NEW IN VITRO METHOD FOR DETERMINATION OF ACARICIDE EFFICIENCY AGAINST DERMANYSSUS GALLINAE MITES

TOMASZ CENCEK, JACEK KARAMON, JACEK SROKA, AND JOLANTA ZDYBEL

Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland
tcencek@piwet.pulawy.pl

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Abstract

The aim of presented investigation was to estimate the usefulness of a new method for determining the efficiency of acaricides against red poultry mites. The special Plexiglas plates were used in order to imitate natural conditions as close as possible. The investigation was conducted on the mites collected from 32 battery cage farms of laying hens. Nine commercial acaricides were used. The efficacy of these acaricides was investigated in four repetitions. The results showed that together with the increasing mite numbers used for the study, a standard deviation (SD) on the average of scores on four test plates underwent a reduction and did not exceed 10 for more than 80 mites per plate. At the same time, it was observed that the SD of fragmentary results was dependent on the effectiveness of the acaricides. Relatively low coefficients for the variation of the obtained results and lack of statistically significant differences for measurements obtained under conditions of repeatability (P=0.415138) and reproducibility (P=0.43594), illustrate that our method is useful for the estimation of efficacy of acaricides against Dermanyssus gallinae mites.

Key words: Dermanyssus gallinae, acaricides, efficiency, in vitro method.

Dermanyssus gallinae (De Geer, 1778) – red poultry mite - is one of the most dangerous external parasites of birds. This mite primarily attacks gallinaceous birds, but also infects other bird species, numerous mammals, and humans. Red poultry mites are typical periodic parasites i.e. they attach to the host mainly in the evening and night, only for the period of essential food uptake - although this behaviour can change under conditions of industrial breeding (5). During the day, they are usually hidden in the slits of cages, on surfaces of feeders, in nesting boxes, and wall crevasses of farm buildings creating colonies into their thousands (2, 6).

The control of red mites is made more difficult because of their concealment in difficult inaccessible places; their exceptional ability to survive in such places over a long period (even one year without food), extraordinary fertility, as well as high and rapid resistance to acaricides insures their proliferation. Therefore, ongoing research into the preparation of new acaricides and using them in the control of the D. gallinae infection are extremely necessary. However, before releasing them onto the market, research on determining their effectiveness is necessary. The Department of Parasitology and Invasive Diseases, National Veterinary Research Institute in Pulawy recognised a need to carry out the investigation under conditions that imitate natural conditions as close as possible, and therefore designed its own method for determining the effectiveness of combined acaricides against red poultry mites. Because there was no reference materials and relatively high dispersal of results, the determination of the control conditions of the examinations and repeatability was necessary.

The aim of this investigation was to estimate the usefulness of the elaborated method for determining the effectiveness of combined acaricides against D. gallinae and estimation of repeatability and reproducibility of this method.

Material and Methods

Mites. The investigation was conducted on red poultry mites collected from 32 battery cage farms of laying hens. The mites were collected from cages into tightly closed plastic containers (volume 150 ml). Once the mites were collected, they were then divided into six containers (about 1-3 g of parasites in each container). The investigations were carried out on living mites 2-3 d after collection from the farm.

Acaricides. The following acaricides were used for the examination: Alfasekt (active substance – α cypermetrin), Blaxime (active substance – phoksim), Bye mite (active substance – phoksim), Ficam (active substance – bendiocarb), Galtox B (active substance –
fenitrothion, dichlorphos, α - cypermetrin), Knox-out (active substance – diazinon – microcapsules form), Master (active substance – chlorpyriphos - microcapsules form), Skorpion (active substance – sevin, α - cypermetrin), Sumition (active substance – fenitrothion - microcapsules form).

**Evaluation of acaricide efficacy.** Investigation into the efficacy of acaricides was carried out on plates presented in Fig. 1.

The investigation was carried out according to the following method: The veneer disc (diameter of 9 cm), imitating the so-called rough surface, was cemented in place with aquarium silicon to the central field of the plate up to the grooves filled with edible oil (substance, which is the barrier for mites). On the surface of the disc a measured amount of acaricide solution was distributed. Solutions and amounts of working liquid were being applied to the surface according to recommendations from the producers. The *D. gallinae* mites were put on the surface of the dripped discs (about 100 mites per disc) after drying (after 24 h). After 24 h, the dead mites were counted (using a stereoscopic microscope and reflected light) - including mites that were incapable of moving (even after delicate touching with the end of the preparation needle).

Next, all the mites were counted for a second time, after immobilising possible living parasites through fastening them to the surface of the disc with transparent shrink-wrap. It was assumed that the number of living mites is the result of a subtraction between the number of dead mites and the total number of mites found on the plate. Each examination was carried out in four replicates (so-called partial examinations). Four plates constituted the control group with moistened discs with water without acaricides. These control plates were treated in the same way as the plates containing discs dripped with acaricide solutions. For each plate containing the disc dripped with a solution of acaricides, a mortality rate of mites was calculated with the correction taking the mortality in the control group into consideration (so-called Abbott correction). An average constituted the final count, around four repetitions.

**Statistical analysis.** Statistical calculations were done with the use of STATISTICA 7 PL. Normal distribution of results was tested by the Shapiro-Wilk test and differences of variation by the Levine test. The statistical significance of differences was estimated by the Friedman ANOVA test and *t*-Student’s test. The charts of dispersion were also analysed.

**Estimation of important parameters for control of correctness of examination.** The influence of two parameters (number of mites used for experiment and mortality in the control group) on the variation of individual examinations, meant by standard deviation, was estimated on the basis of results obtained during the efficacy study of nine acaricides against 32 red poultry mite strains. The calculations based on analysis of dispersion of observation were used.

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**Fig. 1.** Scheme of the plate for examination of acaricide efficacy against *D. gallinae* mites (a – top view, b – profile; 1 – plexiglas plate, 2, 4 – grooves filled with edible oil, 3 – central pole, 5 – veneer disc dripped with an acaricide solution, 6 – silicone glue, 7 – oil).
Estimation of repeatability and reproducibility of the mite counting method. The veneer discs were put on six plates and dripped with insecticides with a mean efficacy of about 30%. Then about 100-150 poultry mites were put on each plate. After 24 h, two examiners counted the living mites and all mites on each plate. Counting was repeated six times for each plate. Statistical significance of differences - difference between results obtained by the same examiner in successive repetitions and difference between results obtained by both of them – were estimated by non-parametric Friedman ANOVA rank test.

Estimation of repeatability of the method for examination of acaricides efficacy against Dermanyssus gallinae. Efficacy study of acaricide (preliminary estimated efficacy was about 30%) was conducted. The examinations were repeated 12 times. The results were then divided randomly in two series (six results in each series). The coefficient of variation (CV%) was calculated for each series of results. Statistical significance of differences of results obtained in both series was calculated by the t-Student test.

Results

The mean results for the efficacy of individual acaricides against 32 D. gallinae strains were presented in Table 1 (because of a large range of results and occurring results, which significantly differed from the average values, median was regarded as more representative).

Estimation of important parameters for control of correctness of examination. It was found that with the use of the Shapiro-Wilk test, the distributions of results for the efficacy examination were not normal distributions. For this reason, during the next analysis, it was decided to base the results mainly on the analysis of the results dispersion.

Dependence between numbers of mites put on the plate and standard deviation of mean results were presented in the Fig. 2.

The presented results show that together with the increasing number of mites used for the effectiveness study, a standard deviation of the average of scores on four test plates underwent a reduction. In case of the number of mites used for the examination, which was higher than 80 per plate, the standard deviations did not exceed 10. Results going beyond the 95% range of the prognosis of the linear regression were obtained for the number of mites on the plate lower than 80 individuals. At the same time, it was observed that the standard deviation of fragmentary results were dependent on the effectiveness of the acaricides, with which the veneer discs were dripped. The relation between the effectiveness of acaricide and the standard deviation of the average fragmentary results was illustrated in Fig. 3.

Data included in the first chart show that the lowest and most concentrated standard deviations were obtained for the results of the examined acaricides with the low effectiveness (<20%) and the acaricides with the high effectiveness (>80%). Dispersion of the results, as well as the 95% range prognosis of the linear regression illustrate that for the majority of examinations, the condition of obtaining a standard deviation lower than 12 can be fulfilled. It was decided to assume this value as a border value for the control of correctness of examination for the effectiveness of acaricides (the highest acceptable standard deviation of fragmentary results). Analysing the relation of the effectiveness of the preparation and standard deviation expressed as a percentage of the average value (coefficient of variation - CV%), it was reported that this coefficient was decreasing together with an increase in the effectiveness of the examined acaricide. This tendency was also observed for the acaricide with the effectiveness higher than >60%, which did not exceed the 20%. To examine the next control parameter of correctness of examination - mortality in the control group, it was decided to use the dispersion of this feature. Average percentages of living mites on control plates in consecutive examinations were presented in the chart.

### Table 1

<table>
<thead>
<tr>
<th>Acaricide</th>
<th>Alfasekt</th>
<th>Blaxime</th>
<th>Bye mite</th>
<th>Ficam</th>
<th>Galtox B</th>
<th>Knox-out</th>
<th>Master</th>
<th>Skorpion</th>
<th>Sumition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal</td>
<td>0.0</td>
<td>71.2</td>
<td>59.0</td>
<td>75.2</td>
<td>17.9</td>
<td>0.0</td>
<td>0.2</td>
<td>8.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Maximal</td>
<td>99.1</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>90.4</td>
<td>75.9</td>
<td>100.0</td>
<td>98.6</td>
</tr>
<tr>
<td>Mean</td>
<td>22.27</td>
<td>96.48</td>
<td>95.97</td>
<td>95.56</td>
<td>66.29</td>
<td>26.04</td>
<td>23.57</td>
<td>74.65</td>
<td>27.56</td>
</tr>
<tr>
<td>Median</td>
<td>7.3</td>
<td>99.0</td>
<td>98.85</td>
<td>98.15</td>
<td>68.9</td>
<td>15.9</td>
<td>15.0</td>
<td>91.55</td>
<td>17.6</td>
</tr>
</tbody>
</table>
\[ \text{SD of partial examinations results} = 4.9524 - 0.0163 \times x; \] 
\[ \text{0.95 Prediction interval} \]

**Fig. 2.** Influence of mite number's on plates on variability of partial efficiency results (expressed as SD).

\[ \text{SD of mean efficiency of partial results} = 5.0711 - 0.024 \times x; \] 
\[ \text{0.95 Prediction interval} \]

**Fig. 3.** Influence of acaricides efficiency on variability of partial efficiency results (expressed as SD and CV%).
It was found, that for the majority of examinations (>95%), the condition of obtaining a higher than the 85% survivability of mites in the control group can be met. It was decided to assume this value as the border value for the estimation of control plates in examinations.

Estimation of repeatability and reproducibility of the mite counting method. In the next stage, the repeatability and reproducibility were determined for the mite counting method on the plates. The average numbers of mites detected on plates in consecutive repetitions counted by both examiners were described in Table 2.

The average coefficients of variation (CV%) for repeated measurements of both examiners amounted to 2.116% and 3.335%, respectively. With the use of the Friedman ANOVA rank test, a lack of statistically significant differences for measurements obtained under
conditions of repeatability was confirmed (between results obtained by the same examiner) as well as for measurements obtained under conditions of reproducibility (between results obtained by both examiners) \(P=0.43594\).

**Estimation of repeatability of the method for examination of acaricides efficacy against *Dermanyssus gallinae*.* On account of finding the greatest variation of the fragmentary results (expressed by standard deviations), in the case of examination of acaricides with the effectiveness fluctuating from 30%-80%, at this stage of experiments acaricide with effectiveness about 30%-40% was chosen. Average effectiveness of acaricide in both repetition series were presented in Table 3.

Data included in the Table, indicate that in both repetition series, close results of the effectiveness of acaricide were obtained. These results were characterised by low coefficients of variation (CV%: 8.148% and 4.907% respectively). Moreover, it was demonstrated using the t-Student test, that the differences between both repetition series were not statistically significant \(P=0.415138\). The obtained results are pointing at the high repeatability of the method.

**Discussion**

The presented method for the determination of the effectiveness of acaricides against *Dermanyssus gallinae* mites in the assumption, may be used for testing the usefulness of combined acaricides (used for control of field *Dermanyssus gallinae* strains), as well as for determination of the susceptibility of mites isolated from poultry farms for available acaricides (choice of preparation for control of the invasion in the farm). On account of the practical character of the method, the fundamental assumption in its elaboration was to mimic the conditions in which acaricides are applied in poultry farms.

During the investigation it was decided not to put mites in closed containers/chambers and to apply the veneer discs to the surface to which the acaricide would be deposited on. The problem of mites leaving the field, on which the acaricide was deposited, appeared to be fundamental for elaboration of this method. This problem was solved by limiting the field, where the mites were moving, by filling the groove with edible oil. On account of the different susceptibility of the different developmental stages of *Dermanyssus gallinae* to acaricides, it was decided to provide all developmental stages in the investigation. It distinguishes the presented method from methods described by Zeman and Zelezny (8) and Thind and Ford (7), which are applied, in particular for comparing the activity of active substances against laboratory *D. gallinae* strains. In these methods, red poultry mites (a few females to a dozen or so copies are starved) are put in closed tight containers - tubes, pipettes or chambers built from two slides of foil or blotting paper, which internal surfaces are covered by acaricide. These methods provide a high repetitiveness of results; however, they are burdened with essential weak points. In particular, these methods do not mimic natural conditions in which mites are normally in contact with acaricides.

Hence, such results cannot be used in practice as an indicator of the real acaricide effectiveness. For example, the mites in the restricted space make the ventilation difficult and can lead to false results because the preparation produces large amounts of fumes. The surface of the test tube or the pipette is a so-called smooth surface, and has an influence in increasing the effectiveness of the preparation administered on it (1, 3, 4). Moreover, applying only one developmental stage of parasites in examinations does not represent the susceptibility of the entire population. In our method such weak points were eliminated and accurate repeatability was assured by the use of significantly more mites and repetition (at least four times) of each examination. Relatively low coefficients of the variation of the obtained results show that our method is useful for the estimation of the acaricide efficacy against *Dermanyssus gallinae* mites.

**References**