An in vitro evaluation of the sensitivity and responses of *Dermanyssus gallinae* to selected acaricides

Sylwia Koziatek-Sadłowska [1], and Rajmund Sokół

Department of Parasitology and Invasive Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

ABSTRACT *Dermanyssus gallinae* is an obligatory ectoparasite of birds which feeds on blood and significantly compromise the well-being of commercially raised laying hens. In this study, the mortality rates and responses of *D. gallinae* to 2 acaricides with a physical mode of action (Dergall and Mite Max) and 2 acaricides with a chemical mode of action (Milben Ex and Bio PK) were evaluated in tree dilutions (S1−3) and compared at 8-time intervals after application. The evaluation involved a novel method that simulates real-world conditions in a commercial poultry farm. Tested products have shown high efficacy (84.3−100%) against *D. gallinae* in the producer recommended solution (S1). Acaricides with a physical mechanism of action were as effective as chemical agents in eradicating poultry red mites. The compared preparations differed only in the onset of action which was longer in acaricides with a physical mode of action (1−6 h for chemical 24 h for physical in S1). An increase in the concentration of the active ingredient did not significantly speed up the onset of action of the evaluated preparations. However, the efficacy of Dergall and Bio PK decreased when the applied dose was halved, to 12% and 0% respectively. A decrease in the dose Mite Max led to a somewhat smaller, but not statistically significant decrease in mite mortality rates (74%). The proposed method for evaluating acaricide efficacy can be helpful in selecting the most effective preparations and the optimal concentration of the working solution to be applied in commercial layer farms, thus reducing the costs associated with the eradication of *D. gallinae*. The developed method enables a reliable evaluation of acaricides with both a physical and chemical mode of action, and it supports observations of the parasites' responses to the applied treatment.

Key words: *Dermanyssus gallinae*, poultry red mite, acaricide, control, laboratory study

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BACKGROUND

Dermanyssus gallinae (De Geer, 1778) (poultry red mite) of the genus *Dermaniside* is an obligatory and transient-feeding ectoparasite of wild birds and poultry. Poultry red mite feed on blood and significantly compromise the well-being of commercially raised laying hens. High and constant temperature, high humidity and unlimited access to hosts promote ectoparasite invasions in hen houses and farms. Mites readily colonize and reproduce inside cracks and crevices in hen houses, and infestations are particularly difficult to eliminate in such structures. Mite infestations cause heightened irritation, anxiety, itch, and chronic stress in the affected birds (Kowalski & Sokół, 2009; Roy et al., 2009; Sokół et al., 2019; Sparagano et al., 2014). Parasite invasions compromise the health and well-being of poultry, decrease hen performance, mainly egg production and egg quality, increase feed consumption and mortality rates (Cencek, 2003; Fiddes et al., 2005; Guy et al., 2004; Hoglund et al., 1995; Koziatek-Sadłowska & Sokół, 2020; Sparagano et al., 2009; 2014). In Europe, the losses caused by *D. gallinae* and the costs associated with eradicating mite infestations are estimated at EUR 231 million per year (Flochlay et al., 2017). Poultry red mite infestations are controlled mainly with the use of synthetic acaricides (approx. 35 chemical substances have been described in the literature, included: organochlorines, organophosphates, pyrethrin, pyrethroids, carbamates, amitraz) as well as natural preparations (Chauve, 1998). Both types of products have certain limitations. Their toxic metabolites can accumulate in eggs and meat (Marangi et al., 2012). For this reason, only three active ingredients (foxime, fluralaner, and spinosad) have been approved for use in poultry

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[1]Corresponding author: sylwia.koziatek@uwm.edu

(Thomas et al., 2018). Some mite strains have developed resistance to multiple pyrethroids due to the widespread and uncontrolled use of acaricides in poultry farming (Abbas et al., 2014; Beugnet et al., 1997; Marangi et al., 2009; Puvača et al., 2019; Tomley & Sparagano, 2018; Zdybel et al., 2011). To minimize the adverse effects of acaricidal substances, their efficacy should be tested before use under conditions that are specific to a given poultry farm and mite population. Alternative methods of controlling mite invasions are also being developed like preparations with a physical mechanism of action (Koziatek & Sokół, 2015). For example products comprising natural or synthetic silica (silicon dioxide, SiO_2). Silica particles adhere to the body shells of *D. gallinae*, absorb lipids from the exoskeleton which leads to drying out, and results in the death of the parasite (Kilpinen & Steenberg, 2009). In recent years net-like polymer structure products have been place on the market. This products immobilize mites. In our opinion these acaricides require new assessment methods that involve observations of the parasites' behavior.

In vitro evaluations of acaricide efficiency focus mainly on parasite mortality rates within a specific period of time. In the present study, a novel method was proposed for assessing acaricide efficacy (time until mite death) and observing the responses and behavior of mites during and after treatment. In our opinion, such observations are essential in analyses of acaricide efficacy. The proposed method involves Petri dishes and parafilm, and it reliably simulates the conditions inside hen houses and, most importantly, facilitates observations in any time interval.

Therefore, the aim of this study was to evaluate in vitro the sensitivity of *D. gallinae* to selected acaricides with a chemical and physical mode of action using a novel method for monitoring mite responses.

MATERIALS AND METHODS

Dermanyssus gallinae *Sampling*

To determine the resistance of *D. gallinae* to the tested preparations, poultry red mites in various stages of development were collected from battery cages in a commercial layer farm where acaricides containing the evaluated active ingredients had not been previously applied (Table 1). Mites were collected with a system of traps (Sokół & Romaniuk, 2007). The trap system relies on the natural behavior of female mites which lay eggs inside cracks and crevices in hen coops that serve as mite refugia. The trap system consisted of calibrated paper tubes that were attached directly to egg conveyor belts under hen cages (5 traps per cage), at an estimated height of 170 cm, in week 36 of the laying season. After 14 d, mites were collected into 1 L glass jars and transported to the laboratory for analysis.

Acaricides Targeting D. gallinae

Acaricides with a chemical (Milben Ex, Schopf Hygiene, Neubeuern, Germany) and Bio PK (Biochem, Trzęsów, Poland) and physical (Dergall, ICBpharma, Jaworzno, Poland) and Mite Max (Barrettine, Bristol, UK) mode of action were evaluated in the study. Each preparation was tested in three concentrations and 5 replicates. The active ingredients, the concentrations of working solutions, and the mode of action are presented in Table 1. Working solutions were prepared directly before the experiment. A measured amount of the tested preparation was poured into a medical atomizer, supplemented with distilled water to a given volume, and the mixture was shaken.

Preparation of Mites and Petri Dishes

Traps containing mites were cooled at a temperature of −18°C for 20 m to induce hibernation and facilitate mite transfer to Petri dishes (with a diameter of approx. 7 cm). Using a brush, around 60 mites (adults and nymphs) were placed in the center of each dish. The analyzed acaricides in each tested concentration were sprayed onto the mites from a distance of around 30 cm with the use of a medical atomizer. Five hundred microliter of the prepared working solution or distilled water was applied to each dish (3 squirts), making sure that the entire surface of the dish was covered. The edges of each dish were wrapped with a 2 cm × 10 cm strip of Parafilm M (PM966, Bemis, Sheboygan Falls, WI), and the dish was covered with a glass lid. The dishes were placed inside a cuvette, and cuvette edges were covered with glycerin to prevent *D. gallinae* from leaving the controlled area. The cuvette was covered with thin black fabric (mites have a preference for darkness) to prevent mites from escaping and to promote adequate ventilation.

Each acaricide was tested at 3 different concentrations labeled S1, S2, and S3. Treatment S1 was a

Table 1. Tested acaricides.

Formulation (Producer)	Active ingredient	Tested solution	Mode of action	Volume of working solution/m^2
Milben Ex (Schopf Hygiene)	Bifenthrin Amitraz	2%*,1%, 4%	Chemical	50 L/1500 m^2
Bio PK (Biochem)	Hydrocarbons	4%*, 2%, 8%	Chemical	-
Dergall (ICBpharma)	Heptamethyltri-siloxane modified with an alkylene oxide	0.5%*, 0.25%, 1%	Physical	50−70 mL/m^2
Mite Max (Barrettine)	Cellulose polymer	11%*, 5.5%, 22%	Physical	9.5 L/200 m^2

*Working solution recommended by the producer.

Table 2. Mortality of *D. gallinae* (%) in response to different concentrations of the tested preparations at various time intervals after treatment.

Treatment	Concentration	Mortality rates (mean ± SD (%))							
		0.5 h	1 h	2 h	3 h	6 h	9 h	12 h	24 h
Milben Ex	S1	88.3 ± 3.4	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0[a]
	S2	51 ± 2.7	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0[a]
	S3	90.3 ± 1.9	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0[a]
Bio PK	S1	50.7 ± 2.1	64 ± 3.0	78.6 ± 3.6	86.3 ± 3.1	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0[a]
	S2	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
	S3	50.3 ± 5.0	53 ± 4.5	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0[a]
Dergall	S1	89.7 ± 3.2	51 ± 4.3	71.7 ± 4.7	97.7 ± 0.9 ±	98.7 ± 0.8	98.7 ± 0.8	98.5 ± 0.8	98.8 ± 0.8[a]
	S2	89.3 ± 5	57.3 ± 4.0	48.3 ± 5.2	50 ± 5.6	49.7 ± 5.5	49.3 ± 5.6	45.7 ± 6.6	12 ± 2.3[b]
	S3	92 ± 2.7	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0[a]
Mite Max	S1	86 ± 2.2	84.3 ± 2.9	84.3 ± 2.9	84.3 ± 2.9	84.3 ± 2.9	84.3 ± 2.9	84.3 ± 2.9	84.3 ± 2.9[a]
	S2	3.7 ± 0.8	7 ± 0.8	42.7 ± 2.0	49.7 ± 3.1	52.3 ± 3.1	60 ± 2.4	74 ± 2.9	74 ± 2.9[a]
	S3	90.3 ± 1.9	99.3 ± 0.6	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0[a]
Distilled water	C1	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	1.7 ± 1.0	2.7 ± 1.5[b]
Not treated	C2	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0[b]

[abc]Different letters denote significant differences between the tested preparations and their concentrations 24 h after application ($P \leq 0.05$).

producer-recommended solution (**PRS**); treatment S2 had a concentration of 50% PRS, and treatment S3 − 200% PRS. Two control groups, C1 and C2, were established. Group C1 mites were sprayed with distilled water only, and group C2 mites were not sprayed with acaricide or distilled water. The study was conducted at a temperature of 21°C and relative humidity of 75%. Dead mites were counted 0.5, 1, 2, 3, 6, 9, 12, and 24 h after treatment under a Leica binocular magnifier (40x). The results were expressed as the percentage of dead mites with standard deviation (mean of 5 replicates) at different time intervals (Table 2). The tested preparations' efficacy was evaluated based on the above values.

Statistical Analysis

The significance of differences ($P \leq 0.05$) in mite mortality rates induced by the tested concentrations of each acaricide 24 h after treatment was determined by Kruskal-Wallis ANOVA. Data were analyzed statistically in Statistica 13 for Windows.

Evaluation of D. gallinae *Behavior*

The responses of *D. gallinae* to various concentrations of the tested preparations were evaluated based on the following behaviors: mite motility (escaping/motile mites), leg movement, and clustering. Each behavior was evaluated on a 4-point scale (−, +, ++, +++), and the analyzed responses were classified as absent (−), weak (+), moderate (++), and strong (+++). Mite behaviors were evaluated immediately after spraying and at each time interval during the experiment.

RESULTS

Mite Sensitivity

The efficacy of the tested acaricides was evaluated based on the percentage of dead mites (%) treated with each concentration of the prepared solutions at each time interval. The results are presented in Table 2. The experiment was conducted for 24 h. The efficacy of Milben Ex at all tested concentrations (S1−S3) was 100% after 24 h. Bio PK was 100% effective at concentrations S1 and S3, whereas treatment S2 was not effective. Twenty-four hours after application, the efficacy of Dergall was 98.8%, 12%, and 100%, and the efficacy of Mite Max was 84.3%, 74%, and 100% at concentrations S1, S2, and S3, respectively.

Milben Ex was characterized by the highest acaricidal efficacy and the most rapid onset of action. Its efficacy reached 100% at all tested concentrations already one hour after application. Bio PK was 100% effective at concentration S1 after 6 h and at concentration S3 after 2 h. Dergall S1 was 100% effective at concentration S1 after 6 h and at concentration S3 after 1 h. However, when applied at concentration S2, mites were only immobilized in the first hours after treatment (mites were not responsive and were classified as dead): 89.3% of mites were classified as dead 1 h after application, but motility was restored in some mites 2 h after treatment, and only 57.3% of mites were classified as dead. Mite Max was 100% effective at concentration S1 after 1 h, at concentration S2 after 12 h, and at concentration S3 after 2 h.

Unlike Bio PK and Dergall which did not exhibit acaricidal activity when applied at concentration S2, a decrease in the dose of Milben Ex and Mite Max did not lead to a significant reduction in mite mortality rates. Bio PK, Dergall and Milben Ex were characterized by faster onset of action when applied at concentration S3.

Mite Behavior

The responses of *D. gallinae* immediately after application (0−0.5 h) of the tested acaricides are presented in Table 3. In control group C1, mites were immobilized in large droplets of water that had formed on the surface of Petri dishes. Rapid leg movements were initially

Table 3. Mite responses to the tested acaricides (0−0.5 h after application).

Mite responses	Group													
			Milben Ex			Bio PK			Dergall			Mite Max		
	C1	C2	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Motility	−	+++	−	−	−	++	+	++	−	++	−	+	+	−
Leg movement immediately after treatment	+++	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Clustering	++	++	−	−	−	−	++	−	−	+	−	+	+	−
Escape outside the dish	++	+++	−	−	−	−	+++	−	−	++	−	+	+	−

+ weak, ++ moderate, +++ strong, − no response.

observed, and they gradually subsided in successive hours of observation. Six hours after treatment, water had completely evaporated, and most mites formed small clusters (several mites each) under the parafilm. Under the influence of light, the few mites remaining on the dish moved up the edges in the direction of parafilm and outside the dish. Water had no significant influence on mite mortality which was determined at 1.7% 24 h after treatment and did not differ significantly from the mortality rate in group C2.

In control group C2, mites moved up the edges of the Petri dish immediately after placement and formed clusters in parafilm folds. The number of mites decreased in successive hours, which suggests that they had continued to escape from the dish. Petri dishes did not contain live or dead mites 24 h after treatment. The mortality rate was determined at 0%.

Similarly, to the observations made in group C1, mites treated with Milben Ex were immobilized in droplets of the solution. Rapid leg movements were observed directly after treatment, but they were weaker after 0.5 h and completely subsided after 1 h when the mortality rate reached 100%. Similar observations were made at all 3 concentrations of Milben Ex.

Bio PK induced different responses at each tested concentration. Treatment S1 produced stains which immobilized mites on the surface of the dish. Around 3 h after application, Bio PK evaporated from the surface of the dish, but it left an oily residue that considerably slowed down mite movement. Treatment S2 produced similar responses, and mortality rates were similar to those noted in control group C1 (Table 2), which implies that this dose did not exert acaricidal effects. Treatment S3 also produced droplets on the surface of the dish, and rapid leg movement was initially observed in mites that had been captured in the droplets. Leg movements subsided completely after 2 h and were not observed in successive time intervals.

Dergall immobilized mites at each tested concentration. Rapid leg movements were observed, and mites appeared to be sliding on the surface of the dish. Unlike the previously described preparations, Dergall did not form droplets or stains. Leg movements were weaker after 30 min and completely subsided in successive hours. Mites that were immobile and did not move their legs were classified as dead. However, mites were only temporarily immobilized under exposure to treatment S2, and motility and leg movements were restored in the following hours. For this reason, mortality rates differed in subsequent time intervals.

Mite Max immobilized all mites when applied at concentrations S1 and S3. Immobilization was considerably delayed in response to treatment S2, but despite the above, this preparation was characterized by high acaricidal efficacy.

DISCUSSION

Live *D. gallinae* are a difficult object of study under laboratory conditions because these mites move rapidly across horizontal and vertical surfaces and are sensitive to light (negative phototropism). The proposed research method relatively accurately simulates real-world conditions in a commercial poultry farm, including smooth surfaces of metal objects, the location of mite refugia (in crevices between the parafilm and the edges of the Petri dish) and ventilation (to facilitate product evaporation). In the authors' opinion, the developed method is an effective tool for monitoring the behavior of *D. gallinae* and evaluating the efficacy of acaricides with both a physical and chemical mode of action. The designed method also relies on widely available materials. Various tools for assessing the efficacy of acaricidal preparations in vitro have been described in the literature. These include paper filters saturated with the tested substance, Petri dishes for immobilizing mites (Baran et al., 2020; Beugnet et al., 1997; Tabari et al., 2020), and a system of glass tubes covered with pesticides (Zeman & Zelezny, 1985). However, none of the described approaches simulate real-world conditions in poultry houses, in addition, mites are unable to escape the applied treatment. According to Thind and Ford (2007), an effective laboratory setup for monitoring mite behavior should be tight, and it should prevent mites from escaping. This approach should be adopted to determine the toxicity of the analyzed acaricidal substance. However, real-world conditions in a commercial farm should be simulated in a laboratory setting to ensure that the results obtained in vitro are consistent with the results of in vivo experiments. For this reason, in the present study, mites were not completely prevented from escaping, but their movement was somewhat restricted with the use of parafilm and a glass lid.

A similar approach had been adopted by Cencek et al. (2011). In the cited experiment, mites were placed on a veneer disc inside a glass plate, and edible oil was poured into a groove surrounding the plate to prevent mites from escaping.

In the current study, the efficacy of acaricides with chemical and physical mode of action was tested at 8-time intervals within 24 h of application. This approach has been rarely used in the research on *D. gallinae* susceptibility to acaricide. In most studies, observations are made only once, usually 24 h after the application of the tested preparation. The method proposed in the present study supports an evaluation of a product's onset of action and efficacy. This study demonstrated that preparations with a physical mechanism of action are as effective as chemical acaricides. The compared products differed only in the onset of action which was longer in acaricides with a physical mode of action. An increase in the concentration of the active ingredient did not significantly speed up the onset of action of the evaluated preparations. However, the efficacy of Dergall and Bio Pk decreased when the applied dose was halved. A decrease in the dose of Milben Ex ad Mite Max led to a somewhat smaller, but not statistically significant decrease in mite mortality rates. These observations indicate that the effective concentration of a given acaricide should be tested by the poultry producers before use because higher doses are not always more effective, but they increase pesticide costs.

CONCLUSIONS

This study demonstrated that acaricides with a chemical and physical mode of action are equally effective in eliminating *D. gallinae* through direct exposure. Their efficacy is determined mainly by the time of exposure, and it is dependent on the concentration of the working solution to a lesser extent. The proposed method for evaluating acaricide efficacy can be helpful in selecting the most effective preparations and the optimal concentration of the working solution to be applied in commercial layer farms, thus reducing the costs associated with the eradication of *D. gallinae.*

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DISCLOSURES

The authors declare that there is no conflict of interest

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