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Laboratory evaluation of diatomaceous earth deposits mined from several locations in central and southeastern Europe as potential protectants against coleopteran grain pests

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ABSTRACT

Diatomaceous earth (DE) deposits from regions of central and southeastern Europe were evaluated for their insecticidal efficacy against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) in comparison with the commercially available DE formulation SilicoSec. The effects of temperature, RH, grain commodity (wheat, barley, maize, rice), application method (spraying vs. dusting) were evaluated. FYROM, a DE from the Former Yugoslavian Republic of Macedonia, was the most effective of the DE deposits for grain treatment, whereas the least effective was from Greece (named Crete). However, for surface treatment, Slovenia was the most effective followed by Elassona 1 and Begora. Increase of temperature increased DE efficacy, while the reverse was noted with the increase of RH. Furthermore, the DEs were more effective in barley or wheat than in maize or rice. Neither the mined DEs nor SilicoSec were able to suppress progeny production of the tested species after previous exposure on the treated commodities. Generally, dust application of DEs was more efficacious than spraying against *S. oryzae* and *T. confusum*. However, spraying of wheat significantly reduced the bulk density (test weight) compared to dusting. For surface treatment, after 1 d of exposure, Slovenia was the most effective of the mined DEs followed by Elassona 1 and Begora, whereas after 6 d of exposure the mortality was almost complete (>99%) with all three DEs. More than 6 d of exposure were required for an effective control of *T. confusum* adults with the remainder of the mined DEs.

1. Introduction

Diatomaceous earths (DEs) are composed by the fossil skeletons of phytoplankton, 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 silicon dioxide (Korunic, 1998). They are natural inert dusts that are efficacious when used as insecticides and are considered among the most promising alternatives to the use of traditional pesticides in stored grain (Korunic, 1998; Subramanyam and Roesli, 2000). DEs act on the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss as long as DE particles are trapped and adhered to insects' body (Ebeling, 1971). They are non-toxic to mammals (rat oral $LD_{50} > 5000$ mg/kg of body weight), leaves no toxic residues on the product and according to the US EPA (Environmental Protection Agency) they are classified in the category of

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GRAS (Generally Recognized As Safe) since they are used as food or feed additives (FDA, 1995). Moreover, they are used in filters, fillers, detergents and deodorizers (Korunic, 1998). DEs can be applied with the same application technology as traditional grain protectants, which means that no specialized equipment is required (Athanassiou et al., 2005a). Moreover, since they are inert materials, no interaction with the environment occurs. DEs persist in the treated substrate, providing long-term protection against stored-products insect pests that is not possible with the use of residual insecticides (Athanassiou et al., 2005b; Vayias et al., 2006b). The DEs mined currently vary remarkably in their insecticidal activity, depending upon the geological and geographical origin as well as certain characteristics, such as SiO_2 content, pH, tapped density and adherence to kernels (Korunic, 1997). Several DEs, based on natural deposits, are now commercially available, and have been proved very effective against stored-grain pests (Subramanyam and Roesli, 2000).

However, the search for newer, naturally occurring DEs that are more effective for insect control is still in progress, especially in areas rich in silicaceous rocks. Korunic (1997, 1998), in an extensive screening of DEs from several parts of the world, found that local DEs from the former Yugoslavia were very effective, and could be used with success against stored-grain pests. Similar results have been reported by Inđić et al. (1999) for certain DEs from the former Yugoslavia. Despite their advantages, the use of DEs in stored-product protection remains rather limited, due to their main drawback: DE application reduces grain bulk density (volume/weight ratio). For a satisfactory level of efficacy, the commercially available DE formulations should be applied at doses between 400 and 1000 ppm (Fields and Korunic, 2000). Many researchers underline the need for using new DEs, which are effective at low dose rates (Arthur, 2003; Athanassiou et al., 2006b, 2007). Some newer DE formulations, combined with low doses of insecticides, have already been evaluated with promising results (Athanassiou and Kavallieratos, 2005; Vayias et al., 2006a, 2006b). For instance, Athanassiou et al. (2006b) noted that two new enhanced DEs were very effective at dose rates as low as 75 ppm.

Considering the increasing global focus on the safety of pesticides on human health and the environment, the development and assessment of suitable replacements for traditional contact insecticides, is of great importance. The development of Integrated Pest Management (IPM) programs based on natural-resources in which new effective DEs are involved, would help the adoption of a judicious control protocol in stored grains. However, the newer DEs besides their effectiveness against stored-product pests, should also be tested as regards their impact on grain properties. In our series of experiments the efficacy of several DEs originating from different geographical locations of central and southeastern Europe was assessed against serious stored-product insect pests such as the primary colonizers: *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and the secondary colonizer, *Tribolium confusum* Jacquelin du Val (Coleoptera: Curculionidae). Furthermore, the progeny production of the above species in substrates treated with the DEs was also evaluated. Factors that affect DE efficacy, such as exposure interval, dose, temperature, RH and grain commodity were also examined. A new alternative application method to dusting was evaluated. The DEs were also assessed for surface treatment.

2. Materials and methods

2.1. Effect of temperature and RH on the efficacy of DEs

Ten DEs, of fresh water origin, which were mined during autumn 2007, originating from Greece (named Crete, Elassona 1, Elassona 2), from Serbia (named Begora, Kolubara, Vranje, Vranje 311207), from Slovenia (named Slovenia), from the Former Yugoslavian Republic of Macedonia (named FYROM), from Germany (named Germany) and one commercially available formulation of fresh water origin, SilicoSec (BioFa GmbH, Munchigen, Germany), were assessed for their effectiveness on hard wheat (var. Mexa), against adults of *S. oryzae*, *R. dominica* and *T. confusum*. The tested wheat was infestation and pesticide free. For all species, unsexed, <21 d old adults were used in the tests. At the beginning of the tests, all DE samples were dried at 40 °C for 24 h to about 6% moisture content (Korunic, 1997). The wheat that was used for experimentation was untreated and had very little dockage (<0.8%). Prior to tests, the grain was kept at 25 °C and 60% RH for 2 weeks to equilibrate with its moisture content. Before experimentation, the moisture content of the grain was determined by a Dickey-John moisture meter (Dickey-John Multigrain CAC II, Dickey-John Co, Lawrence, KS, USA) and ranged between 11.1 and 12.0%. Insects used for the bioassays were obtained from laboratory cultures on hard wheat at 25 °C and 65% RH in the case of *S. oryzae* and *R. dominica* and on hard-wheat flour plus 5% brewers yeast at 27 °C and 65% RH in the case of *T. confusum*.

Eleven 1 kg lots of grain were prepared and each of them was treated with 900 ppm of each of the DE samples. Furthermore, an additional 1 kg lot of grain was left untreated and served as control. The treated wheat lots were shaken manually for approximately 15 min to achieve equal distribution of the DE particles inside the grain mass (Subramanyam and Roesli, 2000). Three 60 g samples were taken from each treated lot as well as from the control and placed separately in 4 glass vials (7 cm in diameter, 12 cm in height). The lid of the vials had a 15 mm hole in the middle, which was covered by gauze, to permit sufficient aeration inside the vial. Subsequently, 30 adults of the tested species were added into separate glass vials and all vials were placed in incubators set at the desired combination of temperature and RH each time. Three temperature levels 20 °C, 25 °C and 30 °C and two RH levels 45% and 65% were tested. Mortality was assessed after 7 d and 14 d of exposure of the individuals on the treated wheat samples or control. The above procedure was carried out separately for each species and was repeated three times, by preparing new lots of wheat each time ($3 \times 3 = 9$ replications per species).

Control mortality was generally low and did not exceed 5% in all of the tested cases. Nevertheless, insect mortality was corrected according to Abbott's formula (Abbott, 1925). Data were analyzed separately for each species according to the Repeated Measures Analysis of SAS (SAS Institute, 2000). The repeated factor was exposure interval, while mortality was the response variable and DE, temperature and RH were the main effects. Also the 2-way associated interactions of the main effects were incorporated in the analysis. To overall assess the efficacy of the tested DEs data were pooled for each exposure interval. Means for mortality counts were separated using Tukey–Kramer (HSD) test at $P = 0.05$ (Sokal and Rohlf, 1995).

2.2. Effectiveness of DEs in different grain commodities

The DEs used in this series of experiments are described in Section 2.1. Before the beginning of experiments all the tested samples were dried out to about 6% moisture content as described in Section 2.1. The DEs were evaluated regarding their effectiveness on four grain commodities against adults of *S. oryzae*, *R. dominica* and *T. confusum* obtained from laboratory cultures as described in Section 2.1. Untreated clean wheat (var. Mexa), maize (var. Dias), barley (var. Persephone) and rice (var. Thaibonnet) were used in the tests. Prior to tests, the grain was kept at 25 °C and 60% RH for 2 weeks to equilibrate with its moisture content. Before the