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Evaluation of three diatomaceous earth and one natural pyrethrum formulations against pupae of *Tribolium confusum* DuVal (Coleoptera: Tenebrionidae) on wheat and flour

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Abstract

Laboratory tests evaluated the effect of three diatomaceous earth (DE) and one natural pyrethrum formulations, against pupae of the confused flour beetle, *Tribolium confusum* DuVal (Coleoptera: Tenebrionidae) on wheat and flour. The formulations were Silicosec®, containing 92% SiO_2, Pyrisec®, which contains Silicosec with 1.2% natural pyrethrum and 3.1% piperonyl butoxide (PBO) and Insecto®, containing 86.7% SiO_2 and 10% food-grade additives. To evaluate the impact of natural pyrethrum alone, PBK® polvere (Copyr S.p.a, Tradate, Italy), containing 0.8% natural pyrethrum and 2.1% PBO was also tested. Pupae were exposed to the above formulations at 25 and 30 °C and 65% rh. The DE formulations were applied at two dose rates 500 and 1000 ppm, and PBK® polvere at 750 and 1500 ppm. Pupae of two ages were examined, 1-d and 5-d old. After 8 d exposure, in the case of 1 d old pupae, and 4 d exposure, in the case of 5 d old pupae, the emergence of *T. confusum* adults was measured. In addition, 7 d after emergence, mortality of the emerged adults was recorded. Temperature had no effect on adult emergence. Silicosec® and Insecto® did not affect emergence, since all pupae on treated commodities successfully emerged into adults. In contrast, the other two formulations significantly decreased adult emergence. Also, with these two formulations, significantly more adults were emerged on flour than on wheat. Furthermore, adult emergence was significantly higher in 5-d old pupae than in 1-d old ones. Mortality of the emerged adults was noted with all formulations tested and ranged between 34% and 100%.

Keywords: Diatomaceous earth; Natural pyrethrum; Piperonyl butoxide; *Tribolium confusum*; Pupae; Emergence

1. Introduction

Nowadays, the most prevailing practice for the protection from arthropods that infest stored products is the use of fumigants (methyl bromide, phosphine) and residual insecticides (chiefly organophosphates and pyrethroids). However, the use and production of methyl bromide has been scheduled to end in Europe within 2005 and it is expected to end worldwide by 2020 (United Nations Environment Programme, 1998). As far as the use of the residual insecticides is concerned, many species have already developed resistance (Zettler, 1991; Arthur and Zettler, 1992; Arthur, 1996; Zettler and Arthur, 1997); thus, higher application dose rates are required for the protection of the stored products. Due to environmental reasons and the consumers' growing demand for residue-free products, research has been led to the development and assessment of alternative protection methods, which should be effective and safe for the environment and public health.

One of the most promising alternative method for control of stored products' pests is the use of diatomaceous earths (DE). DEs are the fossilized remains of phytoplanktons that occurred during the Eocene and Miocene periods (Korunic, 1998). Even though many possible explanations for the mode of insecticidal action of DEs have been proposed by the researchers so far, it is now well documented that the DEs particles are trapped on the insects' body surface inactivating the lipids that exist in the epicuticular

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layer of the cuticula. Therefore, insects die through desiccation due to internal water loss (Korunic, 1998; Subramanyam and Roesli, 2000; Mewis and Ulrichs, 2001). Natural origin, very low mammalian toxicity (Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000) as well as high insecticidal effect of the DEs formulations are factors that make DEs a very promising alternative method for arthropod control in stored products. Furthermore, DEs can be applied on the product with approx. the same technology as residual chemicals, they can be easily removed from the grain before milling, and they do not affect the bread or pasta-making properties of flour (Korunic et al., 1996). On the other hand, the fact that DEs (a) affect the physical properties of stored grains (Korunic, 1998), (b) may cause respiratory problems (silicosis) to the users when applied at high dose rates (Subramanyam and Roesli, 2000) and (c) demonstrate a very low insecticidal effect at high relative humidity levels (Fields and Korunic, 2000; Subramanyam and Roesli, 2000), are some obstacles that limit the use of the DEs formulations, for the time being. Nevertheless, several DE formulations have already been registered, in many countries worldwide, as grain protectants against a wide range of pests infesting bulked grain and inside processing and storage facilities (Korunic, 1998; Subramanyam and Roesli, 2000). Several new formulations of DEs include mixtures of DE with natural origin insecticides (natural pyrethrum) or synthetic ones (synthetic pyrethroids).

The confused flour beetle, *Tribolium confusum* DuVal (Coleoptera: Tenebrionidae) is one of the most serious pests of stored products worldwide. It can feed and develop in an extremely large variety of foodstuffs and it is a highly important pest in cereal products (Daniels, 1956; Howe, 1960; Aitken, 1975; Buchelos and Athanassiou, 1993; Buchelos and Athanassiou, 1998). Although it develops best on broken grain kernels or processed grain products, particularly wheat flour, this species is also able to infest whole kernels (Aitken, 1975). Moreover, this species is now resistant to many residual pesticides and fumigants used for stored-product protection (Zettler, 1991; Arthur and Zettler, 1992; Zettler and Arthur, 1997). Development of the confused flour beetle is possible in a wide range of temperature and humidity values, while the species is very tolerant not only to dry conditions (Howe, 1960) but also to very low temperatures. According to Nagel and Shepard (1934), pupae of the confused flour beetle can survive for 432 h at 7 °C and for 10 h at −6 °C. However, in ambient temperature conditions the duration from pupae to adult is 7 d. The effect of DE formulations against adults and larvae of *T. confusum* has been broadly evaluated by researchers. In particular, this species is considered as one of the most tolerant beetle species in DE (Korunic, 1998; Athanassiou et al., 2004) whilst, larvae of this species are more susceptible to DE than adults (Vayias and Athanassiou, 2004). Although there is adequate information concerning factors that influence the efficacy of several DE formulations against adults and larvae of *T. confusum* (Arthur, 2000a; Mewis and Ulrichs, 2001; Dowdy and Fields, 2002; Vayias and Athanassiou, 2004; Athanassiou et al., 2004) however, the efficacy of DEs against pupae of this species has not been investigated so far.

In the present study, the effectiveness of DE against pupae of *T. confusum* was examined. The influence of the pupal age, source of food and temperature on the exposed pupae of *T. confusum* were also assessed. Moreover, the mortality of emerged adults was also evaluated.

2. Materials and methods

2.1. Formulations and commodities

The DE formulations used in the tests were Silicosec®, Insecto® and Pyrisec®. SilicoSec® (Biofa GmbH, Münsingen, Germany) is a DE formulation of freshwater origin containing 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3, and 1% Na_2O. Insecto® (Insecto Natural Products Inc., Costa Mesa, CA, USA) is a DE formulation of marine origin containing 86.7% SiO_2 and 10% food-grade additives. PyriSec® (Agrinova Gmbh, Obrigheim/Mühleim Germany) contains 1.2% natural pyrethrum (25%), 3.1% piperonyl butoxide (PBO) and 95.7% SilicoSec®. In order to investigate the effect of natural pyrethrum and PBO that PyriSec® contains, we used PBK® polvere (Copyr S.p.a, Tradate, Italy), a dust formulation that contains 0.8% natural pyrethrum (25%), 2.1% PBO and inert ingredients (talc) up to 100%. All formulations were fresh and had been stored at ambient conditions until the beginning of the experiments for approx. 3 weeks. The tested commodities used for bioassays were hard wheat (var Mexa) and flour.

2.2. Products' treatment and bioassays

Each DE formulation was applied at the dose rates of 500 and 1000 ppm. The natural pyrethrum formulation (PBK® polvere) was applied at dose rates 750 and 1500 ppm, in order for the amounts of natural pyrethrum and PBO to be equivalent to the respective ones at 500 and 1000 ppm of Pyrisec®. Sixty 1-d old and 60 5-d old pupae of the confused flour beetle, which were obtained from larvae reared on wheat flour plus 5% brewers yeast at 27 ± 1 °C and 65 ± 5% RH, were separately placed in twelve (six per age) glass cylindrical vials (7 cm in diameter, 12 cm in height) that each contained 60 g of product treated with the above formulations. The closure of the vials had a hole 1.5 cm in diameter, which was also covered with gauze for sufficient aeration. In addition, 60 1-d old pupae and 60 5-d old pupae were separately placed in 60 g pure Silicosec® (six vials per age), 60 g pure Insecto® (six vials per age), 60 g pure Pyrisec® (six vials per age) and 60 g pure PBK® polvere (six vials per age). Moreover, twelve (six per age) vials that contained 60 g untreated hard wheat, twelve (six per age) vials that contained 60 g untreated flour and 12 (six per age) empty vials were used as control. Ten pupae