

## Liquid formulations of monoterpenoids as structural treatments for store rooms

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### Abstract

In the search for natural insecticides based on essential oils, we tested liquid formulations (emulsions) of the monoterpenoids linalool, carvone and anethole (at 2.38% Active Ingredient (a.i.) against *Ephestia kueniella* Zeller (Lepidoptera: Pyralidae), one of the main pests in mills and other food facilities. We also tested the side effects of these compounds on *Venturia canescens*, an important parasitoid of this moth. Laboratory tests were performed by spraying these products in Petri dishes and on the walls of methacrylate cages (30x30x40 cm<sup>3</sup>) and evaluating the mortality of released adults after one day (moth) or one hour (parasitoid) of exposure. Food (a fine layer of flour or a honey solution) and water was added to keep adults alive during testing. The application strategy influenced the efficacy, because 10 µl/ Petri dish painted on the product had no effect on the pest, but spraying it on the walls of the Petri dishes or cages gave a range of mortality from 5–100% depending on the compound considered: carvone or anethole were more toxic (100% mortality) than linalool (5% mortality). When applications were done one week before releasing the insects, mortality decreased but surviving females showed reduced or suppressed fertility. The mortality of *V. canescens* was evaluated one hour after contact exposure with the formulated product. Of the three compounds tested, linalool had the lower toxicity (5% mortality after 24 hour exposure to one-week-old residue) to the parasitoid. Several application strategies can be envisioned to target the pest and minimize the effect on the beneficial insect, such as spot treatments, a delay between spraying and parasitoid release or selecting different products and rates.

Keywords: *Ephestia kuehniella*, *Venturia canescens*, carvone, linalool, anethole, emulsion

### 1. Introduction

The treatment of store room walls, ceiling, floor and other structures is necessary as part of the good hygienic procedures recommended to reduce insect pests. Structural pests hide in the floor, corners, cracks and crevices inside machinery and are always difficult to control. Fumigants (phosphine and sulfur dioxide), dusting or spraying with contact insecticides (organophosphates such as pirimiphos methyl and clorpirphos methyl, pyrethroids like deltamethrin, natural pyrethrins or spinosad) are currently used (Kljajic et al., 2006; Maier et al., 2006; Locatelli et al., 2008; Chloridis et al., 2010). Other control methods used are heat treatments (arthropods have to be exposed to 50–60°C for at least 1 hour), ozonation (Isikber and Oztekin, 2011), mass-trapping (Trematerra and Gentile, 2010) or application of the entomopathogenic fungi *Beauveria bassiana* (Cox, 2002). With the continuous reduction in availability of active ingredients, it is easier for pests to develop resistance to the few insecticide products remaining, and therefore it is relevant to study the potential of alternatives. The use of natural products such as essential oils is one possible alternative when applied on its own or in combination with other methods.

The Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is one of the major pests in cereal stores and food processing industries in Spain. Belda and Riudavets (2013) recorded its presence from March to December with peak populations in summer (July to September). The same authors reported the presence of *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae), a solitary larval endoparasitoid of *E. kuehniella* and other Lepidoptera, mainly from August to October.

According to Navarro et al. (2008), essential oils (EO) may be applied in empty stores where the scent of the volatiles will not present a restriction on treatment, because the absence of the stored commodity will avoid sorption phenomena. In laboratory bioassays the volatiles of caraway (carvone and limonene) proved toxic to *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) and to *Sitophilus granarius* L. (Coleoptera: Curculionidae), while coriander (linalool) was only toxic to *C. maculatus* (Pascual-Villalobos 2002). In further experiments high mortality in *S. granarius* adults was obtained when in direct contact with carvone, linalool or anethole (Pascual-Villalobos et al., 2004; Lopez et al., 2008). Majoram (*Origanum majorana* L.) and lemon EO were shown to be effective against *E. kuehniella* (Karabörkücü et al., 2011), linalool being the major component of majoram EO. In addition, the use of EO is compatible with other methods such as low pressure (Mbata et al., 2012) or modified atmospheres (Pons et al., 2011). However, the side effects of EO on natural enemies have to be considered. Carvone at LC<sub>95</sub> for *Sitophilus oryzae* L. did not kill the parasitoids *Lariophagus distinguendus* Fösster (Hymenoptera: Pteromalidae) or *Anisopteromalus calandrae* Howard (Hymenoptera: Pteromalidae), while linalool was lethal either to the weevil or to the parasitoids (Pons et al., 2011). Tunku et al. (2011), using a Y-tube olfactometer bioassay, found that limonene was repellent to *V. canescens* adults and argued that parasitism of *E. kuehniella* could be reduced if limonene-based insecticides were applied. Encapsulation provides persistence and protection of the active volatile from oxidation or degradation by light or high temperatures. Solid formulations of linalool with the use of glycerol increased the viscosity and improved the stability of the emulsion in beads, giving a better-controlled release of the volatile (Lopez et al., 2012). However, for practical application in structural treatments, liquid formulations are more convenient.

The objective of our work was to study the insecticidal effects of residues of three monoterpenoids applied as liquid formulations to Petri dishes and cages in laboratory assays against *E. kuehniella* and its parasitoid *V. canescens*.

## 2. Materials and Methods

### 2.1. Insects

*E. kuehniella* was reared on a diet of wheat flour and yeast (100:5) under controlled conditions of 24°C, 75% RH and a 16:8 h (light:dark) photoperiod. Adults less than 3 days old were used for the experiments. *V. canescens* was reared on fourth instar larvae of *E. kuehniella* as hosts. Emerged adults of *Venturia* less than 3 days old were used for the experiments.

### 2.2. Liquid formulations

*E*-anethole, *D*-carvone and *R*-(-)-linalool were purchased from Acros Organics. For each product, emulsions were prepared in distilled water containing 2.38% of the active ingredient (a.i.), 2.38% glycerol and 0.47% Tween 80. A control treatment was prepared with the same composition but without the a.i. The liquid formulations were shaken just before the application in the bioassays to assure proper mixing of their components.

### 2.3. Bioassays

*Petri dishes*- Glass dishes 5 cm in diameter were used. The products were applied by painting 10 µl on each side with a paintbrush or with a hand sprayer at 3 bar working pressure; dishes were allowed to dry for 30 min. Ten insects were released immediately or one day later. Insect mortality (%) was recorded after one day of contact with the residues. Two replicates were done for each product and type of application and for each insect species. *Venturia* was provided with a stripe of honey on a filter paper and a 10% sucrose solution to provide moisture.

*Methacrylate Cages*- Small cages (30x30x40 cm<sup>2</sup>) were used as “small store rooms”. The inside was sprayed with a hand sprayer at 3 bar pressure. Ten insects were released immediately either after the product was dry or one week later. Insect mortality (%) was recorded after one day and after six days exposure to the residues. For *E. kuehniella*, two containers containing 3–5 g of diet were provided inside the cages to allow egg laying by the surviving adults, and progeny development was monitored after incubation (at 24°C and 75% RH) during 2 months; the presence of larvae and emerged adults was recorded. For *V. canescens* only adult mortality was recorded. Two replicates were done for each product and each insect species.

## 3. Results

The liquid formulations were stable: the emulsions had a milky appearance with good mixing of their components after shaking.

### 3.1. Effects on *Ephestia kuehniella*

Painting Petri dishes with 10 µl of any of the products tested was not toxic for *E. kuehniella*. However, spraying (that implies a higher amount) had contact insecticidal effects on *E. kuehniella*, causing 70–100% mortality (Figure 1). There was still a residual effect of linalool and anethole on insects released one day after the treatments, with the mortality reduced to 40–50%.

The results with the methacrylate cages are shown in Figure 2, indicating that two monoterpenoids were clearly detrimental to *E. kuehniella*. Fresh residues of carvone and anethol caused total mortality of *E. kuehniella* adults when exposed for either one or six days, and prevented further egg laying (Figure 2 top). In contrast, fresh linalool residue was innocuous when adults were exposed for one day (just 5% mortality), but toxicity increased significantly, to 50%, after 6 days of exposure. Furthermore, surviving adults produced few or no progeny (Table 1). Dry (one week) residues of carvone, anethol or linalool were innocuous when *E. kuehniella* adults were exposed for one day, but, after six days of exposure, 50–70% toxicity was registered for anethol and carvone (Figure 2 bottom).

If biological control is to be applied simultaneously with the application of EO, it is important not to eradicate the pest but leave a small remaining population on which the parasite can reproduce.

**Table 1** Effect of exposing *Ephestia kuehniella* females to EO residues on their fertility after a 2.38 % a.i. formulation was sprayed on cage walls.

Residue	Release of insects	Presence of insects in diet after incubation	
		Larvae	Emerged adults
Linalool	after treatment	Yes	Yes
Linalool	one week after treatment	Yes	Yes
Carvone	after treatment	No	No
Carvone	one week after treatment	No	No
Anethole	after treatment	No	No
Anethole	one week after treatment	No	No
Control	after treatment	Yes	Yes
Control	one week after treatment	Yes	Yes

### 3.2. Effects on *Venturia canescens*

Painting Petri dishes with 10 µl of any of the products tested was harmless for *V. canescens* (0% mortality at 24 h). However, 100% mortality at 24 h was obtained with the three monoterpenoids (Figure 3) after spraying the dishes, with faster effects of carvone and anethole noted just one hour after spraying.

Figure 4 presents the results obtained in methacrylate cages: clear toxic effects of carvone and anethole can be seen. Fresh residues of carvone and anethole caused total mortality of *V. canescens* females when exposed for either one or six days. Fresh residues of linalool were innocuous when females were exposed for a single day (just 5% mortality) but after six days of exposure toxicity was total (Figure 4 top). It is worth noting that mortality in the control treatment was 55% after six days of simple confinement of the parasitoids. With dry (one week) residues, a major reduction in *V. canescens* mortality (0–20% at 24 h) was obtained (Figure 4 lower); when the parasitoid was exposed for six days, total mortality was only observed with carvone, since linalool and anethole were no more toxic than the control treatment.

## 4. Discussion and conclusions

It is feasible to develop monoterpenoid-based liquid formulations for the purpose of reducing *Ephestia* populations, but these compounds are also quite toxic for *Venturia*. Previous studies (Elliot et al., 1983) showed selectivity among pyrethroids against *E. kuehniella*, with some compounds favouring the survival of the parasitoid whilst being very effective against the pest, and others killing both. In addition to further study of the selectivity of monoterpenoids in different types of settings more similar to those occurring in storehouses, we are planning to study the repellent effects on *V. canescens*, because this could serve as a protection (avoidance) mechanism in insecticidal spot applications directed against the pest population.

Carvone and anethole seem more appropriate for spot or structural treatments against *Ephestia* in periods when *Venturia* does not occur naturally (according to Belda et al., 2013, from February to April). A delay in parasitoid release of at least one week is recommended after spraying. Linalool treatment would be recommended for reducing populations of *Ephestia* while not completely eliminating them, because its effect on the natural enemy is likely to be slow whilst allowing *Venturia* to parasitize the larvae.

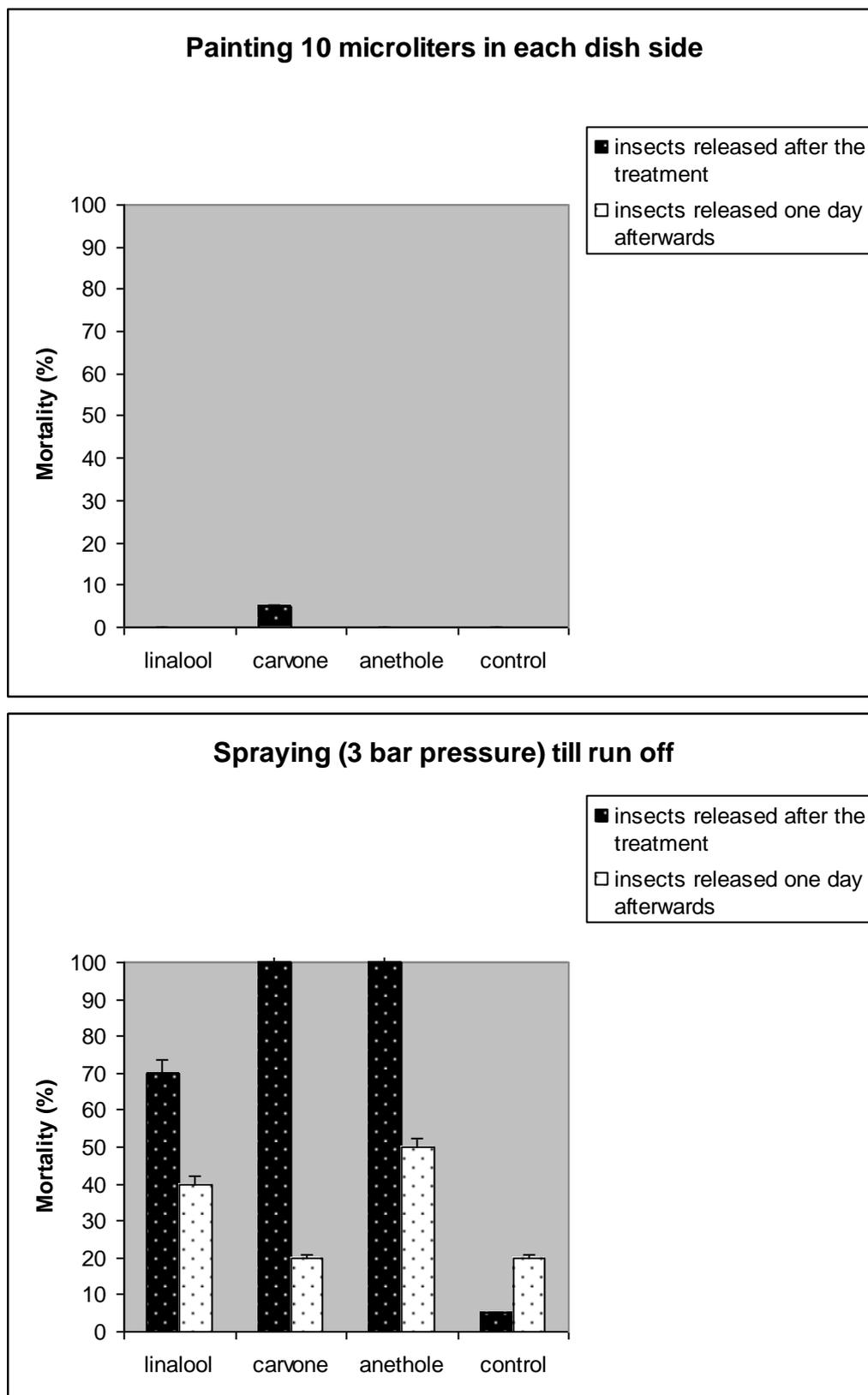
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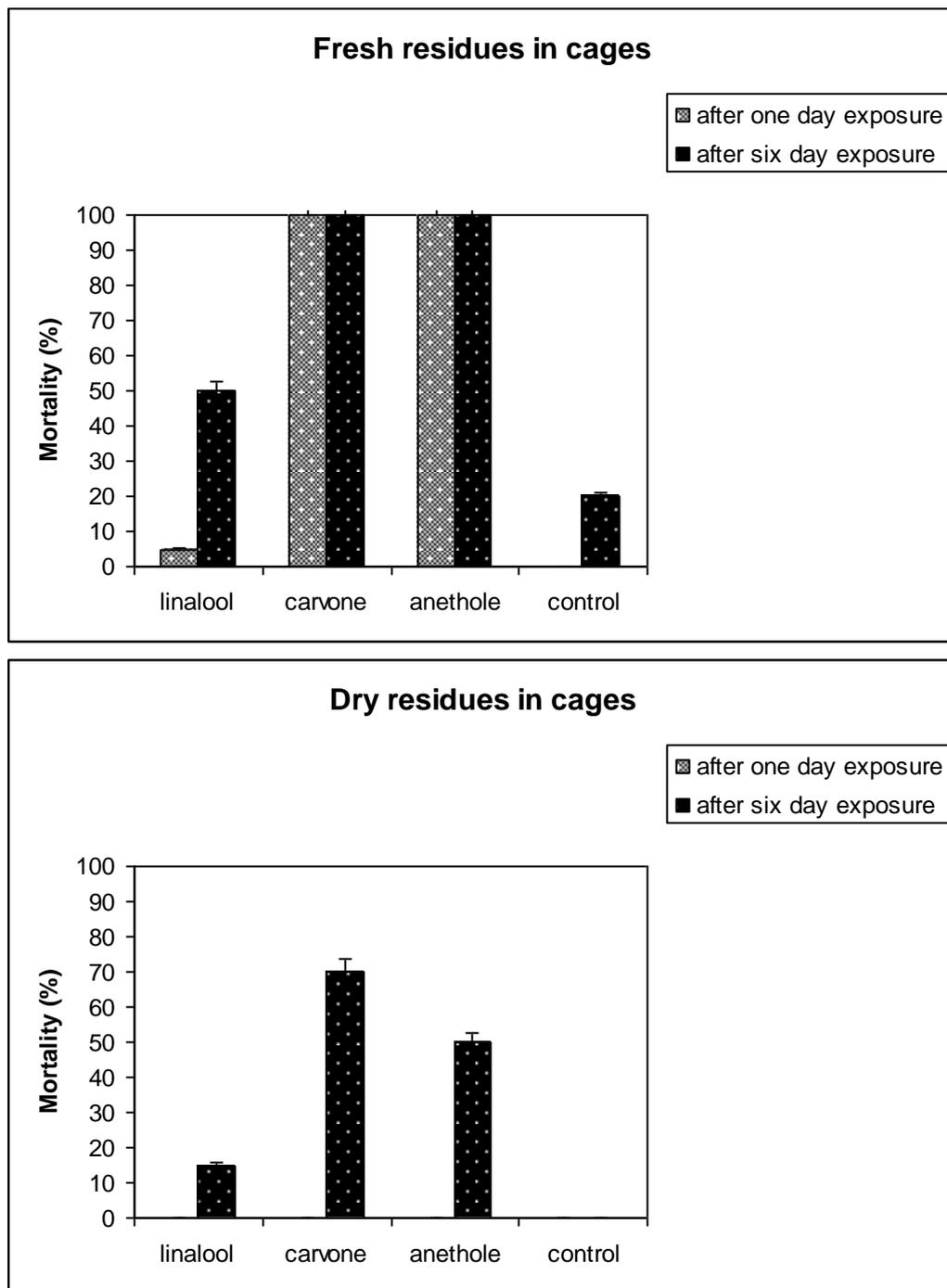
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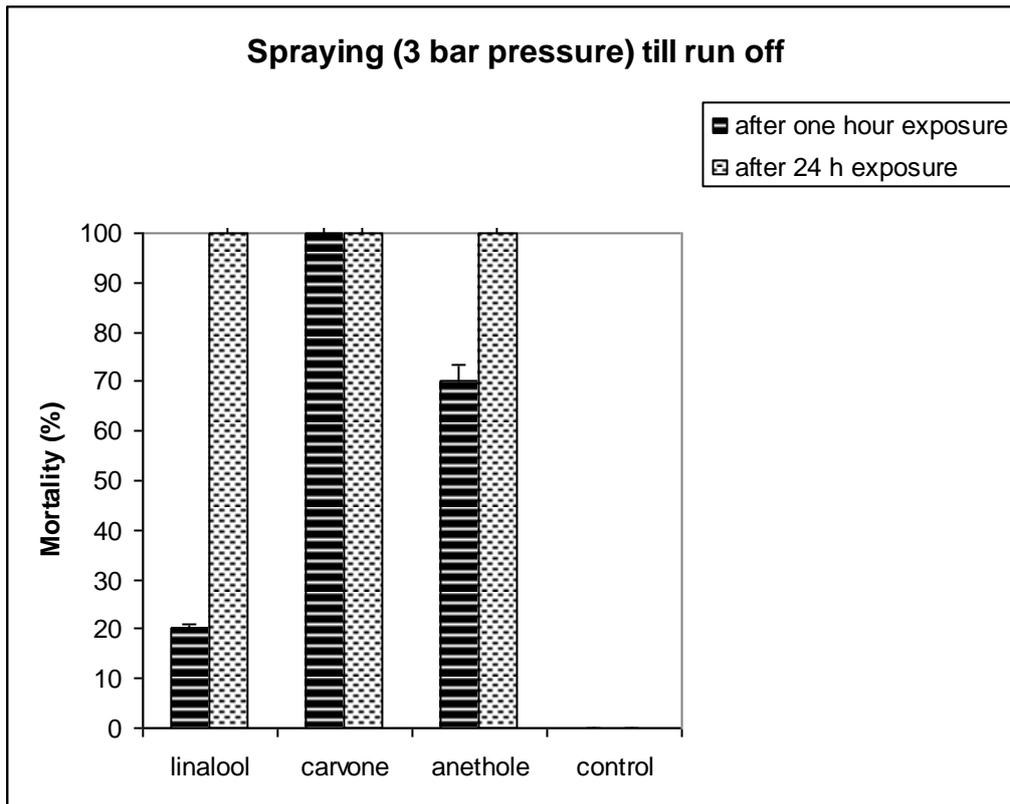
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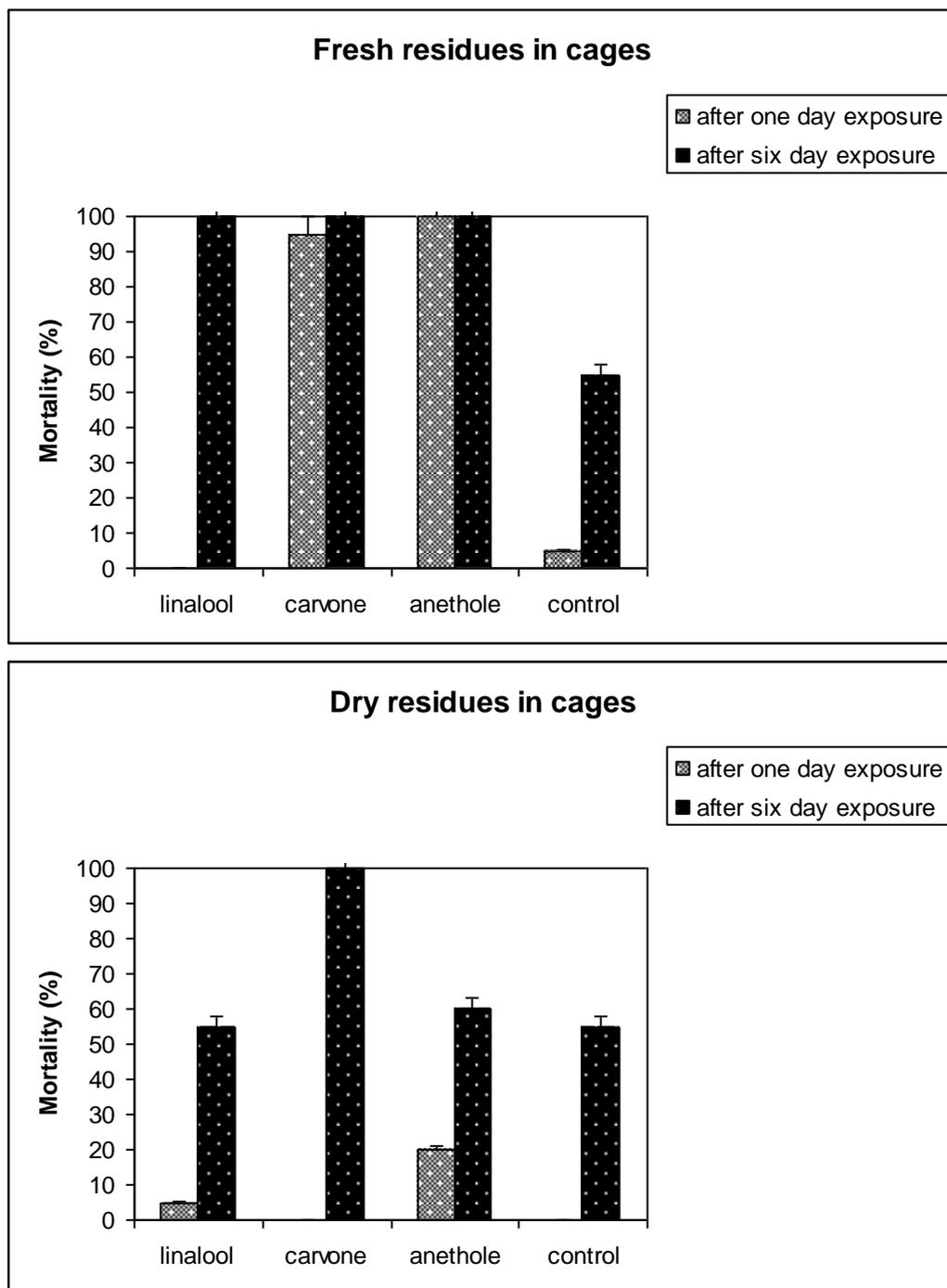
**Figure 1** Pest (*Ephesia kuehniella* adults) mortality after one day of exposure to residues of the different liquid formulations (2.38% a.i.) in Petri dishes.



**Figure 2** Pest (*Ephestia kuehniella* adults) mortality after exposure to fresh or dry (one week) residues of each liquid formulation (2.38% a.i.) in methacrylate cages (30x30x40 cm<sup>3</sup>).



**Figure 3** Parasitoid (*Venturia canescens* adults) mortality after exposure to residues of each liquid formulation (2.38% a.i.) in Petri dishes (painting 10  $\mu$ l/side gave 0% mortality in all treatments and control).



**Figure 4** Parasitoid (*Venturia canescens* adults) mortality after exposure to fresh or dry (one week) residues of each liquid formulation (2.38% a.i.) in methacrylate cages (30x30x40 cm<sup>3</sup>).