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Effects of inert dusts applied alone and in combination with sweet orange essential oil against *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and wheat microbial population

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ABSTRACT

The effects of kaolin and diatomaceous earth applied alone and in combinations with sweet orange [*Citrus sinensis* (L.) Osbeck] peel essential oil against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and wheat microbial populations were evaluated. Adult beetles reared on durum wheat (cv Simeto) were used to carry out the tests. Five formulations at five application rates were tested. Both insect mortality and progeny production were affected by the treatment, the application rate and the exposure time. *C. sinensis* essential oil showed a synergistic effect on the mortality of *R. dominica*, if combined with kaolin, and antagonistic effect when admixed with diatomaceous earth. Yeasts and moulds as well as total mesophilic aerobic bacteria growths were reduced by *C. sinensis* essential oil applied alone more than the other dusts and dust-essential oil-based treatments. Kaolin admixed with *C. sinensis* peel essential oil might be a viable alternative to the chemical pesticides commonly used in wheat pest management.

1. Introduction

Common control strategies to manage wheat pests are mainly based on the use of synthetic insecticides and fumigants. Methyl bromide and phosphine have been used for decades to control insect pests on stored food, feedstuff, and other agricultural commodities (Islam et al., 2010). The use of methyl bromide and phosphine is increasingly being restricted due to ozone-depleting properties and the onset of resistance, respectively (Bell and Wilson, 1995; Schlipalius et al., 2002; Lee et al., 2004). In addition, the use of other organophosphorous and pyrethroid-based insecticides caused the development of resistance phenomena (Edde, 2012) making the pests control still more complex. Consumer's demand for pesticide-free food, and the increasing resistance of pests to traditional insecticides, dictate the need to evaluate alternative methods for the control of stored products pests (Athanassiou et al., 2008). For this purpose, many sustainable alternatives have been tested for the control of the stored product pests such as: extreme temperatures, modified atmospheres, botanical extracts, microbial insecticides and dusts (Subramanyam et al., 1994; Banks and Fields, 1995; Athanassiou et al., 2007; Riudavets et al., 2013; Campolo et al., 2013, 2014a). Furthermore, the development, validation and use of forecasting models could be helpful in increasing the performance of treatments, with a lower environmental and economic impact (Menesatti et al., 2013; Campolo et al., 2014b).

Among the non-chemical control methods, diatomaceous earths and kaolin have been traditionally used as stored grain protectants. Diatomaceous earths (DE), which are made of fossilized skeletons of diatoms, are allowed in the control of stored product pests (Subramanyam and Roesli, 2000). Kaolin is also known as grain protectant, although previous studies reported a good efficacy at high application rates ranging from 5 to 10 g kg^{-1} of seeds (Viado and Labadan, 1959; Permual and Le Patourel, 1992). In the developed countries, such amount of dusts is not well accepted, whereas in underdeveloped regions the research aims to replace it with synthetic silica dusts able to work at rates less than 1 g kg^{-1} of grain (Golob, 1997).

Among bio-pesticides, essential oils showed interesting results not only against insect pests, but also in the control of bacterial and fungal pathogens (Romeo et al., 2008; Ali et al., 2012; Divya and Varadaraj, 2012).

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Essential oils are volatile natural compounds, characterized by a strong odor, and synthesized by many species of plants as secondary metabolites. Essential oils act against insects as larvicidal, antifeedant, development preventive, adulticidal, fertility reducer, deterrent of oviposition and repellent (Ibrahim et al., 2001; Werdin-González et al., 2011; Cardiet et al., 2012).

The lesser grain borer (LGB), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a worldwide pest of stored grains which feed on the germ and endosperm of kernels. *R. dominica* develops 4–5 larval stages, and the post-embryonic development varies from 25 to 65 days depending on temperature and RH%. LGB can also feed on a wide range of materials such as legumes, stored pharmaceuticals, leather stuffing, mud plaster, packaging materials made from wood, paper, bound books, and cork (Edde, 2012).

Grain entering in mills and feed mills carries bacteria, fungi and yeasts; the population structure of these microorganisms depends of field climatic conditions and harvesting processes (Magan and Lacey, 1986; Lacey and Magan, 1991). However, wheat and flour are generally considered as microbiologically safe products due to their low water activity. Moulds' growth is commonly prevented by drying the wheat at usually 8–12% moisture which corresponds to water activity between 0.40 and 0.65 (Petersson and Schnürer, 1995; Berghofer et al., 2003). In these conditions, the growth of the unwanted microbial population is strongly limited, although pathogens may survive for an extended period. In addition, dry procedures are expensive, especially in temperate climatic zones, and are affected by imperfect sealing of the silos, daily temperature fluctuation and removal of grain (Petersson and Schnürer, 1995).

Living insects can also play an important role as vectors of food-borne pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and others (reviewed in Blazar et al., 2011).

This study aimed to evaluate the efficacy of diatomaceous earth and kaolin in combination with *Citrus sinensis* (L.) Osbeck essential oil as grain protectants. The bioassays were performed against the internally feeding key pest of grains *R. dominica*. Mortality and progeny production of treated *R. dominica* were evaluated, and the effect of the treatments on the natural wheat microbial population was also assessed.

2. Material and methods

2.1. Chemicals

The formulation of diatomaceous earth used in the trial was Protector® (purchased from Intrachem Bio, Italy) containing 50% of particles smaller than 9.46 μm, and composed by 69.7% of SiO_2, 5.89% of Al_2O_3, 0.414% of CaO and 1.05% of Fe_2O_3 (Baldassari et al., 2004). Kaolin washed powder [$Al_2Si_2O_5(OH)_4$], purchased from Carlo Erba reagents (Milan, Italy), is a white nonabrasive fine-grained aluminum silicate mineral classified within the kaolinite group. Sweet orange peel essential oil (kindly provided by Ortogel; Belpasso, Italy) was extracted with the cold pressing technique from fruits grown in Sicily and harvested from December 2012 to March 2013.

2.2. GC/MS analysis of the essential oil

The sweet orange essential oil was analyzed by GC/MS using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 Network quadrupole mass selective spectrometer. The separation was achieved using a VF-5ms 5% phenyl 95% methylpolysiloxane capillary column (30 m × 0.25 mm, film thickness of 0.25 μm, Varian). The column temperature was held at 60 °C for 3 min, programmed at rate of 2 °C min^{-1} to 200 °C and held for 2 min, then programmed at rate of 20 °C min^{-1} to 250 °C and held for 1 min. The flow rate of He as a carrier gas was 1 mL min^{-1} kept constant for all the chromatographic run. A quantity of 0.2 μL neat oil was injected in split mode with a split ratio of 1:50 at temperature of 250 °C. The ion source temperature was 230 °C with EI of 70 eV and the transfer line was set at 250 °C. The MS scan range was *m*/*z* 40–400. Retention indices (Kovats, 1958) of compounds of the analyzed oil were calculated respect to a set of even numbered co-injected hydrocarbons (C7–C30). The sample components were identified by matching their mass spectra with those of the library NIST 08, version 2.0 and by comparisons of the Kovats index with those reported in literature.

Table 1
Outline of treatments carried out in the trials. DE = Diatomaceous Earth, K = Kaolin, EO = *Citrus sinensis* essential oil.

Formulation	Application rates (mg × kg^{-1})	Application method
DE	125–250–500–750–1000	Dust
DEEO		
K	250–500–1000–1500–2000	
KEO		
EO	15–30–60–90–120	Emulsion

2.3. Insects, commodities and equipment

The adult specimens of *R. dominica* used in the experiments came from a local mill and were reared for several generations. To obtain adults of the same age, about 500 adults were placed inside 5 L glass containers, each provided with 500 g of non-infested durum wheat (var. Simeto); after 7 days the specimens were removed and the newly emerged adults (1–15 days old) were employed for the trials. Insects were reared at 27 ± 1 °C in a 1400 CFU climatic chamber (Cavallo; Milan, Italy).

Infestation-free wheat with a 12.05 ± 0.05% moisture and <1% of damaged kernels was used for bioassays. The moisture content of wheat was determined by pre-weighing 5 g and heating it at 130 °C in a ventilated oven until reaching of constant weight.

2.4. Treatments

Five treatments (Table 1) [diatomaceous earth (DE), Kaolin (K), *C. sinensis* essential oil (EO), diatomaceous earth +EO (DEEO) and Kaolin +EO (KEO)] at five application rates and two untreated controls (with and without insects) were established, with each treatment replicated four times. The DE were tested using 125, 250, 500, 750 and 1000 mg kg^{-1}, while kaolin was assessed at 250, 500, 1000, 1500 and 2000 mg kg^{-1}.

The DEEO and KEO formulations were obtained by mixing 15, 30, 60, 90 and 120 mg kg^{-1} of essential oil with diatomaceous earth and kaolin at the corresponding application rates of DE and K treatments, respectively. In order to obtain a uniform distribution of EO in KEO and DEEO formulations, 5 mL of acetone were added, and the mixture was shaken with a vortex mixer and then the solvent was left to evaporate under a fume hood. The DEEO and KEO formulations were used at the same application rates of DE and K, respectively.

The EO treatments, with concentrations of 15, 30, 60, 90 and 120 mg kg^{-1}, were dispensed by spraying 1 mL of a water emulsion, containing 0.1% of Tween® 80 (Sigma-Aldrich, Milan, Italy) as emulsifier. To obtain the uniformity of the treatments, the emulsions of essential oil were sprayed on a single layer of wheat seeds using a computer controlled spraying apparatus (Burkard, UK).

2.5. Insecticidal activity and effects on the progeny

Trials were carried out at 30 ± 0.5 °C and 70 ± 5% RH. Fifty grams of treated wheat were placed into 300 mL jars sealed with waxed