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In vitro evaluation of the response of *Dermanyssus gallinae* to products in aqueous suspension

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Abstract
The hematophagous mite *Dermanyssus gallinae* poses a serious sanitary problem in the Brazilian laying poultry industry. Its control is typically performed with acaricides, either in powder or liquid form. However, the intensive use of these products has caused populations of this species to develop tolerance and even resistance. The aim of the present study is to evaluate the response of eggs and adults of *D. gallinae* to products in aqueous suspension according to commercial indication and as per the recommendations of the Brazilian Ministry of Agriculture, Livestock and Supply. The study used four acaricide products (product 1: cypermethrin, chlorpyrifos, and piperonyl butoxide; product 2: alkyl-benzyl-dimethyl ammonium chloride, glutaraldehyde, deltamethrin; product 3: dichlorvos; product 4: fluralaner) tested in vitro using the contact method. Distilled water was used in the control group. The effectiveness of each of the products differed significantly between eggs and adults. Products 2, 3, and 4 caused 100% of adult mortality up to day 5 after start of treatment, product 1 97.5%. The corrected mortality (non-viability) of eggs was 21.4% (product 1) 39.4% (product 2), 47.8% (product 3), and 14.4% (product 4). Although the products evaluated were effective against adults of *D. gallinae*, their effectiveness against eggs was lower under the same conditions. This finding might be directly related to frequent *D. gallinae* reinfestations in poultry houses.

Keywords Resistance · Ectoparasites · Aviculture · Poultry health

Introduction

Various arthropods are a threat to the poultry industry due to the direct and indirect effects they have on bird health and well-being (Sparagano et al. 2009); additionally, commercial laying hens have been affected by mite infestations in Brazil for a long time (Rezende et al.

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2013; Faleiro et al. 2015; Horn et al. 2016). One of the species that poses a threat to the hen population, and that represents a serious sanitary problem for the laying poultry industry, is the hematophagous mite *Dermanyssus gallinae* (De Geer) (Mesostigmata: Dermanyssidae) (Cunha et al. 2009; Cencek et al. 2011).

Dermanyssus gallinae may cause irritation, anaemia, bloodstained eggs, aggressive behaviour, cannibalism, and in some severe cases, even death of laying hens (Chauve 1998; Sparagano et al. 2009; Cunha 2013; Flochlay et al. 2017; Oliveira 2017). This mite is also related to low yield, decreased egg quality, and host immune alterations, which leads this species to attain pest status (Taylor et al. 2007; Oliveira 2017). The high number of specimens of this mite in poultry houses with recurring *Salmonella* infections also raises the issue of the potential role played by *D. gallinae* as a vector for this disease (and other diseases) in poultry farms (Valiente Moro et al. 2007, 2009; Sparagano et al. 2014). *Dermanyssus gallinae* spend the majority of their life cycle away from the host, and they suck blood mostly during the night. When they are not feeding, they form colonies in cracks and crevices that are used as hiding places (Cunha et al. 2009). Adults of this species might survive away from hens without feeding for several months, or even up to a year, which explains their persistence in poultry houses (Taylor et al. 2007; Cencek et al. 2011).

Once *D. gallinae* populations are established, control is in present-day poultry production systems is typically performed with acaricides, in either powder or liquid form (Taylor 2001; Abbas et al. 2014). However, the availability of chemical acaricides has decreased in many countries due to legislation and the options for controlling this mite are somewhat limited due to food safety regulations (Brännström et al. 2008; Abbas et al. 2014; Sparagano et al. 2014). In addition, these chemical compounds have been suffering drawbacks caused by mite resistance and concerns with human, animal, and environmental health (Taylor 2001).

In Brazil, the use of agrochemicals is monitored by the National Plan for the Control of Residues in Products of Animal Origin, which is the risk management tool adopted by the Brazilian Ministry of Agriculture, Livestock and Supply (*in Portuguese*: Ministério da Agricultura, Pecuária e Abastecimento—MAPA). This national plan aims at knowing and preventing the violation of the residual safety levels of authorized substances, as well as monitoring the occurrence of any levels of residues of chemical compounds banned in the country (Brasil 1999).

The repeated use of veterinary pesticides for long periods of time, as well as their incorrect application or application without a clear management program, or even the use of illegal chemical acaricides (*off-label*) have led *D. gallinae* to develop resistance to these compounds, frequently rendering their effectiveness uncertain (Marangi et al. 2009; Sparagano et al. 2009, 2014; Abbas et al. 2014; Gay et al. 2020). Control is also hampered because these mites hide in inaccessible places, due to their ability to remain long periods without feeding, and to their high fertility (Cencek et al. 2011). Therefore, the aim of the present study is to evaluate the response of eggs and adults of *D. gallinae* to acaricidal products in aqueous suspension according to commercial indications and as per recommended by the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA).

Materials and methods

Experimental design

Mites and eggs were collected in a commercial laying poultry house situated in the municipality of Salvador do Sul (RS, Brazil), inserted in plastic bags, which were sealed, and taken to Laboratório de Acarologia/Tecnovates/Univates, where they were immediately screened to begin the experiment, separating eggs and visibly engorged adults; as described by Sparagano et al. (2014), engorged mites are intense red.

Four acaricide products in aqueous suspension were used, tested in vitro through the contact method, according to the amount of product per area indicated on their labels (Table 1). These products are: (1) cypermethrin, chlorpyrifos, and piperonyl butoxide; (2) alkyl-benzyl-dimethyl ammonium chloride, glutaraldehyde, and deltamethrin; (3) dichlorvos; and (4) fluralaner. The total volume applied per arena was 0.5 ml of prepared solution. Distilled water was used in the control group. Eggs and visibly engorged adults of *D. gallinae* were used in the test, and the methodology was adapted from Alves et al. (2019).

Experimental unit

The arenas were comprised of Petri dishes (6 cm diameter, area 28.26 cm^2) with Whatman filter paper discs (80 g/m^2) on the bottom and petroleum jelly on the edges, as a barrier to prevent mites from escaping (Alves et al. 2019). Ten *D. gallinae* adults were distributed on each arena. Five replicates/treatment were performed, with 0.5 ml of solution sprayed on each replicate, using a professional SW-775 airbrush (working pressure of 10 to 45 psi) at a distance of 15 cm inside an Exhaust Cabin. After drying under ambient conditions, the dishes were sealed with plastic wrap and maintained in a climate chamber at 25 ± 1 °C, 70 ± 5% relative humidity, and L14:D10 photoperiod (Alves et al. 2019).

Mites were evaluated on a daily basis for 5 days using a Leica stereomicroscope (S6E—LED 2500; Leica Microsystems, Wetzlar, Germany), and were considered dead if no movement was seen after touching them with a fine-tipped brush. In order to assess ovicidal activity, the same procedure was repeated with eggs of *D. gallinae*, with 0.5 ml of solution applied to each dish. Five replicates were performed for each treatment and for the control. Evaluations were performed on a daily basis for 5 days, counting the eggs that hatched, and live and dead mites (adults) by using a stereomicroscope.

Data analysis

Mite mortality (%) was calculated as: (sum of dead mites/total number of mites) × 100. Corrected mortality (Mc, mortality relative to the control) of adults and eggs was calculated using Abbott's formula (1925):

$$Mc = \left\{ \left[\frac{Mo - Mt}{100 - Mt} \right] \times 100 \right\},$$

where Mo is the observed mortality in each treatment and Mt is the mortality observed in the control (Silva et al. 2007; Locher et al. 2010). Acaricide lethal activity was classified

Table 1 Tested products, their components, and dosage indicated by the manufacturer's label

Product (composition)	Class	Action mode	Pests and diseases	Dosage indicated by the manufacturer (for mite control)	Dosage applied by arena
Product 1 (cypermethrin, chlorpyrifos, and piperonyl butoxide)	Ectoparasiticide	Contact	*Alphitobius diaperinus* (lesser mealworm), *Dermanyssus gallinae* (chicken mite)	Solution: 1 l of product diluted in 400 l of water Application: 1 l of solution for each 1.2 m^2	0.5 ml of solution containing 5.87 µl of product
Product 2 (alkyl-benzyl-dimethyl ammonium chloride, glutaraldehyde, deltamethrin)	Insecticide/acaricide; disinfectant: bactericide, virucide, and fungicide	Contact	*Dermanyssus gallinae*; insects (*Musca domestica*—flies, lesser mealworm, and lice); bacteria; viruses, and fungi	Solution: 300 ml of product diluted in 100 l of water Application: 1 l of solution for each m^2	0.5 ml of solution containing 8.45 µl of product
Product 3 (dichlorvos)	Insecticide	Contact/ingestion/fumigation	Cockroaches, fleas, thumbtacks, woodworm beetles, ants; crawling and flying insects; stored-product pests	Solution: 5 ml of product diluted in 1 l of water Application: 1 l of solution for each 20 m^2	0.5 ml of solution containing 0.702 µl of product
Product 4 (fluralaner)	Acaricide/insecticide	Systemic	Poultry mites (*D. gallinae*)	0.5 mg fluralaner per kg of hen body weight. The product is diluted in hen water	0.5 ml of solution containing 0.25 µl of product

according to Kim et al. (2007), where mortality > 80% is considered strong, 80–61% is moderate, 60–40% is weak, and mortality < 40% is considered little or no activity.

Data analysis was done with two tests using BioEstat v.5.0 (Ayres et al. 2007). Mean corrected mortalities of adults and eggs were compared among treatments using the non-parametric Kruskal–Wallis test, followed by Dunn's test ($\alpha = 0.05$). The Mann–Whitney test was performed on corrected mortalities between adults and eggs within each pesticide.

Results

Effects on eggs

After spraying the products, the mean corrected mortality (non-viability) of eggs was 21.4% with product 1, 39.4% with product 2, and 47.8% with product 3 (Table 2). Product 4 had the lowest egg non-viability after treatment application: 14.4%. Products 1–4 had no significant difference from each other; however, products 2 and 3 differed from the control sample (Table 2).

Effects on adults

Corrected mortality was significantly different from the control with all products tested (Table 2). Products 2–4 caused 100% mortality up to the 5th day, product 1 97.5%. Following Kim et al. (2007), the lethal activity of all products tested was considered strong (> 80%). There was no significant difference among treatments (Table 2). The effectiveness of all four products was clearly higher against adults than against eggs (Table 2).

Discussion

Product 1, composed of cypermethrin, chlorpyrifos, and piperonyl butoxide, had a strong lethal activity (Kim et al. 2007) against adults, but did not show ovicidal action. Considering hens cannot be inside the poultry house when this product is used (Ouro Fino 2020), the low effectiveness against eggs of *D. gallinae* can cause the establishment of new populations of this species even before the chickens repopulate the aviary. Additionally, *D.*

Table 2 Mean (± SD) corrected mortality (%) of adults and eggs of *Dermanyssus gallinae* after spraying one of four products (see Table 1 for composition) or distilled water as a control in Petri dishes

Product	Eggs	Adults
1	21.4 ± 25.77 abB	97.5 ± 5.59 aA
2	39.4 ± 22.86 aB	100 aA
3	47.8 ± 27.61 aB	100 aA
4	14.4 ± 4.35 abB	100 aA
Control	0 bA	0 bA

Means within a column followed by the same lowercase letter, or within a row followed by the same uppercase letter, are not significantly different (lowercase letter: Kruskal–Wallis test followed by Dunn's; uppercase letters: Mann–Whitney test: both $p > 0.05$)

gallinae might survive long enough to infest a new flock, especially because they may live up to several months without feeding (Taylor et al. 2007; Cencek et al. 2011).

Both cypermethrin and chlorpyrifos are widely used to control arthropods and animal parasites. Chlorpyrifos is an inhibitor of acetylcholinesterase (AChE) affinity, whereas cypermethrin blocks sodium channels. Cypermethrin and piperonyl butoxide are classified as synergist components of pesticide formulations, especially pyrethroids (Beckel et al. 2006; Campos et al. 2017). However, the ineffectiveness against eggs of *D. gallinae* might indicate that the product probably does not penetrate the eggshell, but there is no information so far to help understand how the eggs are protected to the products.

Some studies found that even low doses of cypermethrin caused immunotoxicity, oxidative stress, and apoptosis of poultry lymphocytes (e.g., Eraslan et al. 2017; Ambwani et al. 2018). Data such as these show the importance of the correct application of products, in absence of hens and observing the specified withholding period, especially at sites populated by animals that shall subsequently be used for human consumption.

Product 2 is a disinfectant/insecticide composed of alkyl-benzyl-dimethyl ammonium chloride, glutaraldehyde, and deltamethrin. The disinfectant effect of this compound derives from benzalkonium chloride and glutaraldehyde. Deltamethrin is a pyrethroid, which is a synthetic adaptation of pyrethrins, and provides excellent knockdown, despite its low residual activity due to instability (Casida et al. 1983; Taylor 2001; Abbas et al. 2014). Similar to product 1, product 2 cannot be applied while hens are inside the poultry house (Ouro fino 2020; Theseo 2007). *Dermanyssus gallinae* resistance to pyrethroids has already been widely reported and observed in Europe, for instance in UK, Italy, France, and Sweden (Mul et al. 2009; Sparagano et al. 2014). Thomas et al. (2018) reported apparent resistance of *D. gallinae* to deltamethrin (part of product 2) as well as cypermethrin (present in product 1).

Product 3 (dichlorvos) is an organophosphate that acts by inhibiting AChE function, which consequently affects the transmission of nervous impulses and ultimately leads to pest paralysis and death (Taylor 2001). Organophosphates were pioneers among chemical groups used for the control of arachnids, which include bird mites (Beesley 1963; Abbas et al. 2014). Still, studies such as Nordenfors and Höglund (2000) have already mentioned the limited effect of organophosphates, which only provide temporary suppression of mite populations.

The red poultry mites used in the present study proved sensitive: after in vitro application the product caused mortality of 100% of adults. These findings corroborate Beugnet et al. (1997), who found that dichlorvos was effective against adults of *D. gallinae*. Although effective against adults, the dichlorvos-based treatment had much less effect against eggs.

Also product 4 (fluralaner) had strong lethal activity against adults, but was much less effective against eggs (only 14.4% nonviable eggs). Unlike the other poultry acaricides, this product acts systemically and its administration occurs via drinking water. After ingestion by the hen, fluralaner inhibits the mites' nervous system, leading to paralysis and death (Thomas et al. 2018). Thomas et al. (2018) tested three application methods against *D. gallinae* using fluralaner: spray application (used for traditional contact acaricides), immersion, and a feeding test. They found that fluralaner was active in all three methods, although the highest activity was reported in the feeding treatment. They only studied effects on mite adults, not on eggs.

In many countries, the use of synthetic products has been increasingly limited due to a progressively stricter legislation regarding components and their impacts. Restraints to the use of these products also include egg withholding periods or restrictions preventing

treatments while hens are inside poultry houses, in order to mitigate risks of residues in the products, and consequently, minimize risks to human health (Roy et al. 2009; Sparagano et al. 2014).

Although the products evaluated in the current study were effective against adults of *D. gallinae*, their effectiveness against eggs was much lower under the same conditions. This finding might be directly related to frequent *D. gallinae* reinfestations in poultry houses. According to Beugnet et al. (1997), reinfestation in poultry houses occurs within 4–8 weeks after acaricidal treatments, and the apparent treatment failure might be related to rapid parasite reproduction, short interval between depopulating and repopulating the poultry house, or even due to acaricide resistance. The present study corroborates this information, despite the products having an effect on the adults, the eggs showed tolerance to the products tested.

Since the ineffectiveness of acaricides against eggs of these ectoparasites might lead to concerning effects on poultry farm systems and affect their economic viability, further studies aiming to evaluate side effects on immatures mites derived from treated hatched eggs are recommended, as well as field tests in order to confirm the acaricidal activity of these compounds in these environments.

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Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Consent to participate All authors participated voluntarily in the research.

Consent for publication All authors read and approved the final manuscript.

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