

## Research Article

## Influence of grain type on the susceptibility of *Tribolium confusum* adults to three diatomaceous earth formulations

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**Abstract:** Insecticidal efficacy of three diatomaceous earth (DE) formulations, DEBBM, DEA and F2 was evaluated on three different wheat cultivars, Chamran, Verinak and Behrang, against *Tribolium confusum* Jacqueline du Val. DEs were applied at the rates of 100, 200 and 300 mg/kg. Bioassays were carried out on wheat at  $27 \pm 1$  °C and  $55 \pm 5\%$  RH in continuous darkness. Mortality of adults was counted after 2, 7 and 14 days of exposure. Adults of *T. confusum* were the most susceptible to DEBBM in Behrang. So that DEBBM concentration of 100 mg/kg after 7 days of exposure caused 98% mortality which reached 100 after 14 days. Therefore, Behrang was the most tolerant cultivar to *T. confusum* infestations. Furthermore, DEBBM was more effective than the other two tested formulations.

**Keywords:** Diatomaceous earth, Protection, *Tribolium confusum*, Wheat

### Introduction

Insecticide residue and insect resistance to grain protectants increase the tendency to the use of low-risk insecticides (Obeng-Ofori, 2010). Diatomaceous earths (DEs) are promising alternatives to insecticides. They have natural origin and are of low toxicity to mammals. They are registered in some countries for direct application on stored grains. However, DEs have some disadvantages such as reducing grains bulk density and flow-ability which limit their application at higher dose rates (Fields, 1998). To overcome these limitations, modified DE formulations were developed and their commercial application examined. One of the ways of modifying DEs and improving their efficacy is their combination with other compounds (Arthur, 2004; Athanassiou and Korunic, 2007). DE particles absorb the epicuticular lipids of the insect

cuticle, causing death through desiccation and to a lesser degree by abrasion (Ebeling, 1971). However, according to the compounds of formulated diatomaceous earths; they are expected to have different mode of actions.

Several enhanced DE formulation have been developed in order to mitigate the adverse effects of DEs. Arthur (2004) developed a new commercial formulation of F2 and assessed its efficacy as a protectant of stored wheat, maize and paddy rice against *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst).

The enhanced DE formulations of DEBBM and DEA were developed and their toxicity was first examined against *Prostephanus truncatus* (Horn), *R. dominica*, *S. oryzae*, *T. castaneum* (Athanassiou *et al.*, 2006); *S. oryzae*, *R. dominica*, *T. castaneum* and *Cryptolestes ferrugineus* (Stephens) (Athanassiou and Korunic, 2007). In another research, influence of grain type (Barley, wheat and maize) was investigated on *S. oryzae* adults exposed to DEBBM (Athanassiou *et al.*, 2008).

Infestations of grain storage facilities with stored product insect pests reduced the quantity

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and quality of grain and germination rate. In the severe infestations grain warming and mold growth also occur (Hill, 2002). The confused flour beetle, *Tribolium confusum* Jacqueline du Val. (Coleoptera: Tenebrionidae) is one of the common pests of stored grains. It is categorized as a secondary pest feeding on cereal grains that are damaged, flour and related products (Rees, 1996). Flour beetles are the least susceptible stored product insects to DEs (Korunić, 2013). So, the dose rate able to control *Tribolium* spp. populations should be able to control most of the other insect's occurring in food facilities and grain storages (Arnaud *et al.*, 2005).

The aim of the current study was to evaluate the effect of three different wheat cultivars on the efficacy of enhanced DE formulations, DEBBM, DEA and F2, against *T. confusum*.

## Materials and Methods

### Insects and commodity

Adults of *T. confusum* used in the experiment were reared on wheat flour plus 5% brewer's yeast (by weight) at  $27 \pm 1$  °C and  $65 \pm 5\%$  RH. Adults used in the experiments were 7-14 days old of mixed sex. Three wheat cultivars viz. Behrang, Chamran and Verinak were purchased from Safiabad Agricultural Research Center of Dezful for use in the experiments. Clean uninfected wheat kernels were stored at -24 °C for at least 2 days. Before the experiments started, wheat kernels were kept for a week in incubators set at  $27 \pm 1$  °C and  $55 \pm 5\%$  RH to raise the moisture content (m.c.) to near environmental relative humidity. The moisture content of wheat was measured by milling then drying 10 g of wheat in a ventilated oven set at 110 °C. The m.c. of Behrang, Chamran and Verinak was 11.2, 11.5 and 11.6, respectively. Whole plus cracked wheat with the ratio of 9:1 was used for the experiments. Cracked wheat was included to make sure food was accessible for the adults.

### DE formulations

Three commercially available DE formulations were used in the bioassays:

1. DEBBM is a DE developed by Dr. Zlatko Korunic (Diatom Research and Consulting

Inc., Canada). DEBBM is a mixture of 90% DE and 0.05% of the plant extract bitterbarkomycin (BBM) dissolved in a solvent and emulsifier. BBM is a sesquiterpene polyolester, extracted from the roots of the plant *Celastrus angulatus* Maxim (Athanasios and Korunic, 2007).

2. DEA, an abamectin-enhanced DE is a mixture of DE and abamectin (0.25% of active ingredient) dissolved in a solvent and emulsifier (Athanasios and Korunic, 2007).

3. F2 is a mixture of 88.0% DE Protect-It, 0.03% deltamethrin, 0.37% piperonyl butoxide, and 0.95% chlorpyrifos-methyl, plus 10% mineral oil (Arthur, 2004).

### Bioassays

The insecticidal efficacy of three DE formulations, DEBBM, DEA and F2 was assessed on Behrang, Chamran and Verinak against *T. confusum*. Twenty grams of each wheat kernels were poured into glass vials, and treated with 100, 200 and 300 mg/kg of DE formulations. Concentrations were selected from the pre-test. The vials were shaken for 5 min to achieve equal distribution in the entire grain mass. Subsequently, 20 adults of this pest were put into the vials and the vials were covered with muslin cloth for sufficient ventilation. Each treatment was replicated five times and untreated wheat served as the control. The vials were placed in incubators set at  $27 \pm 1$  °C,  $55 \pm 5\%$  RH and continuous darkness. The mortality was counted after 2, 7 and 14 days of exposure. Insects were considered dead when no leg or antenna movements were observed probing with fine brush.

### Data analysis

No mortality was observed in the control group so there was no need to correct the data. Percent mortality was transformed to square root of arcsine to normalize the data, but non-transformed data are presented in tables. The data were analyzed using one way analysis of variance (ANOVA) and Tukey's test was used to determine significant differences between various cultivars exposed to different concentrations and exposure time (SPSS, 2007) at  $P = 0.05$ .

**Results**

The efficacy of DEBBM formulation of diatomaceous earth against *T. confusum* adults is presented in Table 1. The effect of concentrations on three different wheat cultivars after 2, 7 and 14 days of exposure was significant ( $F_{26, 108} = 786.96$ ,  $P < 0.0001$ ). The mortality was about 89, 88 and 98% when the adults were exposed for 7 days to 100 mg/kg of DEBBM in Chamran, Verinak and Behrang, respectively. Results indicated that 200 mg/kg of DEBBM is sufficient enough to cause 100% mortality 7 days after exposure (Table 1).

The effects of DEA formulation on *T. confusum* was significant ( $F_{26, 108} = 463.21$ ,  $P <$

0.0001). The highest mortality level (95%) of insects exposed to DEA after 7 days was recorded in the Behrang cultivar treated with 300 mg/kg DEA. However, after 14 days of exposure to 300 mg/kg DEA, 100% mortality was observed in the adults in both Chamran and Behrang cultivars (Table 2).

In the experiment on F2 formulation, significant differences in *T. confusum* mortality were noted between treatments. Generally after 2 days of exposure, the mortality levels were significantly low. However, after 7 days the mortality exceeded 88% in Behrang which reached 98% after 14 days ( $F_{26, 108} = 343.03$ ,  $P < 0.0001$ ) (Table 3).

**Table 1** The efficacy of DEBBM formulation of diatomaceous earth against *Tribolium confusum* adults.

Wheat cultivar	Exposure time (day)	%mortality ± SE		
		100 (mg/kg)	200 (mg/kg)	300 (mg/kg)
Chamran	2	4.0 ± 1.8g	15 ± 1.5de	18 ± 2.5cd
	7	89 ± 1.8b	93 ± 1.2ab	97 ± 1.2a
	14	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
Verinak	2	6.0 ± 2.4fg	1.0 ± 1.5efg	19 ± 1.8cd
	7	88 ± 3.0b	93 ± 1.2ab	97 ± 1.2a
	14	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
Behrang	2	12 ± 1.2def	19 ± 1.8cd	23 ± 2.5c
	7	98 ± 1.2a	100 ± 0.0a	100 ± 0.0a
	14	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a

Means followed by the same letter are not significantly different using Turkey's Test at  $P < 0.05$ .

**Table 2** The efficacy of DEA formulation of diatomaceous earth against *Tribolium confusum* adults.

Wheat cultivar	Exposure time (day)	%mortality ± SE		
		100 (mg/kg)	200 (mg/kg)	300 (mg/kg)
Chamran	2	0.0 ± 0.0l	1.0 ± 1.0kl	9 ± 1.8jkl
	7	55 ± 2.2h	59 ± 3.6gh	84 ± 1.8cd
	14	88 ± 1.2bcd	94 ± 1.8ab	100 ± 0.0a
Verinak	2	0.0 ± 0.0l	3.0 ± 2.0kl	13 ± 3.0j
	7	43 ± 1.2i	61 ± 3.3gh	74 ± 1.8ef
	14	81 ± 1.8de	92 ± 3.3abc	98 ± 1.2a
Behrang	2	0.0 ± 0.0l	3.0 ± 1.2kl	10 ± 1.5jk
	7	67 ± 1.2fg	72 ± 1.2ef	95 ± 1.5ab
	14	96 ± 1.8ab	98 ± 1.2a	100 ± 0.0a

Means followed by the same letter are not significantly different using Turkey's Test at  $P < 0.05$ .

**Table 3** The efficacy of F2 formulation of diatomaceous earth against *Tribolium confusum* adults.

Wheat cultivar	Exposure time (day)	%mortality $\pm$ SE		
		100 (mg/kg)	200 (mg/kg)	300 (mg/kg)
Chamran	2	1.0 $\pm$ 1.0kl	12 $\pm$ 1.2jk	16.0 $\pm$ 1.0j
	7	54 $\pm$ 1.8hi	67 $\pm$ 2.5fg	79 $\pm$ 1.8cde
	14	90 $\pm$ 1.5abc	97 $\pm$ 1.2ab	100 $\pm$ 0.0a
Verinak	2	1.0 $\pm$ 1.0kl	12 $\pm$ 3.0jk	16.0 $\pm$ 2.4j
	7	46 $\pm$ 1.0i	75 $\pm$ 3.1efg	88 $\pm$ 2.5bcd
	14	94 $\pm$ 1.8ab	98 $\pm$ 1.2ab	100 $\pm$ 0.0a
Behrang	2	0.0 $\pm$ 0.0l	11 $\pm$ 3.3jkl	18.0 $\pm$ 3.7j
	7	65 $\pm$ 2.7gh	78 $\pm$ 2.5def	88 $\pm$ 3.3bcd
	14	95 $\pm$ 1.5ab	98 $\pm$ 1.2ab	98 $\pm$ 1.2ab

Means followed by the same letter are not significantly different using Turkey's Test at  $P < 0.05$ .

## Discussion

The mortality increased with increase in concentration level and time exposed to each concentration (Athanasios *et al.*, 2007; Ziaee and Moharramipour, 2012). Based on the current results, DEBBM was more effective than DEA and F2. However, after 14 days exposure to DE formulations their insecticidal effects were nearly the same. Athanasios and Korunic (2007) stated that the insecticidal toxicity of DEBBM-WP and DEBBM-P after 14 days of exposure were more than those of DEA-WP and DEA-P formulations on *S. oryzae*, *R. dominica*, *T. castaneum* and *C. ferrugineus*. While, after 21 days of exposure, these variations were eliminated and in most cases the mortality reached 100%. Therefore, longer exposure time is necessary for diatomaceous earths formulations to show their performance. In addition, enhancement of diatomaceous earth formulations considerably reduced the required application dose and improved DEs insecticidal efficacy. According to our results, by using 100 mg/kg of DEBBM high adult mortality (89-98%) can be obtained after 7 days of exposure. In contrast, for DE formulations such as SilicoSec<sup>®</sup> about 1000 mg/kg is required for sufficient protection of wheat grains against *T. confusum* (Athanasios *et al.*, 2005). SiO<sub>2</sub> is the main constituent of all

DE formulations that causes insect's desiccation and mortality. Although all DEs have similar mode of action, the different additives change their performance. Additives may increase the effect of DEs which should be tested and approved (Athanasios *et al.*, 2007). Thus, enhanced DEs have more insecticidal potential than other DE formulations. The combination of DEs and different compounds with complementary modes of actions could reduce the dose required for complete protection of stored products, costs of protection and also the development of insect resistance (Korunic and Rozman, 2010). Synergistic interaction effect of *Carum copticum* (L.) essential oil has been proved when combined with Iranian DEs against adults of *T. confusum* and *Sitophilus granarius* (L.) (Ziaee *et al.*, 2014). In our study, DEBBM (a mixture of DE and plant extract), DEA (a mixture of DE and abamectin) and F2 (a mixture of DE and insecticides) were applied in the experiments. Therefore, combinations of physical and chemical performances of materials used in the enhanced DEs were effective. Insects desiccate from the DE and become more sensitive to the action of other compounds (Korunic and Rozman, 2010).

Grain type and properties also influenced the insecticidal efficacy of DE formulations (Korunić, 2013). Results indicated that Behrang

was the most tolerant cultivar to *T. confusum* infestations. Behrang is a new durum wheat cultivar grown in Iran and applied in Macaroni industry for pasta products. Durum wheat is harder and has higher protein than bread wheat grains (Smith, 1995). However, Chamran and Verinak cultivars are bread wheat grains. Therefore, increase of *T. confusum* mortality in Behrang may be attributed to rigid structure and high protein content of the durum wheat. Aldryhim (1993) first reported the importance of varieties of wheat on the insecticidal efficacy of silica dust. He noticed that Silica dust toxicity was highest on durum followed by bread wheat. Athanassiou *et al.* (2008) recorded that *S. oryzae* adults were the most tolerant in barley, whereas populations in maize treated with DEBBM were the most susceptible ones. They concluded that the high oil or lipid content of maize kernels might have increased oil absorption by DE particles and thus DEs had become inactivated.

### Conclusion

Our results showed that DEBBM was an effective DE formulation on *T. confusum*. The cultivar of grain, grain hardness, grain moisture content and other physicochemical characteristics will influence the ability of DEs to protect the grains. However, further studies are required to prepare and optimize new DE formulations by mixing them with other reduced risk materials to enhance their efficiency and make them exploitable for commercial use.

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## اثر نوع دانه بر حساسیت حشرات کامل شپشه آرد، *Tribolium confusum* به سه فرمولاسیون خاک دیاتومه

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**چکیده:** اثر حشره‌کشی سه فرمولاسیون خاک دیاتومه DEBBM، DEA و F2 در سه رقم مختلف گندم، چمران، وریناک و بهرنگ، در برابر شپشه آرد، *Tribolium confusum* Jacqueline du Val بررسی شد. خاک‌های دیاتومه در غلظت‌های ۱۰۰، ۲۰۰ و ۳۰۰ میلی‌گرم بر کیلوگرم مورد استفاده قرار گرفتند. زیست‌سنجی روی گندم در دمای  $1 \pm 27$  درجه سلسیوس، رطوبت نسبی  $5 \pm 55$  درصد و تاریکی انجام شد. مرگ‌ومیر حشرات کامل ۲، ۷ و ۱۴ روز پس از قرار گرفتن در معرض خاک دیاتومه شمارش شد. حشرات کامل بیشترین حساسیت را به فرمولاسیون DEBBM خاک دیاتومه و در رقم بهرنگ از خود نشان دادند. به طوری که غلظت ۱۰۰ میلی‌گرم بر کیلوگرم DEBBM پس از ۷ روز باعث ۹۸ درصد مرگ‌ومیر شد که پس از ۱۴ روز به ۱۰۰ درصد رسید. بنابراین، بهرنگ متحمل‌ترین رقم به تهاجمات *T. confusum* بود. علاوه بر این، DEBBM مؤثرتر از دو فرمولاسیون مورد آزمایش بود.

**واژگان کلیدی:** خاک دیاتومه، حفاظت، *Tribolium confusum*، گندم