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Effect of *Paecilomyces fumosoroseus* (Wise) Brown and Smith (Ascomycota: Hypocreales) alone or in combination with diatomaceous earth against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

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Abstract

The insecticidal effect of the entomopathogenic fungus *Paecilomyces fumosoroseus* (Wise) Brown and Smith (Ascomycota: Hypocreales) was evaluated against adults and larvae of the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and larvae of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). The fungus was added in stored wheat at two dose rates, 200 and 400 ppm, at two temperature levels, 20 and 25 °C alone or in combination with the diatomaceous earth formulation SilicoSec®. Mortality of the exposed individuals was measured after 7, 14 and 21 d of exposure. For both *T. confusum* adults and larvae, mortality was higher at 20 than at 25 °C. In the case of *T. confusum* larvae, after 14 d of exposure, mortality on wheat treated with the highest dose of *P. fumosoroseus* with SilicoSec® was significantly higher than that of SilicoSec® or *P. fumosoroseus* alone. At 20 °C larval mortality was 100% after 21 d of exposure in both fungal doses with SilicoSec®. In contrast, mortality of *T. confusum* adults was low and did not exceed 34% in any of the treatments tested. Finally, mortality of *E. kuehniella* larvae did not exceed 56%, while SilicoSec® alone caused higher mortality in comparison with the other treatments.

Keywords: *Paecilomyces fumosoroseus*; Entomopathogenic fungi; Diatomaceous earth; *Tribolium confusum*; *Ephestia kuehniella*

1. Introduction

The confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) are two of the most destructive stored-product insect pests worldwide (Aitken, 1975; Sedlacek et al., 1995). Both species have a broad range of food preferences, where they can cause serious quantitative losses and qualitative degradations, but they are particularly abundant in amylaceous products (Aitken, 1975; Sedlacek et al., 1995). Their control is currently based on residual chemical insecticides and fumigants (Arthur, 1996; Bell, 2000). However, these substances harm both human health and the environment, and this fact makes essential the evaluation of alternative, non-toxic and ecologically compatible, control methods.

The use of entomopathogenic fungi is among the most promising alternatives, since they combine low mammalian toxicity, high effectiveness and natural origin (Moore et al., 2000). Most studies available are mainly on *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hyphomycetes) (Rice and Cogburn, 1999; Moore et al., 2000; Dal-Bello et al., 2001; Lord, 2001, 2005; Akbar et al., 2004; Vassilakos et al., 2006) and secondarily on *Metarhizium anisopliae* (Metschnikoff) Sorokin (Dal-Bello et al., 2001; Batta, 2004, 2005; Michalaki et al., 2006; Kavallieratos et al., 2006). At present, the only species of entomopathogenic fungus

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registered in the EU according to the Plant Protection Products Directive 91/414/EEC is another fungal species, *Paecilomyces fumosoroseus* (Wise) Brown and Smith (Ascomycota: Hypocreales) which has not yet been tested against stored-product insect species, despite the fact that this fungus has been proved effective against other pests, such as termites (Meikle et al., 2005), moth larvae (Altre and Vandenberg, 2001a,b), thrips (Castineiras et al., 1996), aphids (Mesquita et al., 1996), spider mites (Shi and Feng, 2004) and whiteflies (Poprawski and Jones, 2001; James, 2003).

One other promising alternative is the use of diatomaceous earths (DEs), which are the fossilised remains of phytoplanktons (diatoms). Like fungi, they combine low mammalian toxicity, natural origin and high efficacy against a wide range of stored-product insect pests (Korunic, 1998; Subramanyam and Roesli, 2000; Fields and Korunic, 2000; Athanassiou et al., 2003, 2004, 2005; Vayias and Athanassiou, 2004). Several DE formulations are now commercially available and many of them have been evaluated with success against stored-product insects (Subramanyam and Roesli, 2000).

Lord (2001) first reported that the simultaneous presence of DE synergise the insecticidal effect of *B. bassiana* against larvae of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). The idea of synergism between two IPM-compatible control methods is a very promising alternative to traditional pesticides in a stored-product insect control strategy. Hence, these findings initiated a number of studies on the combination of DEs with *B. bassiana* (Akbar et al., 2004; Lord, 2005) as well as *M. anisopliae* (Michalaki et al., 2006; Kavallieratos et al., 2006). In the present work, we evaluated the insecticidal effect of an isolate of *P. fumosoroseus* and of a DE formulation against *T. confusum* and *E. kuehniella*, in stored wheat.

2. Materials and methods

2.1. Insects

The cultures of both insects used in the tests were kept in the Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, since 2001. *Tribolium confusum* was reared in glass jars containing wheat flour plus 5% brewer's yeast (by weight) and *E. kuhniella* was reared in semolina. Both insects were kept at 25 °C and 70% RH. Adults and larvae of *T. confusum* were used in the bioassays, while in the case of *E. kuehniella* only larvae were used. The larvae of both species used were 2nd/3rd instar, while all beetle adults were $\leqslant$2 wk-old.

2.2. Fungus and DE

A Danish isolate of *P. fumosoroseus* (DPIL isolate 531, originally isolated from a German cockroach, *Blattella germanica* (L.) (Blattodea: Blattellidae), deposited as ARSEF 5855, was used. The fungus was cultured on plates with 2% Sabouraud dextrose agar (SDA) for 2 weeks at 25 °C and 75% RH. Harvesting was achieved by vacuuming conidia into glass tubes through a piece of fine-mesh metal placed on air-dried culture plates. The aerated glass tubes were subsequently stored over silica gel at 4 °C until use. The concentration of fungal conidia was determined by diluting dry conidial powder in a 0.02% Tween 80 solution and counting conidia in a haemocytometer. Based on these measurements, in 0.005 g of conidial dust the conidia concentration was 7×10^{10} conidia. The DE formulation used was SilicoSec® (Biofa GmbH, Münsingen, Germany), which is a DE of freshwater origin containing approx. 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and 1% Na_2O, with average particle size between 8 and 12 μm (Athanassiou et al., 2005).

2.3. Commodity

Untreated, clean, infestation-free soft wheat (var. Dion) was used for experimentation. The moisture content of the wheat, as determined by a Dickey–John moisture meter (Dickey–John Multigrain CAC II, Dickey–John Co., USA), was approx. 13.8%.

2.4. Bioassays

The bioassays were carried out at two temperatures (20 and 25 °C) and one RH level (75%). Samples of wheat (15 g each) were treated with two fungal rates 200 and 400 ppm, alone or in combination with 200 ppm of SilicoSec®, or with this rate of SilicoSec® alone. Although SilicoSec® is recommended at 1000 ppm (1 g/kg of grain) this dose dramatically affect the bulk density of grains (Korunic, 1998) and this is why Subramanyam and Roesli (2000) underline the importance of using low-DE dose rates. All samples were placed in glass capped dishes, 9 cm in diameter and 2 cm in height. Thus, there were five fungal/DE combination treatments (the two rates of fungus alone, the two rates of fungus with DE and DE alone). For each case four dishes were prepared. An additional series of dishes containing untreated wheat was served as a control. After the preparation of the dishes, 10 *T. confusum* larvae were introduced into each dish. The dishes were placed in incubators set at the above conditions. The required relative humidity was maintained by using saturated solutions of sodium chloride, as recommended by Greenspan (1977). Mortality was assessed after 7, 14 and 21 d of exposure in the treated and untreated substrate. The same procedure was performed for *T. confusum* adults and *E. kuehniella* larvae. All the experiments were repeated three times. The germination of the fungal conidia applied was checked during the entire experimental period by placing dry conidia on plates with SDA, and adding sterile water. The percent germination was recorded after 24 h and ranged between 91% and 96%. Temperature and humidity were monitored using HOBO data loggers (HOBO H8, Onset Computers, USA).

2.5. Data analysis

Before the analysis all counts were arcsine transformed to normalize the variances and standardize the means.