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Liquid preparation of diatomaceous earth against the lesser mealworm, *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae)

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ABSTRACT
The lesser mealworm (LMW) is the most important pest in poultry production. Insects are associated to avian pathogens, mainly *Salmonella*. Its control is based on chemical insecticide, with limited efficacy in population reduction. Also, insect populations are resistant to the main active ingredients used. Diatomaceous earth (DE) is a mineral dust that has activity against the LMW when used in dust application. No information is available about DE liquid preparation against this insect. Thus, bioassays were conducted aiming to develop a strategy for DE liquid preparation to this insect control. In laboratory the ideal concentration for poultry house experiment was estimated and the effect of insect contact with poultry litter or chicken feed after application was checked. In the poultry house, DE liquid preparation (10% in water, 1 $L \cdot m^{-2}$) was applied on the dirt soil of a cleaned and empty poultry house. In the control poultry house, a chemical insecticide was used (cypermethrin 15 g + chlorpyriphos 25 g + piperonyl butoxide 15 g) (1 $L \cdot m^{-2}$). DE at all concentrations was efficient mainly when insects were dried for 24 h before contact with a substrate. In poultry house, both treatments obtained similar results at 15 days after treatment (94% of reduction of insect population). At 45 days after treatment, the insect population with DE treatment increased 39% while the insect population remained 17% smaller than the initial population with a chemical insecticide. DE liquid preparation has potential to be used as a safe treatment in LMW population management, as a shock treatment.

Keywords: animal production; inert dust; alternative control; physical control; insect pest.

INTRODUCTION

Alphitobius diaperinus (Panzer, 1797) (Coleoptera: Tenebrionidae) is the most important pest in poultry production worldwide. Larvae and adults are abundant, living in the litter, soil, walls, feeders, curtain (ARENDS et al., 1987; SALIN et al., 2000; UEMURA et al., 2008). This pest plays an important role in the epidemiological chain of poultry pathogens as does *Salmonella* spp., *Clostridium* spp., *Escherichia coli*, virus, fungi, and helminths parasites (CHERNAKI-LEFFER et al., 2002, 2010; VITTORI et al., 2007; HAZELEGER et al., 2008; CRIPPEN et al., 2012, 2018). Furthermore, larvae and adults are consumed as an alternative food, causing nutritional imbalance, influencing the development and weight gain of the birds, and damaging the digestive tract (crop and gizzard) (DESPINS; AXTEL, 1994; 1995; JAPP, 2008).

Chemical insecticides are intensively used to control the lesser mealworm (LMW), mainly cypermethrin. However, their efficacy to suppress LMW outbreaks is limited. The initial impact of the insecticides is followed by the restoration of the population (UEMURA et al., 2008; SANTOS et al., 2009). Some insecticides also affect the health of the birds and humans and are toxic for nontarget animals (GARG et al., 2004).

Another problem is the development of LMW populations resistant to insecticides. Some resistance to insecticides has been founded since 1990s, at United Kingdom, Australia, and USA. In Brazil, the existence of the LMW population resistant to cypermethrin and chlorpyriphos (which have been used for a long time in poultry house) was recently

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proved (COGAN et al., 1996; CHERNAKI-LEFFER et al., 2011; LAMBKIN; FURLONG, 2011; SINGH; JOHNSON 2015; HICKMANN et al., 2018).

Ecofriendly tactics have been sought, including botanical insecticides, biocontrol with nematodes and entomopathogenic fungi, heat, alcoholic solution, gas (ozone and ammonium), and diatomaceous earth (DE) (ALVES et al., 2006, 2012, 2015; FOGAÇA et al., 2017; OLIVEIRA et al., 2017; GEHRING et al., 2019).

DE is a mineral compound, from fossilized algae, composed of amorphous silicon dioxide. The hygroscopicity, abrasiveness, and adsorption of the particles on insect exoskeleton remove the epicuticular wax leading to insect death by dehydration (EBELING, 1971; SUBRAMANYAM; ROESLI, 2000). It is a natural product, which is safe for birds and humans (BERTKE, 1964; OMURA, 1981; KORUNIC, 1998).

Previous laboratory study proved the activity of the DE dust, which was applied and incorporated into the broiler litter and achieved an 80% reduction of the insect population (OLIVEIRA et al., 2017).

Although the pulverization of insecticides on litter is very common; it can reach a small number of the insects which are more superficially located without reaching larvae and pupae, which are in the soil, leading to poultry house reinfestation (DIAS et al., 2013). On the other hand, liquid application of chemical insecticides on the soil and walls before the distribution of the litter for a new flock are efficient and cause a significant reduction of LMW populations (SANTOS et al., 2009; ALVES et al., 2010). The soil treatment with a formulation of *Beauveria bassiana* Unioeste 4 fungus conidia (1000 L, 1×10^9 conidia·mL^{-1}) was also efficient, maintaining the LMW population at low levels for 5 months after treatment (ALVES et al., 2015).

It should be noted that dust applications forms powder clouds, requiring a great amount of DE. This also decreases the efficacy because dry applications in a poultry house cannot reach the insect in wall crevices, litter, and soil. On the other hand, DE liquid preparations are easier to apply and safer than dust in dry applications, both to birds and farm workers (MAURER et al., 2009; MULLENS et al., 2012).

Based on the efficacy of DE against the LMW and aiming to enjoy the benefits of the liquid application on the soil, laboratory and poultry house experiments were carried out to assess the efficacy of the DE liquid preparation to manage LMW in poultry houses.

MATERIAL AND METHODS

Laboratory bioassay

Insects used in experiments were taken from laboratory colony were maintained in plastic boxes with wheat bran, brewer yeast, chicken food, and a piece of apple in a controlled room (30 ± 1 °C, 12 h day: night, 80% relative humidity) (RICE; LAMBKIN, 2008).

The product used was Fisiocontrol (VetScience Bio Solutions, Brazil), previously characterized and evaluated against *Dermanyssus gallinae* (De Geer, 1778) (Mesostigmata: Dermanyssidae) and the LMW (ALVES et al., 2017, 2020). Fisiocontrol was used as a commercially available DE with standardized parameters: mineral particles smaller than 500 μm, 86.2% silica, and 73% suspensibility, according to the Brazilian Association for Technical Standardization (ABNT, 2015). Aiming to confirm the efficacy and to determine the concentration for use in the field, trial bioassays were conducted in two phases as follows.

Insecticidal activity (direct contact)

Fisiocontrol was suspended in distilled water at 1, 2, 5, and 10%. Bottoms of plastic trays (30 × 50 cm) were filled with 150 mL of each DE suspension and insect adults were released in the center of each tray, to simulate the application of DE suspension in the commercial poultry house. After 30 s under slight agitation, the insects were transferred to 9 cm-Petri dishes with the bottom containing a filter-paper to remove the excess of DE suspension on the insects. Finally, insects were transferred to other Petri dishes. Wheat bran as the food was added (0.5 g·plate^{-1}). In the control group, insects were treated with only distilled water. For each treatment and control, 5 trays with 150 insects were prepared.

The insects were kept in a controlled room at 26 ± 1 °C, 12 h day:night, 60% of relative humidity. Surviving insects were counted daily for 10 days. Insects were considered dead if no movement was visible even after being touched with forceps. Each experiment was repeated twice.

Persistence on the insect body

The contact with substrate can affect the DE persistence on insect body (ALVES et al., 2008). Thus, a bioassay was conducted only with DE at 2 and 5% using all the same procedures described before. The lowest concentration (1%) was discarded due to its low insecticidal activity.

After contact, insects were divided in two groups. In the first group, insects were immediately transferred to Petri dishes with bottom filled of chicken food or poultry litter (1 cm deep). In the other group, insects were kept in empty Petri dishes, and after 24 h. After that, insects were transferred to other dishes with the substrates (chicken food or poultry litter). Control insect group was treated with distilled water. For each treatment and control 5 trays with 150 insects were prepared. The experiment was repeated twice.

Field trail

The experiment was carried out in two commercial poultry houses (1200 m^2; compacted soil floor; automated feed and water supply system) in Lindoeste, Paraná, Brazil (25°19’43.13”S, 53°35’36.77”O), with LMW high population history. In the last week of housing of the birds, the insect population was estimated by collecting poultry litter samples (30 × 30 cm at full depth down to the floor) in 18 points in both poultry houses. The samples were individualized in identified plastic bags and frozen for 48 h, then the adults were counted (GODINHO; ALVES, 2009; OLIVEIRA et al., 2019). After the housing period, the empty poultry houses were washed and treated with a disinfectant solution. After 3 days, one of the poultry houses, the earth floor, walls, and curtain were treated with 1000 L of Fisiocontrol 10% suspension in tap water (83 $g \cdot m^{-2}$ of DE). Application was done through the pressure-washing machine used to sanitize the chicken house.

Due to commercial poultry production, it was not possible to design a non-treated control (the owner did not accept the non-treated poultry house, negative control). Thus, the control poultry house was treated by chemical insecticide application on the soil and internal surfaces (cypermethrin 15 g + chlorpyriphos 25 g + piperonyl butoxide 15 g), 1 $L \cdot 1000 L^{-1}$.

Both poultry houses were left empty with the curtains lowered for 48 h after application. Then, curtains were open for ventilation and fresh poultry litter was placed on the dry floor.

The litter sampling procedure was repeated twice as described at approximately 30 and 45 days after housing the new flock in both poultry houses. The first assessment of the insect population before the treatment represented the total insect population in each poultry house (100%). In subsequent evaluations (30 and 45 days) in both poultry houses, the total number of insects sampled was used to estimate the percentage variation to the initial population. This percentage also represented the re-infestation rate (which is the expression of the efficiency of the treatment).

Statistical analysis

Percent mortality data from both laboratory experiments were corrected by Schneider–Orelli formula (http://www.ehabsoft.com/ldpline/onlinecontrol.htm). Means were compared by Kruskal–Wallis test ($p < 0.05$) using the software STATISTICA v.7.0 (STATSOFT, 2004).

Based on OLIVEIRA et al. (2019), the insect number before and after control treatments in each aviary (pre-post experimental design with dependent variable) were compared by Wilcoxon test ($p < 0.05$). In the comparison of the insect population in both poultry houses was used Mann–Whitney test ($p < 0.05$) (SAS, 2003). In order to confirm the population difference before and after treatments, the Friedman’s test ($p < 0.05$) analysis was also performed (R 3.1.2 statistical package).

RESULTS

Laboratory bioassay

Insecticidal activity (direct contact)

DE treatments were efficient in all concentrations ranging from 41% mortality with DE 1% to 99.7% with DE 5 and 10% (Table 1). The increase of DE concentration from 1 to 5% also led to higher mortality up to 99.7%. The activity of the 5 and 10% DE concentrations did not differ (Table 1).

Table 1. Mortality (%) of *A. diaperinus* adults after direct contact with diatomaceous earth (DE) suspension 1, 2, 5, and 10%, in laboratory condition.

Treatment	Mortality (%)
Control	8.0 ± 0.0D
DE 1%	41.3 ± 11.3C
DE 2%	76.4 ± 9.6B
DE 5%	99.7 ± 0.4A
DE 10%	99.7 ± 0.4A

Means (± MSE) followed by the same letter in row are not significantly different (Kruskal–Wallis test; $p < 0.05$).

Persistence on the insect body

The time of contact after application and substrate affected DE activity. Mortality was significantly lower in all treatments with insects in contact with both substrates (chicken food or poultry litter) immediately after the DE application (time = 0), reaching almost 30–40% in both substrates. However, the contact with substrate only 24 h after the treatment did not affect the mortality, which was ranging from 85.6 to 100% (DE 2 or 5%, chicken feed substrate) or 83.9 to 69.6 (DE 2 or 5%, poultry litter substrate). Comparing the effects of substrates on DE activity, mortality was significatively lower when insects were transferred to poultry litter 24 h after DE treatment (69.6%).

Table 2. Mortality (%) of *A. diaperinus* adults after direct contact with diatomaceous earth (DE) suspension (2 and 5%) and transferred to chicken feed or poultry litter, immediately or 24 h after DE treatment.

Treatment	Substrate and time after treatment (h)			
	Chicken feed		Poultry litter	
	0	24	0	24
Control	10.2 ± 0.00Ba	19.7 ± 0.00Ca	10.9 ± 0.00Ca	4.1 ± 0.00Ba
2% DE	5.4 ± 1.42Bb	85.6 ± 9.46Ba	12.4 ± 1.86Bb	83.9 ± 9.46Aa
5% DE	32.3 ± 5.07Ab	100 ± 0.00Aa*	39.2 ± 7.22Ab	69.6 ± 5.36Aa

Means (± MSE) followed by the same upper letter in the column and lowercase in the row (for each type of substrate) do not differ statistically from one another according to the Kruskal-Wallis test ($p < 0.05$); *significantly difference among substrates (chicken feed × poultry litter) at 24 h after DE contact (Mann–Whitney test; $p < 0.05$).

FIELD TRAIL

Both poultry houses had similar insect population previous treatment (almost 10,000 in the samplings). First DE suspension treatment reduced the population by 94.2% at 30 days after the application (DAA). With chemical insecticide (comparison standard) LMW population was also reduced significatively (90.8%) (Table 3). Comparing insect populations in both aviaries, the treatment with DE suspension was significatively most efficient after 30 DAA. Although, at 45 DAA, in DE poultry house the LMW population size was significantly larger than control poultry house, reaching 15,118 insects (39% above the initial population). Meanwhile, with chemical insecticide treatment, the population increase was less intense (8,424 insects; 17.5% below that initially observed) (Table 3).

Table 3. Number of *A. diaperinus* adults and populational variation (%) in poultry houses after soil treatment with diatomaceous earth (DE) suspension 10% or chemical insecticide.

Treatment	Number of insects		
	Pre-application	post-application 1 (30 DAA)	Post-application 2 (45 DAA)
DE suspension	10907 ± 249Ab	632 ± 28.8Ba (−94.2*)	15168 ± 675Ac (+39.1**)
Chemical insecticide[1]	10204 ± 227Ab	943 ± 88.8Aa (−90.8*)	8424 ± 393Bc (−17.5**)

[1]Cypermethrin 5% (1 L/1,000 L); means (± MSE) of individuals at every assessment and respective percentage of infestation, between parentheses, in relation to pretreatment population followed by the same upper letter in the column do not differ statistically (Mann–Whitney test; $p < 0.05$); means (± MSE) followed by the same lowercase in the row do not differ statistically (Wilcoxon test; $p < 0.05$). Means (± MSE) followed by * or ** differ statistically from pretreatment population (Friedman test; $p < 0.05$); DAA = days after application.

DISCUSSION

In a previous study, DE dust led to 80 and 100% mortality in the laboratory corroborating the DE activity against the LMW. This proves the liquid preparation did not affect the efficacy of DE to the LMW (ALVES et al. 2006, 2017).

Furthermore, in a field trial with DE in dust application on the poultry litter, reduced residual activity of DE was observed, which remained up to 30 days. After that both treated and nontreated area presented no difference between insect population (OLIVEIRA et al., 2017).

DE activity is based on direct contact with the insect body surface. Thus, applying a liquid preparation of DE improved the distribution on the surface, which increase the contact and impregnation of the DE particles on the insect body, even in reduced exposure time (MAURER et al., 2009; SCHULZ et al., 2014).

The action of DE is also greater when direct contact with the insect is prolonged, because DE mixed with the substrate on which the insects were present (chicken feed or poultry litter), achieved mortality of 50–70% only after 10 days of contact (ALVES et al., 2006). It should be noted that the contact with DE suspension for only a few seconds followed by contact with the substrate was enough to impregnate the insects' surface and cause 30 to 40% mortality, which proves the susceptibility of the insect to DE even in low concentration.

Adult mortality for a DE dust application and after transferred to chicken feed substrate was higher than the group transferred to poultry litter after treatment. Previously, it was observed in scanning electron microscope that the amount of DE particles on the insect body surface was higher when insects were maintained in chicken feed than when poultry litter was used as the substrate for insects after DE treatment. There was physical removal of DE particles from insect body by poultry litter contact (ALVES et al., 2008). This reinforces the higher mortality in the insect group transferred only 24 h after DE treatment was probably due to the presence of a great amount of DE particles on the insect body.

Soil treatment with liquid insecticide application was efficient and greatly impacted the LMW population. This find corroborates previous studies where the LMW reinfestation at 40 days after soil treatment with chemical insecticide was low (10–20%) (SANTOS et al., 2009; ALVES et al., 2010).

The liquid treatment penetrates the soil until 13 mm depth (varying with soil type) reaching the insects sheltered in tunnels, mainly larvae and pupae. This directly impacts the insect population increases in the subsequent flocks (UEMURA et al., 2008; SANTOS et al., 2009).

However, the poultry house soil is compacted and has heterogeneous porosity and density, which can make the liquid insecticide infiltration irregular along the extension of the poultry house (SANTOS et al., 2009). This could explain the low residual activity of DE, since the irregular infiltration may have reduced its contact with a great number of insects, or DE concentration in the soil was irregularly distributed. This was not observed with chemical insecticide, which even in low amounts can reach the insect body, be absorbed, and immediately acts on the nervous system.

In addition, adults emerge from the soil with body impregnated by DE. Upon contact with the poultry litter, DE particles were probably removed from the insect body, reducing the efficiency of the treatment (ALVES et al., 2008).

The potential of DE as an insecticide is reinforced by its mode of action through contact with the insect's body and can kill insects that are susceptible and resistant to the main active ingredients of chemical insecticides (cypermethrin or organophosphates). Thus, DE can be an alternative to manage insecticide resistance, associated synergistically with another tactic, to reduce the selection pressure of these chemical products (JAPP et al., 2010; DIAS et al., 2013; OLIVEIRA et al., 2016).

Although DE in liquid preparation is known for insect control (ATHANASSIOU et al., 2006) and has been evaluated in the poultry production context, against *Ornithonyssus sylviarum* mites (Canestrini & Fanzago 1877) (Acari: Macronyssidae) and *D. gallinae* (MULLENS et al., 2012; ALVES et al., 2020), there was no record of its activity against the LMW.

DE liquid preparation is a potential tactic to control the LMW mainly if the application is done in empty poultry house in the period of the poultry litter replacement and on all the poultry house internal surfaces—curtains, columns, and other places where insects can take shelter.

CONCLUSIONS

It is possible to affirm that DE liquid preparation has the potential to be used in LMW population management, mainly for shock treatment, considering that the persistence was low in the conditions evaluated here. It can be an important tool in managing insect resistance to chemical insecticides.

AUTHORS' CONTRIBUTIONS

Conceptualization: Alves, L.F.A.; Oliveira, D.G.P. **Data curation:** Alves, L.F.A.; Oliveira, D.G.P. **Formal analysis:** Oliveira, D.G.P. **Funding acquisition:** Alves, L.F.A. **Investigation:** Alves, L.F.A.; Oliveira, D.G.P.; Pares, R.B. **Methodology:** Alves, L.F.A.; Oliveira, D.G.P.; Pares, R.B. **Project administration:** Alves, L.F.A. **Resources:** Oliveira, D.G.P. **Supervision:** Alves, L.F.A. **Validation:** Alves, L.F.A.; Oliveira, D.G.P.; Pares, R.B. **Visualization:** Alves, L.F.A.; Oliveira, D.G.P.; Pares, R.B. **Writing – original draft:** Alves, L.F.A.; Oliveira, D.G.P.; Pares, R.B. **Writing – review & editing:** Alves, L.F.A.; Oliveira, D.G.P.

AVAILABILITY OF DATA AND MATERIAL

Data will be available upon request

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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