In Vitro Evaluation of *Dermanyssus Gallinae* Response to Products in Aqueous Suspension

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

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Abstract

Dermanyssus gallinae are hematophagous mites that pose 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 led populations of this species to develop resistance. The aim of the present study is to evaluate the response of *D. gallinae* eggs and adults to products in aqueous suspension as per the commercial indication and registered at the Ministry of Agriculture, Livestock, and Supply. The study used four different acaricides (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. There was difference between eggs and adults in each treatment ($p < 0.05$, except for control); products had higher efficacy against adult forms. This result might be directly related to frequent *D. gallinae* reinfestations in poultry houses.

Keywords: resistance; ectoparasites; aviculture; poultry health

Introduction

Arthropods are a threat to the poultry industry due to their direct and indirect effects on bird health and well-being (Sparagano et al. 2009). Hematophagous mites are one of the obligatory ectoparasites of domestic, wild, and laying hens (Oliveira 2017), and are considered to be the best adapted to their vectorial role; some species are vectors of pathogen microorganisms (Valiente-Moro et al. 2005).

The control of ectoparasites is essential for maintaining biosafety of poultry farm systems, as lack of control maximizes risks of occurrence of sanitary issues, thus resulting in economic problems (Faleiro 2012). Intensive production inside poultry houses increases the risks of epidemics, reduces egg quality, and causes yield to drop. Therefore, preventive sanitary measures limit the propagation of vectors, and the lack of these measures allows the introduction and dissemination of diseases in poultry farms (Borne and Comte 2003).

Commercial laying hens have been affected by mite infestations in Brazil for a long time (Rezende et al. 2013; Faleiro et al. 2015; Horn et al. 2016). One of the species that threaten the hen population is *Dermanyssus gallinae* (De Geer, 1778) (Mesostigmata: Dermanyssidae), a hematophagous mite that poses a serious sanitary problem for the laying poultry industry (Cunha et al. 2009). Its effects on the production and health of birds places it among the ectoparasites with the greatest economic relevance to egg production worldwide (Chauve 1998).

Dermanyssus gallinae might cause irritation, anemia, blood-stained eggs, aggressive behavior, cannibalism, and in some severe cases, even the death of laying hens (Chauve 1988; Sparagano et al. 2009; Cunha 2013; Flochlay et al. 2017; Oliveira 2017). It is also related to low yield, decreased egg quality, and host unbalance, which causes this species to be deemed as pest, owing to the economic damages it causes to the poultry industry (Oliveira 2017). The high number of specimens of this hematophagous 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; Moro et al. 2009; Sparagano et al. 2014). They spend the majority of their life cycle away from the host, sucking 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; Pereira 2011). Adults of this species might survive away from hens without feeding for several months, which explains their persistence in poultry houses (Taylor et al. 2007), either because they remain inside the poultry facility nearly throughout their cycle (Tucci et al. 2008) or because their control is hampered by their resistance to products (Harrington et al. 2011).

Once *D. gallinae* populations are established, control is typically performed with acaricides, in either powder or liquid form. However, due to food safety regulations, the options for controlling this mite are somewhat limited (Brännström et al. 2008). The most frequently used products are organochlorines, organophosphates, pyrethroids, and carbamates (Marangi et al. 2008; Sparagano et al. 2009; Benítez et al. 2011; Abbas et al. 2014; Sparagano et al. 2014). Their repeated use 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, which often renders their efficacy uncertain (Marangi et al. 2008; Sparagano et al. 2009; Sparagano et al. 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 (up to one year) without feeding and to their high fertility (Cencek et al. 2011). Therefore, the aim of the present study is to evaluate the response of *D. gallinae* eggs and adults to products in aqueous suspension as per commercial indication and as registered at the Ministry of Agriculture, Livestock, and Supply.

Materials and Methods

Experimental design

Mites were collected in a commercial laying poultry house situated in the municipality of Salvador do Sul - RS, inserted in plastic bags, which were sealed and taken to Laboratório de Acarologia/Tecnovates/Univates, where they were screened. After screening, specimens were maintained in plastic bags and stored at ambient temperature until the experiment was performed.

Four different acaricides (Table 1) in aqueous suspension were used in the present study, tested *in vitro* through the contact method, according to the amount of product per area predicted in their labels. The total volume applied per arena was 0.5 ml of prepared solution. Distilled water was used in the control group. *Dermanyssus gallinae* adults and eggs were used in the test, and the methodology used was adapted from Alves et al. (2019).

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
<u>Product 1</u> (Cypermethrin, Chlorpyrifos, and Piperonyl Butoxide)	Ectoparasiticide	–	<i>Alphitobius diaperinus</i> (lesser mealworm) and <i>Dermanyssus gallinae</i> (chicken lice)	Broth: 1 liter of product diluted in 400 liters of water. Application: 1 liter of broth for each 1.2 m ² to be treated.	0.5 ml of broth containing 5.87 µl of product.
<u>Product 2</u> (Alkyl-benzyl-dimethyl ammonium chloride, glutaraldehyde, deltamethrin)	Insecticide – Acaricide Disinfectant: bactericide, Virucide and fungicide	–	<i>Dermanyssus gallinae</i> (chicken lice) Insects (<i>Musca domesticae</i> - flies, lesser mealworm, and lice); bacteria; viruses, and fungi.	Broth: 300 ml of product diluted in 100 liters of water. Application: 1 liter of broth for each m ² to be treated.	0.5 ml of broth containing 8.45 µl of product.
<u>Product 3</u> (Dichlorvos)	Insecticide	C/I/F	Cockroaches, fleas, thumbtacks, woodworm beetles, ants Crawling, flying insects, and stored product pests.	Broth: 5 ml of product diluted in 1 liter of water. Application: 1 liter of broth for each 20 m ² to be treated.	0.5 ml of broth containing 0.702 µl of product.

Product 4 (Fluralaner)	Acaricide Insecticide	S	Poultry mites <i>Dermanyssus gallinae</i>	0.5 mg Fluralaner per kg of hen body weight. The product is diluted in hen water.	0.5 ml of broth containing 0.25 µl of product.
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Source: Elaborated by the author (2020).

*Action mode: C - contact / I - ingestion / S - systemic / F - Fumigation

_ Manufacturer does not provide information in product instructions

Experimental Unit

The arenas used were comprised of Petri dishes ($a= 28.26 \text{ cm}^2$) with qualitative filter paper discs (80 g/m^2) at the bottom and vaseline on the edges. Vaseline was used 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 broth sprayed on each replicate, using a professional SW-775 airbrush (working pressure 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 $25 \pm 1^\circ \text{C}$, $70 \pm 5\%$ relative humidity, and 14 hours photophase (Alves et al. 2019).

Mites were evaluated on a daily basis for five days using a Leica stereoscope microscope (S6E - LED 2500) (Leica Microsystems, Germany), and were considered dead if no movement was visible after touching them with the fine-tipped brush.

In order to evaluate ovicidal activity, the same procedure was repeated with *D. gallinae* eggs, with 0.5 ml of broth applied to each dish. Five replicates were performed for each treatment and for the control. Evaluations were performed on a daily basis for five days, by counting the number of eggs hatched, and live and dead mites by observation under a stereoscopic microscope.

Data analysis

In order to calculate mortality, the following formula was used:

$$\text{Mortality (\%)} = \text{sum of mites} \times 100 / \text{total mites}$$

Corrected mortality of adults and eggs, on the other hand, was calculated using Abbott's formula (1925), where Mt is Total Mortality, Mc is Corrected Mortality relative to control, and Mo is the observed mortality in each treatment (Silva et al. 2007; Locher et al. 2010).

$$Mc = \{[(Mo - Mt)/(100 - Mt)] \times 100\}$$

Acaricide lethal activity was classified 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.

Mean corrected mortalities of adults and eggs in the treatments were compared statistically using *Kruskal-Wallis* non-parametric test, followed by Student *t*-test, with significance of $p \leq 0.05$. Corrected mortalities against adults and eggs using each pesticide tested were compared using the Mann-Whitney test, with significance of $p \leq 0.05$. All tests were performed using Bioestat 5.0 (Ayres et al. 2007).

Results

Effects on adults

Corrected mortality was significant for all products tested (Table 2). Products 2 (Alkyl-benzyl-dimethyl ammonium chloride, glutaraldehyde, deltamethrin), 3 (Dichlorvos), and 4 (Fluralaner) had 100% of mortality up to the 5th day. Product 1 (Cypermethrin, Chlorpyrifos, and Piperonyl Butoxide), on the other hand, had 97.5% of mortality. Following Kim et al. (2007), the lethal activity of all products tested was considered strong, as it was $>80\%$. There was no significant difference between treatments ($p > 0.05$); however, all treatments were different from the control sample ($p < 0.05$).

Table 2: Corrected mortality of *Dermanyssus gallinae* adults and eggs after spraying the products tested, and lethal activity against adults (Kim et al. 2007).

Product	Eggs	Adults	Lethal activity against adults
<u>Product 1</u>	21.39±25.77 abB	97.5±5.59 aA	> 80% - Strong
<u>Product 2</u>	39.44±22.86 aB	100±.0 aA	> 80% - Strong
<u>Product 3</u>	47.78±27.61 aB	100±.0 aA	> 80% - Strong
<u>Product 4</u>	14.44±4.35 abB	100±.0 aA	> 80% - Strong
<u>Control</u> (water)	0 bA	0 bA	--

Source: Elaborated by the author (2020).

*Means inside the same column followed by the same lowercase letter are not significantly different ($p > 0.05$).

*Means inside the same row followed by the same uppercase letter are not significantly different ($p > 0.05$).

Effects on D. gallinae eggs

After spraying the products tested, the corrected mortality (nonviability) of eggs was 21.39% with the first product tested (Cypermethrin, Chlorpyrifos, and Piperonyl Butoxide); 39.44% with Product 2 (Alkyl-benzyl-dimethyl ammonium chloride, glutaraldehyde, deltamethrin); and 47.78% with Product 3 (Dichlorvos). Product 4 (Fluralaner) had the lowest egg nonviability after treatment application; 14.44%. Products 1, 2, 3, and 4 had no significant difference from each other ($p > 0.05$). However, products 2 and 3

differed from the control sample ($p < 0.05$). There was difference between eggs and adults in each treatment ($p < 0.05$, except for control), indicating a higher efficacy of the products against adult forms.

Discussion

Dermanyssus gallinae can be found throughout the year (Sparagano et al. 2014), and its life cycle (egg to egg) can be completed in up to 7 days under optimal conditions (Chauve 1998). Poultry houses are considered sites with suitable conditions for the growth and development of populations of this species, with temperatures ranging from 10°C to 35°C, and relative humidity higher than 70% (Nordenfors et al. 1999; Sparagano et al. 2014).

The results obtained here showed that the products tested were not efficient against both *D. gallinae* life stages (eggs and adults) evaluated in the present study. Product 1, made of Cypermethrin, Chlorpyrifos, and Piperonyl Butoxide had a strong lethal activity $> 80\%$ (Kim et al. 2007) against adults. However, there was a significant difference between eggs and adults. Considering that hens cannot be inside the poultry house during spraying (Ouro fino 2020), the low efficacy of the product against *D. gallinae* eggs might become an even greater issue, as mites of this species might have already established new populations once the hens repopulate the house. Additionally, *D. gallinae* might survive long enough to infest a new flock, especially because they may live up to several months without feeding (Chauve 1988; Sparagano et al. 2014).

Regarding Product 1 formulation, both cypermethrin and chlorpyrifos are widely used to control arthropods and animal parasites. Chlorpyrifos is an inhibitor of acetylcholinesterase (AChE) affinity. The action mechanism of cypermethrin, on the other hand, occurs via sodium channel blockade. Cypermethrin and Piperonyl Butoxide are classified as synthetic pyrethroids, and their efficacy is due to synergy of their active principles (Beckel et al. 2006; Oliveira-Sequeira et al. 2014; Campos et al. 2017). However, the variation used in hens and evaluated in the present study indicated *D. gallinae* egg resistance.

It is worth noting that in some studies, e.g., Ambwani et al. (2018), even low doses of cypermethrin caused immunotoxicity, oxidative stress, and apoptosis of poultry lymphocytes. When its toxicity is taken into consideration, the effects of this compound are deemed as detrimental (Eraslan et al. 2017). Data such as these show the importance of the correct application of products, without the presence of hens and observing the specified withholding period, especially at sites populated by animals that shall be used subsequently for human consumption.

The second product tested (Product 2) is an Alkyl-benzyl-dimethyl ammonium chloride-, glutaraldehyde-, and deltamethrin-based disinfectant/insecticide. The disinfectant effect of this compound is due to benzalkonium chloride and glutaraldehyde. Deltamethrin, on the other hand, is part of the group of pyrethroids, which are synthetic adaptations of pyrethrins and which provide excellent knockdown, despite their low residual activity due to their instability (Casida 1983; Taylor 2001; Abbas et al. 2014). Similar to Product 1, Product 2 application cannot occur in the presence of animals inside the poultry house (Ouro fino 2020; Theseo 2020).

The compound (Product 2) was effective against 100% of adults that underwent the treatment (100±0). Hence, its lethal activity was considered strong >80% (Kim et al. 2007). However, its action was significantly different against eggs, with a nonviability of 39.44%. *D. gallinae* resistance to pyrethroids has already been widely reported and observed in the United Kingdom and European countries such as Italy, France, and Sweden (Mul et al. 2009; Sparagano et al. 2014). Thomas et al. (2018) also reported apparent resistance of *D. gallinae* to deltamethrin, which is present in Product 2, as well as to Cypermethrin, present in Product 1.

The composition of the third product tested (Product 3) is based on dichlorvos, an organophosphate that, similar to other insecticides of its class, acts by inhibiting acetylcholinesterase (AChE) function, which consequently affects the transmission of nervous impulses and leads ultimately 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). Despite that, studies such as Nordenfors and Höglund (2000) have already mentioned the limited effect of organophosphates, with only temporary suppression of mite populations.

After it was applied *in vitro* to *D. gallinae*, the product had a strong lethal activity and mortality of 100% on adults. These findings corroborate Beugnet et al. (1997), who found that dichlorvos was effective against *D. gallinae* adults. Nevertheless, despite its efficacy against adults, our results showed that the dichlorvos-based treatment had a significant difference against eggs, as 47.78% of them were nonviable. On the other hand, this product had higher nonviability of egg hatching than the other products tested in the present study.

The last product tested, comprised of Fluralaner (Product 4), showed strong lethal activity against adults, reaching corrected mortality of 100%. When applied to eggs, its efficacy was not the same, with only 14.44% of nonviable eggs; this number was the least significant among the treatments tested against eggs in the present study.

It is important to consider that the mode of action of Fluralaner is different from any other poultry acaricides. This product acts systematically and its administration occurs via drinking water. Once it is ingested by the hen, Fluralaner inhibits parts of the mite nervous system, leading to paralysis and death (Thomas et al. 2018).

Working with *D. gallinae*, Thomas et al. (2018) tested three different methods using Fluralaner: one corresponding to spray application, used for traditional contact acaricides; an immersion test; and a feeding test. According to the authors, Fluralaner was active in the three tested methods, although the highest activity was reported in the feeding treatment. This study counted only adults of the species, and eggs were not evaluated.

Although the availability of this type of product has decreased in many countries due to legislation (Sparagano et al. 2014), *D. gallinae* control in current poultry production systems is difficult to achieve without the use of chemical products of any kind (Taylor 2001; Abbas et al. 2014). However, these chemical compounds have been suffering drawbacks such as mite resistance and concerns with human, animal, and environmental health (Taylor 2001).

In many countries, the use of synthetic products has been increasingly limited due to a progressively stricter legislation regarding components and their impacts. The restraints to the use of these products also include egg withholding periods or restrictions that prevent treatments from occurring with the presence of hens 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).

Conclusions and Perspectives

The data provided here show that, although the products evaluated were effective against *D. gallinae* adults, their efficacy was not the same against eggs under the same conditions. This finding might be directly related to frequent *D. gallinae* reinfestations in poultry houses, corroborating the initial hypothesis that *D. gallinae* is resistant to the products tested, which are frequently applied in laying poultry houses.

According to Beugnet et al. (1997), reinfestation in poultry houses occurs four to eight weeks after acaricidal treatments, which might be related to treatment failure, rapid parasite reproduction, short period of time without hens in the poultry house, or even resistance to the products. The present study corroborates this information, as mites of this species showed resistance to the products tested.

Data such as these indicate the need for further studies and for improving these products and their components (Taylor 2001), as well as the need for studies that focus on the biological control of this species, using either microorganisms or predators. It is worth emphasizing that the data shown here report a laboratory situation, where products are applied directly on the area with the mites under evaluation. In poultry houses, it is harder for products to come into direct contact with mites, especially due to the large number of cracks and crevices that exist at these sites, which work as hiding places for this species (Nordenfors and Höglund 2000).

Since developing resistance to these ectoparasites might lead to concerning effects on poultry farm systems and affect their economic viability (Beugnet et al. 1997), further studies considering the efficacy of products also against larvae and adults 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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Data availability All data and materials are available for publication.

Compliance with ethical standards

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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