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Evaluation of three novel diatomaceous earths against three stored-grain beetle species on wheat and maize

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ABSTRACT

The insecticidal effect of three diatomaceous earths (DEs), that contained different active ingredients, was evaluated under laboratory conditions for the control of adults of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). The tested DEs were DEBBM, which is a mixture of two natural compounds: bitterbarkomycin (BBM) and DE, DEA which is a mixture of abamectin and DE and DESgBAIT which is a mixture of DE, silica gel Sipernat 50S and food grade bait. They were tested at 200, 400, 600, 800 and 1000 ppm on wheat, *Triticum durum* Desf., and maize, *Zea mays* L. DEA and DEBBM were generally more effective than DESgBAIT, for all species and commodities. For *R. dominica*, mortality was high and exceeded 90% for both commodities treated with DEA even at 200 ppm, after 14 d of exposure. Similarly, for the same DE, all adults of *S. oryzae* were dead on wheat after 14 d for all doses, but mortality was considerably lower on maize. *T. confusum* was the least susceptible species to all three DEs, as compared with *R. dominica* and *S. oryzae*. For this species, doses >400 ppm were needed to obtain a satisfactory level of control. The results of the present study indicate that the simultaneous use of a low mammalian toxicity active ingredient with DEs are notably effective against major stored-product insect pests.

1. Introduction

Diatomaceous earths (DEs) are very promising alternatives to traditional residual grain protectants. They are of natural origin, non-toxic to mammals and can be applied with approximately the same technology as the residual pesticides (Korunic, 1998; Subramanyam and Roesli, 2000). In addition, DEs can be easily removed from the grain before milling and they do not affect the bread or pasta-making properties of flour and semolina (Korunic et al., 1996; Korunic, 1998). Several DEs are commercially available (Subramanyam and Roesli, 2000; Fields and Korunic, 2000; Arthur, 2000a,b; 2002; Athanassiou et al., 2014), and many studies document that they are very effective against a wide range of stored-product insect species (Fields and Korunic, 2000; Subramanyam and Roesli, 2000; Arthur, 2000a,b; 2002; Arthur and Throne, 2003; Athanassiou et al., 2003, 2005a,b, 2011; 2014). Although many DEs are effective at doses ≥1000 ppm or more (Aldryhim, 1990; Fields and Korunic, 2000; Fields et al., 2003; Arthur, 2003; Athanassiou et al., 2003, 2004, 2005a,b) these doses should be considered as high (Subramanyam and Roesli, 2000; Athanassiou et al., 2013). In this regard, it is essential to evaluate the use of novel DEs, that are effective against insects at lower doses. One of the solutions suggested in this implication is the combined use of DEs with other substances, such as botanicals

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and other insecticides that have low mammalian toxicity, if possible, in one single binary DE (Athanassiou and Korunic, 2007; Athanassiou et al., 2007, 2008, 2009; Vayias and Stephou, 2009).

Although there are reports on the use of enhanced DEs (Wakil et al., 2010, 2011; Riasat et al., 2013), little information is available on the simultaneous evaluation of such DEs as grain protectants on both parental adult mortality and progeny production of major stored-product insects. In the present work, we evaluated the insecticidal effect of three new DEs, that combine DE with low doses of other substances that have insecticidal activity, in an effort to reduce the required dose rates for stored-product insect control. This was done for three of the most important stored-grain species worldwide, two internal feeders, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and one external feeder, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). The influence of the commodity in the insecticidal activity of these DEs and the progeny production of the above species on the substrates treated with the DEs were also evaluated.

2. Materials and methods

2.1. DEs

Three DEs were used in the tests: a) DEA, b) DEBBM, and c) DESgBait. DEA is a mixture of two natural compounds; abamectin (0.25% of active ingredient) (CAS number 71751-41-2) (MSD Agvet Division Merck and Co, Rahway, NJ, USA) and 90% DE and was used as a powder in the tests. Abamectin is a mixture of avermectins (80% avermectin B1a and 20% avermectin B1b), which are produced by the soil bacterium *Streptomyces avermitilis* (ex Burg et al.) Kim and Godfellow (Actinomycetales: Streptomycetaceae). DEBBM is also a mixture of two natural compounds; 0.05% of BBM (bitterbarkomycin) active ingredient and 90% DE and was used as a powder in the tests. BBM is a sesquiterpene polyol ester, extracted from the roots of *Celastrus angulatus* Maxim. (Celastrales: Celastraceae) (Habei Guangshenk (HK) Biotech Limited, Shijiazhuang, Habei, China). DESgBait is a mixture of 85% DE, 12% silica gel Sipernat 50S (particle size is 18 μm; absorption capacity is 290 ml/100 g; specific surface area is 475 m^2/g; pH is 6; CAS number 1343-98-2) (Evonik Degussa Corporation, Parsippany, NJ, USA) and 3% food grade bait (non activated yeast and icing sugar) and used as powder in the tests. For all DEs, a freshwater DE was used. It is white in color, pH is 7.5, moisture is 0.5%, particle size distribution is between 10 and 12.27 μm, specific gravity is 2.1 and surface area is 37.5 m^2/g. It contains 92.4% SiO_2, 1.1% Al_2O_3, 1.6% Fe_2O_3, 0.5% CaO, 0.1% Na_2O and K_2O, <1% crystalline silica.

2.2. Commodities

Untreated, clean and free of infestation and pesticides hard wheat, *Triticum durum* Desf. (var. Mexa) and maize, *Zea mays* L. (var. Dias) were used in the tests. The moisture content of the tested grain commodities was 13.2% as determined by a moisture meter (mini GAC plus, Dickey-John Europe S.A.S., Colombes, France) at the beginning of the tests.

2.3. Insects

The insects used in the tests were reared at the Laboratory of Agricultural Entomology, Benaki Phytopathological Institute, Greece, under continuous darkness. The cultures, initially collected from Greek storage facilities, have been kept at Benaki Phytopathological Institute for more than 10 years. The *R. dominica* and *S. oryzae* individuals were reared on whole hard wheat at 27 °C and 65% relative humidity (RH). The *T. confusum* individuals were reared on wheat flour plus 5% brewers yeast (by weight) at 28 °C and 65% RH. In the experiments, only unsexed adults <2 weeks old were used.

2.4. Bioassays

Exposure studies were carried out in incubators set at 25 °C, 65% RH and continuous darkness. For each DE and grain, five 3 kg lots were prepared, one for each DE application dose. The application doses were 200, 400, 600, 800 and 1000 ppm. The lots were placed in glass jars and, in order to achieve an equal distribution of the DE, the grain was shaken manually for approx. 5 min. An additional series of untreated wheat or maize lots were used as controls. Six wheat or maize samples, of 100 g each, were taken from each lot and placed into six glass vials, of 200 ml capacity each, with a different scoop that was inside each jar. The quantity of 100 g was weighed with a Precisa XB3200D compact balance (Alpha Analytical Instruments, Gerakas, Greece). The closure of the vials had a number of small holes covered with filter paper for ventilation. Then, 50 adults of *R. dominica* were introduced into each vial. The same procedure was repeated for *S. oryzae* and *T. confusum*. The internal "necks" of the vials were covered by Fluon (Northern Products Inc., Woonsocket, USA), to prevent insects from escaping. In the case of *T. confusum*, the lots contained 1% cracked kernels, in order to enhance progeny production. The mortality of each species was measured after 7 and 14 d of exposure on the treated commodity. For each exposure, separate series of vials were used. The death of the exposed adults was determined by prodding each of them with a brush to detect movement under an Olympus stereomicroscope (Olympus SZX9, Bacacos S.A., Athens, Greece). The brush was carefully washed after the examination of each vial. All bioassays were repeated three times, by preparing new lots each time. After the 7 d and 14 d counts, all vials were remained at the same conditions as above. Then, 50 d later for *S. oryzae* and 60 d later for the other two species, the vials were opened and the numbers of progeny were counted. In the cases of *R. dominica* and *S. oryzae*, all progeny individuals recorded were at the adult stage, given that the immature development of these species takes place in the internal part of the kernel (Aitken, 1975). In the case of *T. confusum* larvae and pupae were also found, since this species is a secondary colonizer (Aitken, 1975). Thus, progeny production was expressed as number of individuals per vial. However, the majority of individuals were adults (>85% of the total).

2.5. Data analysis

Control mortality was low (<5%) so no correction was considered necessary (Abbott, 1925). Before the analysis, all data were arcsine transformed to standardize means and normalize variances. For each species, DE, commodity and exposure interval the mortality and progeny data were submitted to a one-way ANOVA, for DE dose, by using the statistical package JMP 10 (SAS Institute Inc., 2012). Progeny production in the control vials were not included in the analysis, since a preliminary ANOVA indicated that significantly more progeny were found in the control vials than in the treated ones ($P < 0.01$). For the comparison of the means the Tukey–Kramer honestly significant difference (HSD) test was used, at 5% significance level (Sokal and Rohlf, 1995).

3. Results

3.1. Mortality caused by DEA

In the case of *R. dominica*, significant differences were noted only in the case of 7 d counts on wheat (Table 1). In this exposure,