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The Laboratory Efficacy of Cypermethrin Dust Against Lesser Mealworm Larvae and Adults, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)

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Abstract: Cypermethrin dust was evaluated as a tool for the integrated management of lesser mealworms (also called the darkling beetle), *Alphitobius diaperinus* (Panzer). This experiment examined the efficacy of the cypermethrin against adult and late instar lesser mealworms under laboratory conditions. Two bioassay methods were evaluated, using either a petri plate or a covered plastic container simulating poultry house conditions. In the simulated conditions, two different samples were used and cypermethrin was either dusted onto the surface of the container or was directly dusted onto the bottom. The LC_{50} for adults was 636.6 ppm, however, 929.7 ppm of cypermethrin dust was needed to achieve a 50% mortality rate in late instar larvae 24 h after the administration of the insecticide. A similar trend was observed in the simulated poultry houses when the adult mortality was >90% while effectiveness in late instar larvae was decreased, i.e., between 50 and 85%. Significant differences in the toxicity profiles were observed in larvae mortality when cypermethrin it was dusted directly onto the litter surface, compared to the bottom of the container. We have verified that cypermethrin dust is available for use in poultry houses however, toxicity profiles of lesser mealworm may depend on the beetle's stage of development and method of application.

Key words: Lesser mealworm, cypermethrin, avian production, aviary simulation, efficacy, Brazil

INTRODUCTION

The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) is one of the primary pests in the poultry industry (Tomberlin *et al*., 2008). *Alphitobius diaperinus* may cause considerable economic losses if not adequately controlled because it can rapidly reach pest status in a poultry house. When adult and larval beetles are consumed, the feed conversion and weight gain of broilers and turkey poults have been shown to decrease (Despins *et al*., 1994). In addition, the lesser mealworm can cause structural damage when last instar larvae tunnel into insulation and structural materials (Weaver, 1996). It is estimated that these insects were responsible for $4,516,000 worth of poultry production losses and $7,548,000 in control costs in the US State of Georgia alone (Guillebeau *et al*., 2006).

In addition, *A. diaperinus* has long been recognized as a potential mechanical vector of foodborne and animal pathogens such as *Salmonella enteritidis* (Crippen and Sheffield, 2006; Crippen *et al*., 2009; Leffer *et al*., 2010) and *Campylobacter jejuni* (Hazeleger *et al*., 2008). The lesser mealworm is a human allergen and is still considered to be a source for occupational exposure (Schroeckenstein *et al*., 1988). Finally, the attempt to attract lesser mealworms away from poultry houses and towards outdoor locations with lights at night has led to heavy infestations in residential communities and businesses and this may result in lawsuits (Gall, 1980). There are many factors involved in the emergence of *A. diaperinus* in poultry houses. However, the most important factors are higher bird densities, improved ventilation patterns within poultry housing and the fact that all life stages of *A. diaperinus* can live in poultry litter and manure which this insect uses as a food source (Rueda and Axtell, 1997).

Pest management in poultry production relies heavily on the broadcast application of broad-spectrum residual organic insecticides, primarily pyrethroids, usually applied every production cycle. In the United States insecticides

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currently registered for application in poultry facilities include pyrethroids, such as β-cyfluthrin, λ-cyhalothrin, permethrin and bifenthrin (Stringham and Watson, 2009; Hinkle, 2010). The organophosphates used include tetrachlorvinphos, dichlorvos and chlopyrifos as well as carbaryl spinosad, pyriproxyfen and imidacloprid (Tomberlin *et al*., 2008; Lambkin and Furlong, 2011).

The control practices for *A. diaperinus* within broiler industries in Brazil primarily includes the application of contact pyrethroids (cypermethrin and cyfluthrin) or the combination of pyrethroids and organophosphates. In addition, the insect growth regulator triflumuron is also used (Chernaki-Leffer *et al*., 2011). Due to the increased use of organophosphate and pyrethroids insecticides, a loss of field efficacy in insect control has been repeatedly reported in the United States (Steelman, 2008), the United Kingdom (Cogan *et al*., 1996), Australia (Lambkin, 2005; Lambkin and Rice, 2006; Lambkin and Furlong, 2011) and Brazil (Chernaki-Leffer *et al*., 2011).

Spray-formulated insecticides are commonly applied by poultry producers to control the lesser mealworm. However, an alternative and more desirable method of treatment is the use of a dust formulation. Insecticides formulated as dust can be advantageous to poultry house managers because they are easy to handle and apply (Khan *et al*., 1998). Moreover, unlike liquid formulations, dust formulations do not require dilution with water and a power supply for the sprayer and they can be directly mixed to poultry litter.

Thus, the goal of this study was to assess the efficacy of the cypermethrin dust formulation for controlling lesser mealworms.

MATERIALS AND METHODS

Alphitobius diaperinus larvae (≈1.2 cm long) and adults (sex and age unknown) were collected in a naturally infested poultry farm in Louveira (23°6'S, 46°'W, Sao Paulo, Brazil). Insects were submitted to the Research Center for Veterinary Toxicology (CEPTOX), Department of Pathology, Faculty of Veterinary Medicine and Animal Sciences, University of Sao Paulo, Pirassununga, SP, Brazil and reared on chicken food (Broilil, Socil®) at 28°C and 65±5% relative humidity in the dark.

The commercial product evaluated was Vetancid (Cypermethrin 1 kg/300 m^2, 5% cypermethrin, Vetanco, Argentina, AR). About 1 g sterilized wood shaving samples were placed in 3.5 cm diameter petri dishes.

Doses of cypermethrin were determined with preliminary tests and wood shavings were mixed with a dust formulation to obtain at least four concentrations of the insecticide with an expected range of 10-90% mortality of *A. diaperinus*. Eighty adult beetles (adults or larvae) were placed into groups of either 20 larvae or 20 adults per dish. A range of concentrations of cypermethrin was tested against lesser mealworm with four to six concentrations for adults (100-1800 ppm) and four to six concentrations do larvae (300-9000 ppm). Dishes without cypermethrin were used as a control group.

(a)

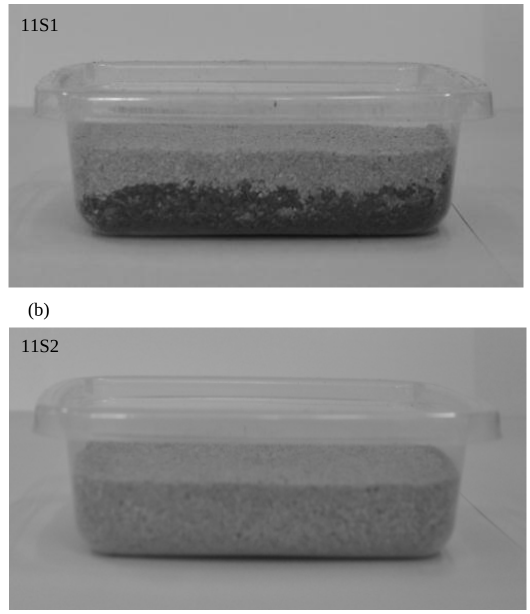

Fig. 1: Plastics containers (ca. 171.96 cm^2) covered with 2 cm layers of different Samples (S); a) S1: Soil floors+wood shavings; b) S2: Wood shavings

All dishes were then placed in incubators set at 28°C and 60±5% RH in the dark. The dishes were evaluated 24 h after the treatment. Adults and larvae were considered dead if they were ataxic. Mortality data were analyzed by probit analysis (Finney, 1971) using the POLO PLUS Software (Robertson *et al*., 2002), to obtain the lethal concentration, the 95% confidence intervals and the slopes of the dose-mortality curves. The criterion for a significant difference was nonoverlapping fiducial limits of the LD_{50} values.

Simulated poultry house bioassay: The efficacy of the cypermethrin dust was evaluated against adults and larvae in plastic containers (ca. 171.96 cm^2) using different habitat-treatment combinations 2 cm deep. Treatment groups consisted of surface treatments with following substrates: S1-soil floors+wood shavings+5 g of chicken feed pellets; S2-wood shavings+5 g of chicken feed pellets (Fig. 1). Soil floors were collected from the poultry house in Louveira, SP. Cypermethrin was applied to the surface of wood shavings or directly to the bottom of the container (except sample containing soil floors). The surface of the wood shavings was treated with cypermethrin 2 h later using 0.0573 g cm^{-2} which corresponds to the dose recommended by the manufacturer. In the control group, cornstarch was used as a placebo in all samples. Insects were placed in groups of either 20 larvae or 20 adults per container and per sample (S) and this was repeated four times (total 200 adults and 200 larvae include control group). Mortality was recorded after 48 h and insects were considered dead if they were ataxic. The method of repeated measures analysis used to simulated poultry house effects was based on the mixed model (PROC MIXED; SAS Institute, 1995) and data were fitted to a model that included the effects of beetle stage (adults or larvae); application (bottom or surface) x beetle stage; soil floors (presence or absence) x beetle stage x application. The model for the design is as follows:

$$y_{ijklm} = +F_i+L(F)_{j(i)}+T(FxL)_{k(ij)}+S(FxLxT))_{l(ijk)}+e_{ijklm}$$

Where:

y_{ijklm}	=	Percent mortality in the transformed scale for the mth replication
lth	=	Soil floors
jth	=	Application
ith	=	Beetle stage
μ	=	The overall mean
F_i	=	Effect of ith beetle stage with i = 1 (larvae) and 2 (adults)
$L (F)_{j(i)}$	=	Effect of jth application level x ith stage level with j = 1 (bottom) and 2 (surface)
$S (FxLxT))_{l(ijk)}$	=	Effect of the lth soil floor level, within the ith-jth-kth combination of the stages and application with 1 = 1 (presence) e 2 (absence)
e_{ijklm}	=	The error term, assumed Normally Identically and Independently Distributed (NIID) 0, $^2{}_e$

Efficacy data were analyzed by a one-way Analysis of Variance (ANOVA) for a randomized complete block design with mean separation determined by the Student's t-test (p = 0.05) (SAS Institute, 1995). To equalize variances, the percent mortality of adults and larvae on poultry litter were transformed using the square root of the arcsin. For presentation, the results were returned to the original scale (Banzatto and Kronka, 2006).

RESULTS AND DISCUSSION

Bioassays in the petri plate: Dose-response assays showed that adult beetles were more susceptible than the larvae. The LC_{50} for adults was 636.6 ppm but 929.7 ppm of the insecticide was needed to achieve a 50% mortality rate in larvae after 24 h (Table 1). Comparison of the LD_{50} values of different isolates did show significant differences among larvae and adults.

Table 1: Predicted LC_{50} and LC_{90} values, expressed as ppm (μg g^{-1}), from dose-mortality bioassays of adults and larvae of *A. diaperinus* populations exposed to cypermethrin dust

Beetle stage	n	Slope±SEM	LC_{50} μg g^{-1} (95% FL)[a]	LC_{90} μg g^{-1} (95% FL)[a]
Adult	462	3.21±0.24	636.6 (568.6-707.0)	1597.4 (1391.3-1897.5)
Larvae	640	1,35±0.09	929.7 (610.2-1456.3)	8265.5 (4414.9-22485.8)

n = total insects treated. SEM = Standard Error of the Mean; LC = Lethal Concentration; FL = Fiducial Limits; [a]Concentration that produces 50% mortality in the population relative to the untreated controls calculated using a generalized linear model

Table 2: Mean mortality (%) ±SEM of *A. diaperinus* adults (n = 80) and larvae (n = 80) 48 h after of exposure to cypermethrin dusted on surface and bottom of wood shavings

Beetle stage	Application	Mortality (%) (±SEM)
Adults	Surface	98.75±3.89[a]
Adults	Bottom	91.25±5.47[a]
Larvae	Surface	85.00±3.87[a]
Larvae	Bottom	50.00±5.47[c]

Means in rows with different lowercase letters indicate significant difference (p = 0.05); SEM = Standard Error of the Mean

Simulated poultry house bioassay: Significant differences were observed when compared to all of the associated interactions. The effect of beetle stage (adults or larvae) (F = 32.68, df = 36, p<0.0001) and application (bottom or surface) x beetle stage (F = 12.10, df = 36, p<0.0001) were all significant. No significant differences were observed when compared to the effects of soil floors (presence or absence) x beetle stage (F = 0.55, df = 36, p = 0.69). No significant differences (p<0.01) were observed in adult beetles when insecticide was dusted onto the surface bottom. On the other hand, with respect to the larvae, the efficacy of cypermethrin was significantly increased when insecticide was dusted onto the surface (Table 2).

While several laboratory tests have been performed to evaluate the susceptibility of *A. diaperinus* to spray-formulated insecticides, few studies have evaluated the efficacy of dust formulations. The efficacy of the organophosphate dust tetrachlorvinphos, against lesser mealworms in commercial broiler chicken barns has been studied (Khan *et al*., 1998). However, the present study is the first laboratory test employing cypermethrin dust anecdotal reports indicate that this compound is the main insecticide formulation used currently for lesser mealworm control in the Brazilian poultry industry.

In general, studies on the topical application of insecticides reveal that adult *A. diaperinus* are more tolerant than late instar larvae. However, irregular responses of the late instar were observed. For example, Vaughan and Turner (1984) reported that adults were more tolerant than larvae to the insecticides permethrin, famphur, tetrachlorvinphos, dimethoate, carbaryl and propoxur. On the other hand in relation to malathion this same study showed the opposite effect (LD_{50} for adults was 13.93 versus 30.97 μg g^{-1} for late instar larvae). Similarly, a study conducted by Kaufman using cyfluthrin against lesser mealworm larvae and adults, demonstrated that the mortality of larvae was considerably lower (87.7%) than adults (100%).

The results show that last instar larvae were more tolerant than adult beetles when treated with cypermethrin dust in the bioassay test using a petri plate and in the simulated poultry house.

Several factors may be involved in the variable response of the late instar larvae and adult beetles. First, according to Vaughan and Turner (1984) the variable response of the late instar larvae may be attributed to behavioral resistance or avoidance to insecticides. Second, a previous study documented that there are significant variations in susceptibility between lesser mealworm strains to insecticides (Hamm *et al*., 2006). Vayias and Athanassiou (2004) verified that the susceptibility variation may be related to insect agility. These researchers related that young larvae are particularly agile when compared to older larvae stages. This facilitates increased contact between the older larvae stages and the insecticide dust particles. Indeed, researchers observed this in the present study, noticing that compared to last instar larvae, adults are more agile.

CONCLUSION

Based on experiments in simulated poultry house conditions, we verified that for the adults tested in several combinations, cypermethrin dust efficacy did not vary significantly. On the other hand, considering the last instar larvae, the efficacy of the cypermethrin dust formulation was highly dependent on the application type (surface and bottom). This result seems to be associated to an interesting behavior of beetles' larvae. Late instar larvae preferred to remain on the wood shavings surfaces when containers remain on plastics. Consequently, it could be hypothesized that the late instars' behavior increases their contact with the cypermethrin dust when it was dusted onto wood shavings.

This finding demonstrates the excellent performance of cypermethrin dust, at the manufacturer's recommended dose, to control of *A. diaperinus* adults. However, toxicity profiles for late instar larvae depend on the type of application.

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REFERENCES

Chernaki-Leffer, A.M., D.R. Sosa-Gomez, L.M. Almeida and I.O.N. Lopes, 2011. Susceptibility of *Alphitobius diaperinus* (Panzer) (Coleoptera, Tenebrionidae) to cypermethrin, dichlorvos and triflumuron in southern Brazil. Rev. Bras. Entomol., 55: 125-128.

Cogan, P., D. Webb and M. Wakefield, 1996. A comparison of four residual insecticides for the control of the lesser mealworm beetle *Alphitobius diaperinus* (Panzer) in Turkey broiler houses in the UK. Int. Pest Control, 38: 52-55.

Crippen, T.L. and C.L. Sheffield, 2006. External surface disinfection of the lesser mealworm beetle, *Alphitobius diaperinus*, (Coleoptera: Tenebrionidae). J. Med. Entomol., 43: 916-923.

Crippen, T.L., C.L. Sheffield, S. Mowery, R.E. Droleskey and J. Esquivel, 2009. The acquisition and internal carriage of *Salmonella* by lesser mealworm beetles, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Vector Borne Zoon. Dis., 9: 65-71.

Despins, J.L., R.C. Axtell, D.V. Rives, J.S. Guy and M.D. Ficken, 1994. Transmission of enteric pathogens of turkeys by darkling beetle larva (*Alphitobius diaperinus*). J. Applied Poult. Res., 3: 61-65.

Finney, D.J., 1971. Probit Analysis. 3rd Edn., Cambridge University Press, Cambridge, UK., pp: 76-80.

Gall, A., 1980. Are lesser mealworms worth the trouble they may cause?. Poult. Digest., 22: 76-77.

Guillebeau, P., N. Hinkle and P. Roberts, 2006. Summary of losses from insect damage and costs of control in Georgia 2004.. University of Ga. CAES, Miscellaneous, pp: 106.

Hamm, R.L., C.A. Kaufman, C.A. Reasor, D.A. Rutz and J.G. Scott, 2006. Resistance to cyfluthrin and tetrachlorvinphos in the lesser mealworm, *Alphitobius diaperinus*, collected from the eastern United States. Pest. Manag. Sci., 62: 673-677.

Hazeleger, W.C., N.M. Bolder, R.R. Beumer and W.F. Jacobs-Reitsma, 2008. Darkling beetles (*Alphitobius diaperinus*) and their larvae as potential vectors for the transfer of *Campylobacter jejuni* and *Salmonella* enterica serovar Paratyphi B Variant Java between successive broiler flocks. Applied Environ. Microbiol., 74: 6887-6891.

Hinkle, N.C., 2010. Georgia pest handbook. Poultry Housing Parasite Control, Litter Beetles.

Khan, B.A., P.A. Day, L.A. Goonewardene, M.J. Zuidhof and G. Hawkins, 1998. Efficacy of tetrachlorvinphos insecticide dust against darkling beetles in commercial broiler chicken barns. Can. J. Anim. Sci., 78: 723-725.

Lambkin, T.A. and M.J. Furlong, 2011. Metabolic mechanisms only partially explain resistance to pyrethroids in Australian broiler house populations of lesser mealworm (Coleoptera: Tenebrionidae). J. Econ. Entomol., 104: 629-635.

Lambkin, T.A. and S. Rice, 2006. Baseline responses of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) to â-cyfluthrin and detection of strong resistance in field populations in eastern Australia. J. Econ. Entomol., 99: 908-913.

Lambkin, T.A., 2005. Baseline responses of adult *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) to fenitrothion and susceptibility status of populations in Queensland and New South Wales, Australia. J. Econ. Entomol., 98: 938-942.

Leffer, A.M., J. Kuttel, L.M. Martins, A.C. Pedroso, C.S. Astolfi-Ferreira, F. Ferreira and A.J. Ferreira, 2010. Vectorial competence of larvae and adults of *Alphitobius diaperinus* in the transmission of *Salmonella* Enteritidis in poultry. Vector Borne Zoon. Dis., 10: 481-487.

Rueda, L.M. and R.C. Axtell, 1997. Arthropods in litter of poultry (broiler chicken and turkey) houses. J. Agric. Entomol., 14: 81-91.

SAS Institute, 1995. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC USA.

Schroeckenstein, D.C., S. Meier-Davis, F.M. Graziano, A. Falomo and R.K. Bush, 1988. Occupational sensitivity to *Alphitobius diaperinus* (Panzer) (lesser mealworm). J. All. Clin. Immunol., 82: 1081-1088.

Steelman, C.D., 2008. Comparative susceptibility of adult and larval lesser mealworms, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), collected from broiler houses in arkansas to selected insecticides. J. Agric. Urban Entomol., 25: 111-125.

Stringham, M. and W. Watson, 2009. News and notes on insect control. http://www.thepoultrysite.com/articles/1289/news-and-notes-on-insect-control

Tomberlin, J.K., D. Richman and H.M. Myers, 2008.. Susceptibility of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) from broiler facilities in Texas to four insecticides. J. Econ. Entomol., 101: 480-483.

Vaughan, J.A., E.C. Jr. Turner, 1984. Residual and topical toxicity of various insecticides to the lesser mealworm (Coleoptera: Tenebrionidae). J. Econ. Entomol., 77: 216-220.

Vayias, B.J. and C.G. Athanassiou, 2004. Factors affecting the insecticidal efficacy of the diatomaceous earth formulation SilicoSec7 against adults and larvae of the confused flour beetle, *Tribolium confusum* Du Val. (Coleoptera: Tenebrionidae). Crop Prot., 23: 565-573.

Weaver, J.E., 1996. The lesser mealworm, *Alphitobius diaperinus*: field trials for control in a broiler house with insect growth regulators and pyrethroids. J. Agric. Entomol., 13: 93-97.