous earth (DE) formulations against *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) (rice weevil), a major pest of the stored rice. Biological tests were carried out at 30°C and 75% RH on rice treated with DE formulations. In the biological tests, different concentrations of Protector® (DE formulation) and DEA-P [mixture of natural DE (83%) and 0.25% abamectin (w:w)] were used. After DE treatment, dead adults were counted once a week for a month and percentage mortalities were determined. In order to determine progeny production (F_1), rice was also incubated under the same conditions for a 60-day period. Protector® gave 100% mortality to *S. oryzae* adults at 1000 ppm after 14 d, and DEA-P gave the same mortality rate at 75 ppm at the same time. In studies to determine progeny (F1) emergence, Protector® resulted in 100% mortality at 1750 ppm after 60 d of storage, while DEA-P caused 100% mortality at 50 ppm concentration. In conclusion, the protective effect of both DE formulations was confirmed, however DEA-P was more effective against *S. oryzae* at lower concentrations than Protector®.

Keywords: Diatomaceous earth, physical control, *Sitophilus oryzae*, stored rice

Özet

Bu çalışma 2012-2014 yılları arasında laboratuvar koşullarında yürütülmüştür. Bu çalışma ile diyatom toprağı (DT) formülasyonlarının, önemli bir depo zararlısı olan *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) (Pirinç biti)'ye karşı depolanmış çeltikte koruyucu etkisi araştırılmıştır. Çalışmalar 30°C sıcaklık ve %75 orantılı nemde, DT uygulanmış çeltikte yürütülmüştür. Denemelerde Protector® (DT formülasyonu) ve DEA-P (DT ile abemectin karışım formülasyonu) formülasyonlarının farklı konsantrasyonları kullanılmıştır. Çalışmalarda DT uygulamasının ardından birer hafta ara ile 4 kez ölü ergin birey sayımı yapılmış ve ölüm oranları belirlenmiştir. Ayrıca *S. oryzae*'nin F_1 çıkışını (birinci nesil erginleri) belirlemek amacı ile çeltik tekrar aynı koşullarda 60 gün süre ile bekletilerek yeni nesil ergin sayıları hesaplanmıştır. Protector®, *S. oryzae* erginlerinde 1000 ppm konsantrasyonunda 14. günde %100 ölüm sağlarken DEA-P aynı ölüm oranını 75 ppm konsantrasyonda aynı sürede sağlamıştır. F_1 çıkışlarını belirlemek için yapılan çalışmalarda ise Protector® 60 günlük depolama süresi sonunda 1750 ppm konsantrasyonda %100 etkili olurken, DEA-P ise 50 ppm konsantrasyonda %100 ölüme neden olmuştur. Sonuçlar değerlendirildiğinde, her iki diyatom toprağı formülasyonunun ürünü koruyucu etkisinin olduğu, ancak DEA-P' nin *S. oryzae*'ye karşı Protector®'unkilerden daha düşük konsantrasyonlarda etkili olduğu saptanmıştır.

Anahtar sözcükler: Diyatom toprağı, fiziksel mücadele, *Sitophilus oryzae*, depolanmış çeltik

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Introduction

Rice, *Oryza sativa* L. is a staple food for 1.5 billion people around the world. Global rice production is around 741 Mt annually, of which Turkey produces 830 kt (FAO, 2014). Rice has an important place in human nutrition due to its high protein, starch, vitamin (B1, B3, B6 and E) and mineral (K, P, Fe and Mg) (Özder et al., 2013) content. To maintain its nutritive quality, rice, like other cereal grains, should be dried and stored under the proper conditions, and special precautions against the pests must be taken. For the latter, continuous pest monitoring and timely applications of control measures are very important.

About 10-30% of annual global grain production is damaged by storage insect pests (Singh et al., 2009). Chemical insecticides are currently the main way of managing insect pests of stored grains. However, environmental, ecological and health effects of pesticides have lead researchers to seek safe alternatives (Zettler & Keever, 1994; Benhalima et al., 2004; Isikber & Oztekin, 2009; Pimentel et al., 2010; Alkan & Gökçe, 2012; Kepenekçi et al., 2013; Alkan et al., 2015). One of these alternatives is diatomaceous earth (DE), which is used as a physical control measure against stored grain insects. Diatomite rocks (kieselgur) that are used for a wide range of purposes from filter aid to filling materials and from food additives to refractories, are the fossilized silica remains of one-celled microscopic algae which are known as diatoms (Özbey & Atamer, 1987). DE is the only mineral that is of organic origin. In USA, silica-based DEs are rated by the Food and Drug Administration as GRAS (generally recognized as safe) for human consumption and are also registered as animal feed additives (Banks & Fields, 1995). DEs are currently used against stored product pests in the EU. Water loss in the DE-exposed insects is the main cause of mortality (Ebeling, 1971).

Due to their low mammalian toxicity (in rats, oral LD_{50} >5000 mg/kg body weight) (Subramanyam et al., 1994), stability, efficacy and lack of toxic residues, DEs have been the subject of considerable research (Ebeling, 1971; Banks & Fields, 1995; Golob, 1997; Korunic, 1998; Fields & Korunic, 2000; Subramanyam & Roesli, 2000). DEs can be used alone or in combination with insecticides of various origin (Athanassiou et al., 2005; 2006). DEA-P, for example, used in the current study is a mixture of DE and abamectin, an insecticidal/acaricidal toxin produced by fermentation by *Streptomyces avermitilis* (Burg et al., 1979) Kim and Goodfellow 2002, a soil-inhabiting bacterium (Athanassiou & Korunic, 2007).

Although numerous and versatile DE formulations have been tested against pests of various stored products (Korunic, 1998; Athanassiou et al., 2005; Ferizli & Beris, 2005; Ziaee & Khashaveh, 2007; Kavallieratos et al., 2007; Kostyukovsky et al., 2010; Doğanay, 2013; Ertürk & Emekci, 2014), there is no literature on the long-term efficacy of DEs against rice weevil, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae). Therefore, this study aimed to evaluate the efficacy of two DE formulations, Protector® and DEA-P, against rice weevil.

Material and Methods

Insect rearing

Sitophilus oryzae were reared in 1-L glass jars on whole grain soft wheat in incubators (Nüve ID 501, Ankara, Turkey) maintained at about 30°C and 75% RH. The mouth of each jar was covered with a perforated lid with inner surface were with US standard sieves mesh #120 to facilitate ventilation and prevent escape of insects. To obtain 1-2-wk-old adults in sufficient numbers, about 700-1000 adults were transferred into glass jars having approximately 500 g of wheat and left to oviposit for 48 h. After oviposition, adults were removed and after 30 d newly emerged adults were sieved off every 3-4 d to use in the experiments.

Diatomaceous earth formulations

Two DE formulations, DEA-P (supplied by C.G. Athanassiou, University of Thessaly, Greece) and Protector® (Intrachem Bio Italia, Grassobbio, Bergamo, Italy) were used in the experiments. DEA-P is a DE formulation composed of a mixture of natural DE (83%) and 0.25% abamectin (w/w). Abamectin is a mixture of avermectins containing >80% avermectin B1a and <20% avermectin B1b. These two components have very similar biological and toxicological properties. The avermectins are insecticidal or anthelmintic compounds derived from the soil bacterium *S. avermitilis*. Abamectin is a natural fermentation product of this bacterium (Lankas & Gordon, 1989; Hayes & Laws, 1990). Its DE component is of freshwater origin and contains 89% amorphous SiO_2, 4% Al_2O_3, 1.7% Fe_2O_3, 1.4% CaO, <1% MgO and K_2O and 3% water (w/w) (Athanassiou & Korunic, 2007).

The other DE formulation was natural DE sold as Protector® and is composed of 69.7% SiO_2, 5.89% Al_2O_3, 0.414% CaO and 1.05% Fe_2O_3, and, half of its particles were below 9.46 µm. (Baldassari et al., 2008).

Experimental protocol

A Turkish rice variety, *Oryza sativa* cv. Osmancık-97, with 9.8% moisture content (Multi-Grain Moisture Tester, Dickey John, Auburn, IL, USA) was used in the experiments. DEA-P was used at the concentration of 25, 50, 75, 100, 150, 175 and 200 ppm, and Protector® at 250, 500, 750, 1000, 1500, 1750 and 2000 ppm (i.e., mg DE/kg rice). Untreated rice (0 ppm) was used as the control for both DEs. DE required for four replicates of each concentration was weighed and added to 280 g of rice in 1-L plastic bags. The plastic bags were sealed inflated, then thoroughly shaken by hand for 4-5 min to ensure even distribution, and left for 10 min to allow dust settle before dividing into 225-mL PVC test vials (3 x 8 cm). Each vial was fitted with a plastic lid prepared as for the rearing jars. The vials were then filled with 70 g of DE treated rice and with 50 adult weevils each. Test vials were put into large PVC boxes containing KOH (22.25 g KOH, 77.75 g distilled water) solution to maintain the humidity at about 75%. The PVC boxes were closed tightly and then placed in an incubator (Binder KB 720, Tuttlingen, Germany) adjusted at 30±1°C. To determine mortality, live and dead adults were counted after 7, 14, 21 and 28 d. On day 28, all the live insects were removed. The vials were incubated for another 60 d and F_1 progeny counted.

Statistical analysis

Results were analyzed by factorial design repeated measures ANOVA (Gürbüz et al., 2003) using Statistica 8 (Weiß, 2007), with observation time as the repeated-measures factor and DE formulation and DE concentration as categorical predictor variables. Mortality data were arcsine transformed before analysis. The differences among the treatment means were analyzed by Tukey's HSD test at 5% significance level (Sokal & Rohlf, 1995).

Results and Discussion

Studies with Protector®

Mortality of rice weevil increased with the increase of both concentration and exposure time, and complete mortality had occurred by 14 d at 1000 ppm ($F = 23.6$; df = 21,576; $P < 0.05$) (Table 1). Similar results for rice weevil were reported by other researchers using various DEs. Kostyukovsky et al. (2010), reported complete mortality after 21 d in wheat treated with DDDE (Detia Degesch diatomaceous earth, Laudenbach, Germany) at 1000 ppm at 28°C and 65% RH. Athanassiou et al. (2004), who worked with various cereals treated with Insecto, SilicoSec, and PyriSec, obtained complete mortality of *S. oryzae* after 7 d. Matti & Awaknavar (2009) reported that Protect-It applied to sorghum at 1000 ppm for 7 d caused complete mortality of *S. oryzae* adults at 30°C with RH up to 90%; however, decreased temperatures of 25 and 20°C restricted the RH range at which complete mortality was observed, to 50 and 30%, respectively. The differences in the reported times to reach complete mortality is considered to be due to be the differences in experimental conditions, such as DE, crop/cultivar, temperature and RH (Korunic, 1998; Fields & Korunic, 2000; Baldassari et al., 2008; Matti & Awaknavar, 2009).

Table 1. Mortality of *Sitophilus oryzae* adults exposed to rice treated with Protector® at 30°C and 75% RH

Concentration (ppm)	Mortality±SE (%)							
	Exposure time (day)							
	7		14		21		28	
Control	2.0±0.23	aC*	3.1±0.24	aB	4.1±0.30	aC	6.5±0.45	aC
250	1.5±0.27	cC	24.5±2.57	bcB	53.0±2.97	abB	80.0±0.27	aB
500	23.0±1.54	cBC	80.5±0.83	bA	97.5±1.81	aA	100.0±0.00	aA
750	74.5±2.93	bA	98.0±1.20	aA	100.0±0.00	aA	100.0±0.00	aA
1000	78.0±1.80	bA	100.0±0.00	aA	100.0±0.00	aA	100.0±0.00	aA
1500	93.5±0.27	aA	100.0±0.00	aA	100.0±0.00	aA	100.0±0.00	aA
1750	99.0±0.16	aA	100.0±0.00	aA	100.0±0.00	aA	100.0±0.00	aA
2000	96.0±0.80	aA	100.0±0.00	aA	100.0±0.00	aA	100.0±0.00	aA

*Means followed by the same lowercase letter within a row or the same uppercase letter within a column are not significantly different (P ≤ 0.05).

F_1 progeny suppression is as important as the immediate mortality in population suppression of the pests. Fewer adults than that of the beginning of the experiment developed with both DEs applied at ≥750 ppm (Table 2). Similarly, Ferizli & Beris (2005) reported that in *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae) increase in dosage resulted in fewer F_1 progeny than at the start of exposure. Kavallieratos et al. (2007) also reported enhanced and natural DEs, such as Pyrisec, Insecto and Protect-It, had a similar effect in decreasing in population growth in *Tribolium confusum* Jacqueli du Val, 1863 (Coleoptera: Tenebrionidae) with increasing concentration from 500 to 1000 ppm due to the increased effects on larvae.

Table 2. Mean number and mortality of F_1 progeny of *Sitophilus oryzae* exposed to rice treated with Protector® at 30°C and 75% RH

Concentration (ppm)	F_1 Progeny (Mean)	Mortality±SE (%)	
Control	49.00	26.0±0.41	d*
250	62.75	47.6±3.17	cd
500	54.5	46.5±1.95	cd
750	43.25	49.6±1.52	cd
1000	19.75	82.4±2.16	bc
1500	22.75	90.1±1.19	ab
1750	11.00	100.0±0.00	a
2000	5.25	100.0±0.00	a

*Means followed by the same lowercase letter are not significantly different (P ≤ 0.05).

Studies with DEA-P

As with Protector®, adult mortality increased with the increase of concentration and exposure time, but complete mortality occurred after 7 d at ≥100 ppm ($F = 6.09$; df = 21,288; $P < 0.05$) (Table 3). Since DEA-P is an enhanced DE formulation combined with abamectin, complete mortality was obtained at lower concentration and shorter time than with Protector®. This can be helpful to overcome obstacles associated with natural DEs applied at higher concentrations. Athanassiou et al. (2006), in order to eliminate decrease in bulk density and flowability caused by high concentrations of DEs, used DEA-P against *Prostephanus truncatus* (Horn, 1878) (Coleoptera: Bostrichidae) and *R. dominica* at a concentration of 75 ppm and obtained complete mortality after 14 d in corn and wheat. The differences in time to achieve complete mortality in various reports is thought to be a consequence to different pest species investigated. Among insect pest species, there are differences in tolerance to DEs, from least to most tolerant being *Cryptolestes* spp., *Sitophilus* spp., *Oryzaephilus* spp., *R. dominica, Tribolium* spp. and *P. truncatus* (Maceljski & Korunic, 1971; Desmarchelier & Dines, 1987; Subramanyam et al., 1998; Fields & Korunic, 2000).

Table 3. Mortality rate of *Sitophilus oryzae* adults exposed to rice treated with DEA-P at 30°C and 75% RH

Concentration (ppm)	Mortality±SE (%)			
	Exposure time (day)			
	7	14	21	28
Control	26.5±0.20 bB*	45.0±0.34 aB	51.8±0.21 aB	54.0±0.29 aB
25	91.5±0.50 aA	78.1±1.61 aA	75.0±6.12 aA	75.0±6.12 aA
50	95.8±0.12 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
75	99.8±0.12 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
100	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
150	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
175	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA
200	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA

*Means followed by the same lowercase letter within a row or uppercase letters within a column are not significantly different (P ≤ 0.05).

Progeny studies showed that complete F_1 mortality was obtained at all concentrations except 25 ppm (F = 23.1; df = 7,96; P < 0.05) (Table 4). Similarly, Athanassiou et al. (2006), using the same DE, reported that 125 ppm is required to get a complete mortality of F_1 progeny of *S. oryzae* at 27°C and 65%. The difference between the two studies probably due to the differences in temperature and RH.

Table 4. Mean number and mortality of F_1 progeny of *Sitophilus oryzae* exposed to rice treated with DEA-P at 30°C and 75% RH

Concentration (ppm)	F_1 Progeny (Mean)	Mortality±SE (%)
Control	44.50	27.2±1.87 b*
25	13.75	57.0±2.38 b
50	5.25	100.0±0.00 a
75	2.25	100.0±0.00 a
100	1.50	100.0±0.00 a
150	0.75	100.0±0.00 a
175	2.25	100.0±0.00 a
200	0.25	100.0±0.00 a

*Means followed by the same lowercase letter are not significantly different (P ≤ 0.05).

This study aimed to introduce DEs to Turkish rice sector as efficient and safe protectants posing the least risks for human health and the environment. Both DE formulations, Protector® (natural DE) and DEA-P (enhanced DE) were shown to be effective in protecting rice against rice weevil. As reported by several authors, natural DEs when used at high concentrations have some disadvantages, such as reduced grain flow ability and bulk density, abrasion of machines parts and workplace health concerns (Subramanyam et al., 1994; Golob, 1997). Therefore, enhanced DEs can address these limitations by using lower concentrations and thus broaden the adoption of DEs for the control of storage pests (Subramanyam & Roesli, 2000; Athanassiou et al., 2006; Athanassiou & Korunic, 2007; Kavallieratos et al., 2007; Vayias & Stephou, 2009; Wakil et al., 2010). As exemplified in the present study, DEs hold considerable promise for reducing the need for synthetic pesticides by effectively suppressing pest populations. However, to encourage their use more works are needed regarding the efficacy of DEs in other situations, such as with other pest species, temperature, moisture content and crop/cultivar.

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