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Laboratory evaluation of efficacy of several formulations to control the lesser mealworm - *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae)

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ABSTRACT

The lesser mealworm *Alphitobius diaperinus* (Panzer, 1797.) (Coleoptera: Tenebrionidae) is a cosmopolitan and the most common pest in the global commercial poultry industry and is present in a growing number of poultry facilities in Croatia. This study presents the results of laboratory research into the sensitivity of the adult population of *A. diaperinus* collected in poultry facilities in the area of Međimurje County (Croatia) to the following insecticide formulations: Solfac 050 EW containing 50 g/L of cyfluthrin active ingredient (a.i.), naturalit Laser SC (a.i. spinosad 240 g/L) and essential oil cineole (eucalyptol) over 10 days. The population of *A. diaperinus* collected in poultry facilities in the area of Međimurje County has never been exposed to insecticides. The tested insects showed a very high sensitivity to the insecticide Solfac 050 EW at a dose of 2×10^{-2} g/m^2 and naturalit Laser SC at a dose of 2.4×10^{-3} g/m^2 (100% mortality 24 hours after exposure). Essential oil cineole showed very high efficiency at a dose of 60 µL/350 mL^{-1} vol (174 g/m^3) (100% mortality 24 hours after exposure). These research results could be used as guidelines in creating a management strategy for lesser mealworm in poultry facilities in Croatia.

Key words: *Alphitobius diaperinus,* cyfluthrin, spinosad, eucalyptol, effectiveness

Introduction

The lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) is a major cosmopolitan pest in poultry facilities (PFEIFFER and AXTELL, 1980). *A. diaperinus* was imported into temperate regions from West Africa and introduced into animal production systems by means of contaminated food stuff (SALIN et al., 2000b).

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In Croatia *A. diaperinus* was described by KORUNIĆ (1990) as a storage pest that causes significant damage in poultry facilities, especially in broiler breeding. All development stages of the lesser mealworm live in the litter of poultry houses.

Larvae enter the insulation material of buildings where they develop into a pupal stage (SALIN et al., 2000b).

Lesser mealworm *A. diaperinus* is a potential reservoir for important poultry pathogens, including bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Campylobacter jejuni*), viruses (Newcastle disease, Marek´s disease, Gumboro disease, Turkey coronavirus, Avian influenze) and fungi (*Aspergillus flavus*) (DES LAS CASAS et al., 1972; DES LAS CASAS et al., 1973; McALLISTER et al., 1994; McALLISTER et al., 1995). Lesser mealworm has been involved in transmission of Protozoa (GOODWIN and WALTMAN, 1996), Nematoda (KARAUNAMOORTHY et al., 1994) and Cestoda (ELOWNIE and ELBIHARI, 1979) to chickens. The significant economic damage is the result of the temperature decreasing in poultry facilities caused by larvae and adults of lesser mealworm entering into insulation material (VAUGHAN et al., 1984). Chickens feeding on larvae of the lesser mealworm show poor weight gain and increased mortality (DESPINS and AXTELL, 1995).

Biosecurity programs implemented by poultry companies should include some measures to control *A. diaperinus* in poultry houses (CHERNAKI-LEFFER et al., 2007). Regular cleaning of the facility after emptying litter can greatly reduce the population of the lesser mealworm, especially if it is performed in winter time when most of the insects can be eliminated by exposure to low temperatures (MUSTAČ and MERDIĆ, 2012). Insecticides used for chemical control of *A. diaperinus* in poultry houses include boric acid, carbaryl, iodofenifos, malathion, permethrin, ravap (tetrachlorvinphos + dichlorovs), cyfluthrin and bifenthrin (TOMBERLIN et al., 2008). In Australia spinosad-metabolite of soil bacteria *Saccharopolyspora spinosa* was proposed as a potential insecticide for control of lesser mealworm (LAMBKIN and RICE, 2007). Biological control of *A. diaperinus* include protozoans (APUYA et al., 1994), fungi *Beauveria bassiana* (GEDEN et al., 1998) and nematodes (GEDEN et al., 1985). Plant extracts and neem oil (MARCOMINI et al., 2009) are a potential alternative to control the lesser mealworm.

In the present study, the adults of *A. diaperinus* collected in poultry farming facilities in the area of the Međimurje County (Croatia) were examined for susceptibility to three insecticides: Solfac 050 EW (a.i. cyfluthrin 50g/L), naturalit Laser SC (a.i. spinosad 240 g/L), and essential oil cineole (eucalyptol).

Materials and methods

Tested insects. The population of *A. diaperinus* adults was collected from poultry farming facilities in the area of Međimurje County (Croatia) which has never been exposed to insecticides. To obtain the first generation of insects (F1) for use in bioassays they were reared in the laboratory under controlled conditions: 30 ºC, 70% RH and in darkness on a maize flour. Adults used in the experiments were 2-3 weeks old and included males and females. Fifty (50) individuals were placed into the glass containers in 4 repetitions.

Tested compounds. Adults of *A. diaperinus* were examined for susceptibility to three insecticides: Solfac 050 EW (a.i. cyfluthrin 50 g/L), Bayer, naturalit Laser SC (a.i. spinosad 240 g/L) Chromos - agro and essential oil cineole (eucalyptol) SIGMA®.

Treatments. The compound Solfac 050 was tested in 3 doses EC (0.3, 1 and 2×10^{-2} g/m^2) and naturalit Laser SC was tested in 3 doses (0.8, 2.4 and 4.8×10^{-3} g/m^2). The tested insecticides were applied with a Kartell digital micropipette the surface of the wall and bottom of the glass containers, which were then tightly sealed. The compound Solfac 050 EW was applied in the concentration of 0.8% in doses of 0.1, 0.3 and 0.6 mL/1.2×10^{-2} m^2, naturalit Laser SC in a concentration 0.04% in doses of 0.1, 0.3 and 0.6 mL/1.2×10^{-2} m^2. The compound cineole was tested in 3 doses (10, 60 and 120 μL/350 mL^{-1} vol) and they were applied with a Kartell digital micropipette on to filter paper attached to the lids of the glass containers, which were hermetically sealed for 10 days. Reading the insect daily mortality in samples with cineole was performed without opening the container, viewed using a light-heated surface. The tested insects and control samples were kept in the laboratory under controlled conditions: 30 °C, 70% RH and in darkness. All the samples for determination susceptibility were observed daily over the exposure period of 1-10 days, or until 100% mortality of the test insects, and compared to the untreated control samples.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) for insect's mortality according to the GLM (general linear model). Significant differences were shown by LSD test (least significant difference) and entered in table. Data processing was conducted by SAS/STAT software 9.1.3.

Results

After ten days of the exposure mortality was highly variable across the tested compounds with significant ($P<0.05$) differences (Table 1).

Solfac 050 EW (2×10^{-2} g/m^2) and Laser SC (2.4 and 4.8×10^{-3} g/m^2) had already caused 100% mortality of test insects after 24 hours of the exposure time. Solfac 050 EW in a dose of 0.3×10^{-2} g/m^2 was not effective at killing *A. diaperinus* adults even after ten days of exposure.

Essential oil cineole (60 and 120 μL/350 mL^{-1} vol) caused 100% mortality of test insects after 24 hours of the exposure time. The dose of 10 μL/350 mL^{-1} vol of cineole was not effective and the mortality of the test insects was not significantly different to the results generated in untreated replications.

Table 1. *A. diaperinus* mortality (%) during 10 days of laboratory treatment

Treatment	Dose	Mean percent *Alphytobius diaperinus* mortality ± S.E. after days*									
		1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days
Solfac 050 EW (cyfluthrin)	2×10^{-2}g/m^2	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	1×10^{-2}g/m^2	27.0 ± 8.6^{bc}	92.5 ± 6.8^{a}	96.5 ± 3.5^{a}	98.0 ± 2.0^{a}	99.0 ± 1.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	0.3×10^{-2}g/m^2	2.0 ± 0.8^{d}	3.0 ± 1.2^{d}	9.5 ± 1.7^{cd}	20.0 ± 10.0^{c}	26.0 ± 14.0^{c}	34.0 ± 22.0^{b}	36.5 ± 26.0^{b}	38.5 ± 20.6^{b}	45.0 ± 18.4^{b}	49.5 ± 16.8^{b}
Laser SC (spinosad)	4.8×10^{-2}g/m^2	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	2.4×10^{-2}g/m^2	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	0.8×10^{-2}g/m^2	5.5 ± 1.5^{cd}	11.0 ± 1.7^{c}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Cineole (eucalyptol)	120 µL/350 mL^{-1} vol	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	60 µL/350 mL^{-1} vol	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	10 µL/350 mL^{-1} vol	2.5 ± 0.9^{d}	7.5 ± 2.5^{cd}	15.0 ± 4.4^{c}	15.5 ± 4.1^{c}	15.5 ± 4.1^{c}	15.5 ± 4.1^{c}	17.0 ± 3.0^{c}	20.5 ± 2.5^{c}	23.0 ± 3.0^{c}	23.3 ± 3.3^{c}
Untreated	-	0.0 ± 0.0^{d}	1.0 ± 1.0^{d}	1.0 ± 1.0^{d}	1.0 ± 1.0^{d}	1.0 ± 1.0^{d}	1.5 ± 0.9^{d}	1.5 ± 0.9^{d}	1.5 ± 0.9^{d}	1.5 ± 0.9^{d}	1.5 ± 0.9^{d}

* means followed by the same letters are not significantly ($P>0.05$) different as determined by the LSD-test

A 2×10^{-2} g/m^2 dose of Solfac 050 EW after 24 hours of exposure caused greater mortality with a significant ($P<0.05$) difference to the 1×10^{-2} g/m^2 dose. When the adult population of *A. diaperinus* was exposed for 48 hours, mortality was achieved with no significant ($P>0.05$) difference between the concentrations. A 0.3×10^{-2} g/m^2 dose of Solfac 050 EW was not enough for killing *A. diaperinus* adults in the 10 day period and mortality of the test insects was only 49.5% compared to the control.

The insecticide Laser SC in doses of 2.4 and 4.8×10^{-3} g a. t. per m^2 caused 100% mortality after 24 hours, but the dose of 0.8×10^{-3} g a. t. per m^2 caused 100% mortality after 72 hours.

Discussion

The lesser mealworm, *Alphitobius diaperinus* (Panzer), is the most common pest in poultry production worldwide and is widely distributed in an increasing number in the poultry facilities in Croatia (MUSTAČ et al., 2011). The results from the susceptibility research of the adult population of *A. diaperinus* collected in poultry facilities in the Međimurje County could be used as a guideline for controling *A. diaperinus*.

The methods most often used for controling lesser mealworm in poultry facilities are chemical measures. Chemical control of *A. diaperinus* is generally accomplished by spraying insecticide on the walls and floors, after removal of the litter and before the new breeding cycle (SALIN et al., 2003). In poultry facilities in Texas (USA) the newer generation of pyrethroid insecticides especially cyfluthrin have proved to be very effective in controling *A. diaperinus* (TOMBERLIN et al., 2008). According to the results of our research, in order to achieve 100% mortality of the adult population of *A.diaperinus*, collected from poultry facilities in the Međimurje County and 24 h after exposure, the required dose was 2×10^{-2} g/m^2 Solfac 050 EW (a.i. cyfluthrin 50g/L), while doses of 0.3×10^{-2} g/m^2 were not effective at killing *A. diaperinus* adults after ten days of exposure. Under field conditions, good results are achieved with the application of a combination of cyfluthrin (0.2 g/50 mL/m^2) with insect growth regulator triflumuron 25% (2 g/200 mL/m^2) (SALIN et al., 2003). The advantage of chemical agents is their relatively fast action on insects, but their weakness is their negative effect on the environment and frequent use precipitates the occurence of resistant populations.

In Australia during 2004-2006 strong resistance to cyfluthrin was discovered in many of populations of lesser mealworm (LAMBKIN and RICE, 2006). As a chemical potential control of lesser mealworm in chicken fattening facilities in 2007 natural spinosad was suggested. However**,** due to the level of resistance to spinosad, which is detected in other harmful insects, the use of spinosad was suggested in combination with chemical methods as a part of an integrated prevention program (LAMBKIN and RICE, 2007). The results of our research showed that a dose of 2.4×10^{-3} g a. t per m^2 Laser SC (a.i. spinosad 240 g/L) was enough to cause 100% mortality of the tested population *A.diaperinus*, 24 h after exposure and a dose 0.8×10^{-3} g a. t. per m^2 Laser SC caused 100% mortality of the tested insects after 72 h of exposition.

Plant active components have an insecticidal (contact and fumigant) effect on insects. They act as repellents and have a negative effect on feeding, hatching eggs, growth and reproduction and may cause the appearance of adultoids and deformed units (LIŠKA et al., 2011) which, besides low toxicity and rapid degradation in the environment, gives them an advantage over the use of synthetic insecticide (ROZMAN et al., 2006). Our research results showed that the tested populations of *A. diaperinus* were highly susceptible to cineole and a dose of 60 μL/350 mL^{-1} vol was enough to achieve 100% mortality 24 h after exposure. Botanical insecticides achieve the highest mortality if they were directly applied to adult lesser mealworms, while having less effect if the lesser mealworms are exposed to treated litter, and further research is required related to their application within poultry facilities (MARCOMINI et al., 2009).

The adult population of *A. diaperinus* collected from poultry facilities of Međimurje County (Croatia) showed very high susceptibility to the insecticide Solfac 050 EW in a dose of 2×10^{-2} g/m^2 and naturalit Laser SC in a dose 2.4×10^{-3} g/m^2. Essential oil cineole was effective as a fumigant in a dose of 60 μL/350 mL^{-1} vol and could be part of an integrated prevention program for *A. diaperinus*.

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SAŽETAK

Manji brašnar *Alphitobius diaperinus* (Panzer, 1797.) (Coleoptera: Tenebrionidae) najrasprostranjeniji i najčešći štetnik u svjetskoj komercijalnoj peradarskoj proizvodnji prisutan je u sve većem broju peradarskih objekata u Hrvatskoj. U radu su prikazani rezultati laboratorijskog istraživanja osjetljivosti populacije odraslih insekata *A. diaperinus* prikupljenih u peradarskim objektima na području Međimurske županije sa sljedećim formulacijama insekticida: Solfac 050 EW (a.t. ciflutrin 50 g/L), naturalit Laser SC (a.t. spinosad 240 g/L) i eterično ulje cineol (eukaliptol) tijekom 10 dana. Populacija vrste *A. diaperinus* na području Međimurske županije nikada nije bila obrađena insekticidima. Testirani insekti pokazali su vrlo visoku osjetljivost (100% mortaliteta 24 sata nakon izloženosti) na insekticid Solfac 050 EW u dozi od 2×10^{-2} g/m^2 i naturalit Laser SC u dozi od $2,4\times10^{-3}$ g/m^2. Eterično ulje cineol, kao moguća alternativa za kontrolu manjeg brašnara vrlo je učinkovito u dozi od 60 µL/350 mL^{-1} vol 24 sata nakon izloženosti. Rezultati ovog istraživanja mogli bi poslužiti kao smjernice u izradi strategije suzbijanja manjeg brašnara u Hrvatskoj.

Ključne riječi: *Alphitobius diaperinus,* ciflutrin, spinosad, eukaliptol, učinkovitost