Archives of Veterinary Science **ISSN 1517-784X**
v.24, n.2, p.81-92, 2019 www.ser.ufpr.br/veterinary

ACARICIDAL ACTIVITY OF INERT POWDERS AGAINST THE POULTRY RED MITE *Dermanyssus gallinae* (DE GEER, 1778) (*Mesostigmata: Dermanyssidae*)

Ação acaricida de pós inertes contra o ácaro-vermelho Dermanyssus gallinae (De Deer, 1778) (Mesostigmata: Dermanyssidae)

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ABSTRACT: *Dermanyssus gallinae* is one of the most important ectoparasite of laying hens. The intensive use of chemicals has led to the selection of resistant mite populations. The difficulty in controlling the mites has promoted the search for alternative products. Thereby, the aim of this study was to assess in laboratory the acaricidal activity of inert mineral powders against *D. gallinae*, in dust and water suspensions (5 and 10%) treatments. The products were tested against eggs, nymphs and adults of the mite. There were differences in the activity of tested products. Some products based on diatomaceous earth, kaolin and talc, applied in powder form on adults of the mite were more efficient and selected for subsequent tests. In liquid suspension bioassay it was observed no ovicidal activity of any of the tested products. However, the products were efficient on the nymphs with no difference between both products and concentrations. The activity of diatomaceous earth and Kaolin 2120 in 5 and 10% water suspension on adults was significatively higher than other treatments against adults. There was no difference between the treatments in residual activity. The morphological characterization and identification of diatom frustules present in the product PosturaSec® indicated prevalence of the genus *Brachysira* Kützing, density of 5.7 x 10^7 valves/g, 200 µm size particles and 86.2% of silicon.
Keywords: animal production, alternative control, ectoparasite.

RESUMO: *Dermanyssus gallinae* é um ácaros de maior importância na postura comercial. O uso intensivo de produtos químicos tem levado a seleção de populações do ácaro resistentes. A dificuldade em controla-los faz com que se busquem produtos alternativos. Assim, o presente trabalho teve por objetivo avaliar, em laboratório, a ação acaricida de pós minerais inertes contra *D. gallinae*, em aplicações em pó e em suspensão aquosa (5 e 10%). Os produtos foram testados contra ovos, ninfas e adultos do ácaro. Houve diferença na atividade dos produtos testados. Alguns deles, à base de terra de diatomáceas, caulim e talco, aplicados em pó sobre os ácaros adultos e selecionados para testes seguintes. Nos bioensaios com suspensão em água, não foi observada ação ovicida de nenhum dos produtos testados. Contudo, todos os produtos foram ativos contra ninfas, não havendo diferença significativa entre eles. A atividade da terra de diatomáceas e do caulim 2120 nas concentrações de 5 e 10% em água foi significativamente maior que os outros tratamentos. Não houve diferença entre os tratamentos no teste residual. Na caracterização morfológica e identificação das frústulas de diatomáceas presentes no produto à base desse mineral, verificou-se prevalência do gênero *Brachysira* Kützing, com densidade foi de 5,7 × 10^7 valvas/g, partículas

Recebido em 12/11/2018
Aprovado em 20/05/2019

com 200 μm e 86,2% de sílica.
Palavras-chave: produção animal, controle alternativo, ectoparasita.

INTRODUCTION

The poultry red mite *Dermanyssus gallinae* (De Geer, 1778) (Mesostigmata: Dermanyssidae) is a cosmopolitan hematophagous ectoparasite of laying hens. It is frequently found in aviaries throughout the year (Pritchard et al., 2015). *D. gallinae* leads to anaemia, behavioural changes, skin irritation, feather loss, excessive pecking, and dermatitis in hens. Egg production is also affected, both in quantity and quality. Premature bird deaths have also been observed. In addition, it can constitute a vector of pathogens of veterinary importance, including *Salmonella enterica* serotype Gallinarum and *Salmonella enterica* serotype Enteritidis. There are also reports of discomfort and dermatitis in workers of highly infested aviaries (Rosen et al., 2002; Moro et al., 2009; Pereira, 2009; Cunha, 2013; Pritchard, 2015).

Chemical acaricides are used with relative efficacy in mite control. However, indiscriminate use has made populations of mites resistant to pyrethroid and organophosphorous pesticides, as well as to other compounds (Nordenfors et al., 2001; Abbas et al., 2014), and promoted the presence of residues of these products in eggs (Romão, 2007; Ciscato, 2008; Liebisch et al., 2011; Sparagano et al., 2014; Pritchard et al., 2015). In addition, the production of poultry outside cages has been encouraged, either by the market or by law (Murillo and Mullens, 2016), which, together with restrictions on the use of chemicals in organic production systems, and increased demand for eggs, has reinforced the need for safe and more effective control of poultry mites.

Inert powders are present in the poultry industry, especially hydrated lime and diatomaceous earth. They are used as sanitizers for poultry houses, reducing ammonia production and proliferation of lesser mealworms *Alphitobius diaperinus* Panzer, 1797 (Coleoptera: Tenebrionidae) (Watson et al., 2003; Oliveira et al., 2017). Also, inert powder-based products have been marketed in Europe for over 40 years (Ebeling, 1971; Pritchard et al., 2015) in liquid and powder formulations, interfering with reproduction and survival of mites (Kilpinen and Steenberg, 2009; Maurer et al., 2009; Shulz et al., 2014; Alves et al., 2017).

The acaricidal activity of powders occurs physically by abrasion of the cuticle after first contact, which removes the lipid layer and causes excessive water loss and desiccation (Ebeling, 1971; Kilpinen and Steenberg, 2009; Maurer et al., 2009; Schulz et al., 2014, Amalin et al., 2015). It should be noted that powder application leads to loss of material by dispersing in the environment with formation of dust clouds. For this reason, some products are available in liquid formulations, because they are more advantageous. These products minimise the problems abovementioned, especially pulmonary silicosis (Santesson, 2013; Sparagano et al., 2014). With respect to *D. gallinae*, Maurer and Perler (2006) and Schulz et al. (2014) observed that different silica-based dry and liquid products and preparations exhibited variations in control efficiency. These authors highlighted the acaricidal activity of liquid preparations.

Given that these products are of mineral origin, there is great variation in the physicochemical composition and the morphology of the particles. The effects of these variations on the insecticidal activity have been proven (Korunic, 1997, 1998, 2013). The goals of the present study were to assess the acaricidal activity of inert powders of Brazilian origin used in dry and liquid applications against different development stages of poultry red mite *D*. *gallinae*, and perform a morphological

characterisation of silica-based products.

MATERIALS AND METHODS

Mites

The mites (eggs, nymphs, and adults) were collected in a commercial poultry house. In the laboratory, the eggs were transferred to Petri dishes and the nymphs and fed adult females were placed in glass tubes sealed with voile fabric and kept for 1 day at climate room (25±1°C, 14-h photophase, 70±5% RH) to be used in bioassays.

Products

Twelve products of mineral origin were tested (Table 1). The product PosturaSec®, based on micronized diatomaceous earth - Vet Science Bio Solutions Ltd. (www.vetscience.com.br), was used as a comparison standard. The following controls were used: a group treated with commercial corn starch (in powder or in water suspension) and other group untreated (total control group).

The study was divided into two phases. In the first one, all the products were assessed through dry applications in *D. gallinae* adults. The products that caused a minimum mortality of 90% were selected. In the second phase, these products were suspended in distilled water at 5 and 10% and used against eggs, immature and adult mites.

Acaricidal activity of products against Dermanyssus gallinae

Dry products

Fifteen fed adult female were transferred into glass tube (Ø 2 × 7 cm) sealed with Parafilm® membrane containing sterile filter paper (7 × 7 cm) previously treated with 14 mg (2.8 g/m^2) of each product on the surface. The tubes were kept at room (25±1°C, 70±5% RH, 14 h photophase). In all treatments and controls, seven tubes were used as replicate.

Table 1 - Products assessed *in vitro* against red poultry mite *Dermanyssus gallinae*: composition and manufacturer.

Product	Composition	Manufacturer
A-Bind[1]	Thermo activated natural bentonite	Vet Science
Kaolin[2]	Hydrated aluminium silicates	Vet Science
PosturaSec®[3]	Diatomaceous earth (Diatomite)	Vet Science
Vermiculite DM 430[2]	Vermiculite	Gold Star Brasil
Vermiculite DM 440[2]	Vermiculite	Gold Star Brasil
Calcium carbonate OB 1000[2]	Calcium carbonate	Minérios Ouro Branco
Calcium carbonate OB 1130[2]	Calcium carbonate	Minérios Ouro Branco
Calcium carbonate OB 1170[2]	Calcium carbonate	Minérios Ouro Branco
Kaolin OB 2120[2]	Hydrated aluminium silicates	Minérios Ouro Branco
Kaolin OB 2130[2]	Hydrated aluminium silicates	Minérios Ouro Branco
Talc OB 3130[2]	Magnesium silicate	Minérios Ouro Branco
Talc OB 3510[2]	Magnesium silicate	Minérios Ouro Branco

Note. [1]product indicated as aflatoxin adsorbent for use in aviaries; [2]there is no indication for field use; [3]recommended for faecal drying and environment sanitation in commercial poultry houses.

The mites were daily observed by using a stereomicroscope for five days. Mites were considered dead if no movement was visible after a with a paint brush. The experiment was repeated twice.

Products in aqueous suspension

Direct activity

Ovicidal activity. Mite eggs obtained in the laboratory (24 hours after oviposition) were distributed in Petri dishes and sprayed with 2 mL of the aqueous suspensions (5 and 10% in distilled water) using a Potter tower (0.70 kgf/cm^2). After drying under ambient conditions, Petri dishes were sealed with Parafilm® membrane and kept at the same condition before. Five replicates and control were conducted. The assessments were performed daily, for three days, counting the number of hatched, whether dead or alive, by using a stereomicroscope.

Activity against nymphs and adult D. gallinae. The procedure described above was repeated but applying 1 mL of the suspensions of each product directly to non-sexed nymphs in Petri dishes. To avoid escaping mites, petroleum jelly was applied as a barrier to the edges of the dishes. After drying, the mites were transferred with a paint brush to glass tubes sealed with voile

fabric and kept under the same condition as the eggs.

The same procedures were performed in the test with adults; but 2 mL of suspensions were applied on the mites.

Residual activity

All the same procedure of the tests with the dry products was repeated with suspension of each product, except that the filter paper (7 × 7 cm) was immersed for 10 seconds in the suspensions of each product with concentrations of 5 and 10%.

Morphological characterisation and identification of diatom species in silica-based products

For genus identification and size determination of diatom frustules (whole or fragment), a sample of 1 g of product was suspended in 50 mL of distilled water, and 0.1 mL aliquots were used in permanent slides. This permanent slides were made using Naphrax® as mountant (refractive index 1.74; Brunel Microscopes Ltd.). Photomicrographs were obtained in a microscope coupled digital camera. Diatom frustules were measured (greater and smaller length) with Axio Vision 4.8 software (Carl Zeiss Vision\AxioVision 4\AxioVs40). The identification of genera was based on Krammer and Lange-Bertalot (1991), Round et al. (2005), Metzeltin et al. (2005), Metzeltin and Lange-Bertalot (2007) and Costa et al. (2017).

Relative abundance and density was obtained by counting valves in transects at 1000 X magnification. The minimum valve count threshold was 400 in total, and minimum counting efficiency was 90% as Pappas and Stoermer (1996): Efficiency = 1 - (number of species/total number of valves).

Density was determined using the sedimentation method (Battarbee, 1986). The results of density of diatomaceous earths were expressed in individuals/g and obtained using the formula below:

$$N = (n \times \frac{AL}{(Nc \times Ac)} \times \frac{V}{v}) / M$$

where: N = no valves/g dry weight; n = number of counted valves; AL = cover slip area; Nc = number of fields counted; Ac = field area (mm^2); V = volume of the initial sample; v = volume of the subsample (mL); M = mass of the initial sample (g).

Statistical Analysis

The experiments were performed according to a completely randomised design. Mortality values were corrected using Schnider-Orelli formula (Püntener, 1981), and subsequently analysed for homogeneity using the Cochran test. For the parametric data, the statistical analysis to detect significant differences between treatments (comparison of the activity of the products and concentrations was conducted with one-way ANOVA and ANOVA Factorial with Tukey HSD test ($p \leq 0.05$)). For the non-parametric data, Kruskal-Wallis test ($p \leq 0.05$) using STATISTICA 7.1 (StatSoft Inc., USA, 2005).

RESULTS AND DISCUSSION

Acaricidal activity of the products against Dermanyssus gallinae

Dry products

All products showed activity against adult mites, ranging from 39 to 100% of mortality. The products Kaolin 2120, Kaolin 2130, PosturaSec®, and Talc 3130 were the only ones to cause mortality above 95%. So, they were selected for the next step (Table 2).

Previous studies proved the susceptibility of *D. gallinae* to some kind of silica-based and others mineral powders, with a great variation in the mortality (Kilpinen and Steenberg, 2009; Maurer et al., 2009). In this sense, it was observed in a comparison of acaricidal

activity of 12 silica-based products that morphological characteristics of the particles were determinant for the greater activity of some products to the red mite, reinforcing the physical basis of the acaricidal activity of silica (Schutz et al., 2014).

As observed for diatomaceous earth, hygroscopicity has also been showed by other minerals and, in this sense, kaolins possess adsorptive and abrasive activity. They are largely used as vehicles for insecticides and, in addition, some of them have insecticidal activity according to their granulometric ranges (Glenn and Puterka, 2005; Amalin et al., 2015; Sarwar, 2016).

Even though acaricidal activity of Talc 3130 (98.1%) and Talc 3510 (39% mortality), in others study (Schulz et al. 2014; Kilpinen and Steenberg, 2009), talc and kaolin were considered to be less active against mites. It is generally accepted that besides minerals, other molecules may be present as adjuvants in the formulations, which have acaricidal activity that may affect the mites. Similarly, it is known that acaricidal activity is influenced by the origin, morphological characteristics, chemical composition of the mineral particles, surface chemistry in contact which can explain the activity of some talc and kaolin tested in this study (Japp, 2008; Schulz et al., 2014).

Products in aqueous suspension
Direct activity

Ovicidal activity. None of the tested products exhibited ovicidal activity against *D. gallinae*. Hatching values ranged from 84 to 90%. These corroborate some of the results of the study conducted by Schulz et al. (2014) with powdered and liquid minerals used against eggs of *D. gallinae*, mainly which based on natural silica, with 90% of hatching. In addition, as in the present study, it was observed that there was mortality in newly hatched larvae (76 to 91% without significative difference) when they came in contact with the applied products (Table 2).

Activity against nymphs and adult D. gallinae. Mortality obtained with nymphs was higher than with the adults. The products Kaolin 2130 and PosturaSec®, both at 5% concentration, stood out causing nymphs mortality of 95.7 and 98.9%, respectively. There was no significant difference between the products at 10% concentrations (mortality ranging from 89 to 100%). Regarding adults, the values obtained with a 1% concentration were significatively lower than obtained for the others concentrations. Besides, only PosturaSec® was different from control (49.5% mortality). The mortality obtained at 5 and 10% concentration was not different from each other, ranging from 67% (Kaolin 2120) to 100% (PosturaSec®) (Table 2).

In the most treatments, for both nymphs and adults, increasing the dose results in higher mortality values. This fact was attributed to the greater coverage of the mites, and the abrasive and lipophilic character of the products, mainly PosturaSec®, which is based on silica (Harrington et al., 2011). Even with the lowest concentration, mortality achieved by PosturaSec® can be attributed to the porosity of the diatom frustules that produce high hygroscopicity. This product is able to absorb water up to four times its weight and, thus, cause the death of the mites by dehydration (Horn-Veiga and Son, 1980).

As there was no significant difference between them, the concentration of 5% can be recommended for the control of the mites, representing efficiency at low cost to the producer. Diatomaceous earth is highly absorbent and abrasive due to the amount of silica (95% silica [SiO_2], alumina (2 to 4%), and hematite (0.5 to 2%) (Khraisheh et al., 2004; Wu et al., 2005). Its mode of action is

characterised by physical control and does not depend on metabolic pathways. This way, mites can hardly develop resistance. However, is it possible they may develop behavioural mechanisms in order to avoid contact with these powders (Ebeling, 1971; Chauve, 1998; Maurer et al., 2009). For this reason, it can be inferred that, in addition to reducing the amount of product applied (less waste), the liquid form does not harm the health of the animals and the individuals, and allows greater coverage of the treated surface.

Table 2 - Mortality of nymphs and adult of *Dermanyssus gallinae* after spraying of inert powder suspensions at different concentrations.

Treatment	Concentration	
	Immature	
	5%	10%
Control	0.0±0.0 Ea	0.0±0.0 Ca
Starch	9.4±5.0 Da	6.6±9.1 Ba
Postura Sec®	98.9±2.8 Aa*	98.9±2.8 Aa*
Kaolin 2120	54.8±27.8 Cb*	95.7±8.5 Aa*
Kaolin 2130	95.7±5.9 Ba*	100.0±0.0 Aa*
Talc 3130	58.1±20.8 Cb*	89.2±15.0 Aa*
	Adult	
	5%	10%
Control	0.0±0.0 Ca	0.0±0.0 Ca
Starch	5.9±8.7 Ca	0.7± 1.1Cb
PosturaSec®	98.8±3.1 Aa*	100.0±0.0 Aa*
Kaolin 2120	67.4±11.5 Ba*	72.1±6.2 ABa*
Kaolin 2130	77.1±25.2 ABa*	73.3±18.2 ABa*
Talc 3130	81.4±16.8 ABa*	84.9±20.7 Ba*

Means (±SE) followed by a lowercase letter in the row for each life stage of development and upper case letter in the column did not differ from each other according to the Kruskal-Wallis test; *Differed significantly from the respective control according to the Dunnet's test ($p<0.05$).

In addition, it was observed that liquid formulations of diatomaceous earth had a higher residual effect in comparison to powder applications, causing above 90% mortality (Maurer and Perler 2006; Maurer et al., 2009). Recently, Schulz et al. (2014) demonstrated that liquid formulations were more effective against *D. gallinae*, exhibiting lower mean lethal time in comparison to powder applications. Also, the authors highlighted the fact that instantaneous death of mites occurred by drowning in the droplets applied onto them and an additional acaricidal effect by the silica present in the product.

The activity of diatomaceous earth depends on a great number of factors which include environmental conditions (such as air humidity), particle size and shape, diatomaceous origin, type and purity of the formulation, as well as diatomaceous pores that may be related to a higher adsorptive capacity of lipids and water (Korunic, 2013, 2016). In this sense, significant differences in mortality of *Alphitobius diaperinus* were observed when submitted to diatomaceous earth from four regions of Argentina (Japp, 2008).

Based on these characteristics that may influence the insecticidal activity of diatomaceous earths, studies to their characterisation are important in order to know the morphological profile of the components.

Residual activity

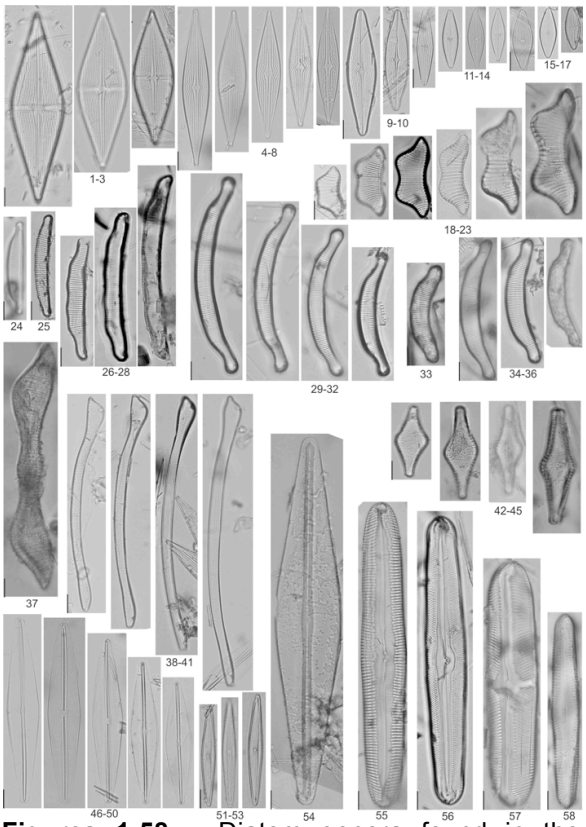
All products selected sprayed on the filter papers exhibited high acaricidal activity mainly PosturaSec® that was the most effective, leading to 100% mortality in both concentration. Although there was not statistical difference between them (85 to 100% mortality). This confirms that mites were able to picks up enough product to be killed. This also suggests the need of thoroughly treatment of all surfaces in a shed and also the need for reapplication where the treated surfaces become covered by debris and dust (Kilpinen and Steenberg, 2009).

Morphological characterisation and identification of diatom species in the product PosturaSec®

There was predominance of the classes Bacillariophyceae (six genera) and Fragilariophyceae (one genus). *Brachysira* Kützing stood out with an

occurrence of 56.8% (Table 3, Figure 1). The density was 5.7 x 10^7 valves/g and the maximum size was 200 µm for the frustules, and 3 µm for non-identifiable fragments. Still, according to the manufacturer, PosturaSec® has 86.2% of silicon.

Such results are similar to those obtained by Alves et al. (2017), who assessed a similar product to control the lesser mealworm. The authors found eight genera of the class Bacillariophyceae and one of the class Fragilariophyceae. As observed in the present study, they also found predominance of the genus *Brachysira* Kützing. The size of the diatom frustules was also similar, varying from 3 to 120 µm.



Figures 1-58 – Diatom genera found in the diatomite sample: 1-17 - *Brachysira* Kützing; 18-37 - *Eunotia* Ehrenberg; 38-41 - *Actinella* Lewis; 42-45 - *Fragilariforma* Williams and Round; 46-50 - *Frustulia* Agardh; 51-53 - *Encyonopsis* Krammer; 54 - *Frustulia* Agardh; and 55-58 - *Pinnularia* Ehrenberg; Scale = 10 µm.

However, Japp (2008) analysed samples of diatomaceous earth from different origins in Argentina and found greater diversity of algae of the genus *Fragilaria*, present in all samples analysed. The genus *Aulacoseira* was found in only one sample, proving the existing relationship between the origin of the mineral and its morphological characteristics.

Morphological characterisation is important to explain the activity of inert powders, given that it is directly related to the physical properties of the particles, such as size, pore diameter, and moisture content (Schulz et al., 2014). With respect to diatomaceous earth, it is also necessary to assess silicon contents, given that the direct relationship of the activity with the amount of silicon dioxide, and the inverse relationship with particle size and uniformity have been proven (ideally less than 10 µm) (Korunic, 2013).

Table 3 – Identification and determination of diatom frustules present in the product Postura Sec®.

Genus/Family	Number of Valves	%
Brachysira Kützing / Bacillariophyceae	300	56.8
Frustulia Agardh / Bacillariophyceae	156	29.5
Eunotia Ehrenberg / Bacillariophyceae	31	6.0
Encyonopsis Krammer / Bacillariophyceae	20	3.7
Pinnularia Ehrenberg / Bacillariophyceae	14	2.6
Actinella Lewis / Bacillariophyceae	5	1.0
Fragilariforma Williams and Round / Fragilariophyceae	2	0.4

Specifically for *D. gallinae*, It was found that the smaller the size of the diatomaceous earth particles, the greater the acaricidal activity. This fact is due to the ease of adhesion, abrasion, and desiccation (Schulz et al., 2014). Other effects of diatomaceous earth include the asphyxia caused by the closure of the spiracles due to the presence of small particles (Korunic, 2016).

CONCLUSION

Based on our results, it can be affirmed that PosturaSec® exhibits

desirable characteristics and can be used against *D. gallinae*, both in the dry powder and liquid formulations. Tests performed in poultry houses are recommended in order to confirm the acaricidal activity of the product and its capacity to reduce mite populations.

Regarding the other products tested in the present study (Kaolin 2120, Kaolin 2130, and Talc 3130), a more detailed analysis is required to assess their composition, particle size, and mode of action. Also, field tests should be performed to determine the efficiency of the products.

ACKNOWLEDGMENTS

To CNPq, CAPES and Vetscience Biosolutions for financial support to this research.

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