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SHORT COMMUNICATION

In vitro efficacy of ByeMite® and Mite-Stop® on developmental stages of the red chicken mite *Dermanyssus gallinae*

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Abstract The present in vitro study shows the efficacy of two antimite products (ByeMite® = phoxim, Mite-Stop® = neem seed extract) against all developing stages of the important red chicken mite *Dermanyssus gallinae* (obtained at two farms in France and Germany). While permanent contact with the active compound led to an efficacy of 100% in the case of Mite-Stop® on mites in both farms, there was only a 96.2% killing effect of ByeMite® on the mites of the French farm. Even short contacts of only 4 s killed 100% of mites in the case of Mite-Stop® at the French farm and only 84.5% in the German farm. ByeMite®, on the other hand, killed only 27.8% (Germany) and 30% (France) when mites got the chance to escape from the treated grounds to untreated ones. When using only the half doses of both products, Mite-Stop® still reached, after permanent contact, 100% activity on the German farm and 98.2% in France, while ByeMite® killed 93.8% (Germany) and 90.6% (France). Short contact to half doses of course reduced the activity of both products (Mite-Stop®=59.3% in France, 22.1% in Germany; ByeMite®= 28.8% in France, 18.8% in Germany). With respect to the fumigant activity of the products, the strains of *D. gallinae* reacted differently. While Mite-Stop® showed a clear fumigant activity in the case of the German mites, this product did not affect the French mites by air distribution, neither did ByeMite® in both cases. Therefore, mites have to come in contact with both products. Against Mite-Stop®, there was apparently no resistance and low doses have high efficacy after even short contacts, which regularly occur in a treated stable, where mites have the chance to leave treated places to untreated hidden spots.

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Red chicken mites (*D. gallinae*) are worldwide important agents of diseases in poultry, threatening the health and productivity of many farmed birds (Sparagano et al. 2009; De Luna et al. 2008). Large amounts of such mites may cause enormous losses of blood (anemia) and diseases by transmitted viruses, bacteria, and/or parasites (Zeman et al. 1982; Hoglund et al. 1995; Vreeken-Buijs et al. 1998; Valiente-Moro et al. 2007; Abdel-Ghaffar et al. 2008a, b; Mehlhorn 2008). Large amounts of such mites may also stain the surface of eggs (by their bloody feces) in poultry farms, leading to a problem in selling them (Chauve 1998).

Massive infestations occur especially in giant chicken houses where inspections by naked eye are done only at some selected places, so that growing populations of mites may be overlooked or only noted at a late stage. Furthermore, in the last years, the control of *D. gallinae* mites became more and more problematical due to increasing resistances to pyrethroids and others (Marangi et al. 2009). The problem of control is also enlarged by the fact that the use of some chemical products needs the discharge of eggs laid during the spraying period or the request of a rather long waiting period before the chicken may be slaughtered (Hamscher et al. 2007). Thus, a beneficial control of the development of *D. gallinae* mites in chicken houses is highly needed. Most antimite products are acting exclusively by contact, which is reduced at many

hidden places around cages or even in chicken houses with floor breeding.

One chemical product (ByeMite®, Fa. Bayer) and one biological product (Mite-Stop®, Fa. Alpha-Biocare) were proven to be active when used in chicken houses (Meyer-Kuehling et al. 2007; Abdel-Ghaffar et al. 2008a; Locher 2009). The present in vitro study compares both products with respect to their needed dosage and the duration of the needed contact of mites to the applied product. The studied *Dermanyssus* mites (Fig. 1, 1 and 2) were collected at a commercial egg production farm with caged poultry close to Nancy (France) and at a scientific rearing farm close to Rommerskirchen (Germany) where poultry was kept free in and outside of stables. Collected mites were brought to the institute and exposed to the compounds in three repeated series on 3 days following the day of collection.

Mite-Stop® is a patented special formulation of an extract of the seeds of the neem tree (*Azadirachta indica*), which are eaten by humans since centuries in India. The product was obtained from Fa. Alpha-Biocare (Düsseldorf, Germany) and was freshly prepared before each application by diluting the seed extract with tap water.

ByeMite® (the content is identical with the pig acaricide Sebacil®, containing the organophosphorous compound phoxim) was obtained from the veterinarian trade in France and used according to labeling or diluted as described below.

The mites (Fig. 1, 1 and 2) were collected in the farms, brought to the institute, kept at 25–27°C, and the experiments were started immediately afterwards. Under the dissection microscope, groups of 100 mites were formed (containing all stages of the life cycle—having sucked blood or not). The experiments were done by exposing the mites to the two products under the conditions described below.

The first two series of experiments aimed to evaluate the principal susceptibility of the mites to each of the two acaricide formulations and in order to find out whether the mites (1) are killed upon permanent contact with the biocide or (2) die even after short contact with the biocide. This could occur, if a mite was hit by a sprayed droplet, but then could crawl to an untreated site and eventually recovers. The third trial aimed to see whether the biocides also exert a toxic effect in the vapor phase, i.e., work as fumigants and thus reach and kill mites hidden in crevices.

In the experiments of type 1, the mites were kept sealed in 8.5-cm Petri dishes on a filter paper wetted with 400 µl biocide solution, giving a dosage of 7 µl/cm^2. In the experiments of type 2, the mites were immersed with the biocide solution on a small piece of filter paper (area of 2 cm^2) for 4 s and then this small piece of filter paper was transferred to a Petri dish lined with an untreated, dry filter paper. Any mite still mobile could leave the treated area. In the experiments of type 3, the mites were encased in small vials (1 cm diameter, 2 cm in length, one side covered with a fine nylon mesh to permit gas exchange). The vials were transferred into Petri dishes, dosed with 400 µl acaricide as described above, and then the dishes were tightly sealed. All treated groups of mites were observed during the next hours after treatment. Results of mortality rate in percent were recorded 24 and 48 h after application.

Sebacil® (identical to ByeMite®) was used in the dilution recommended by Bayer for use in poultry, which

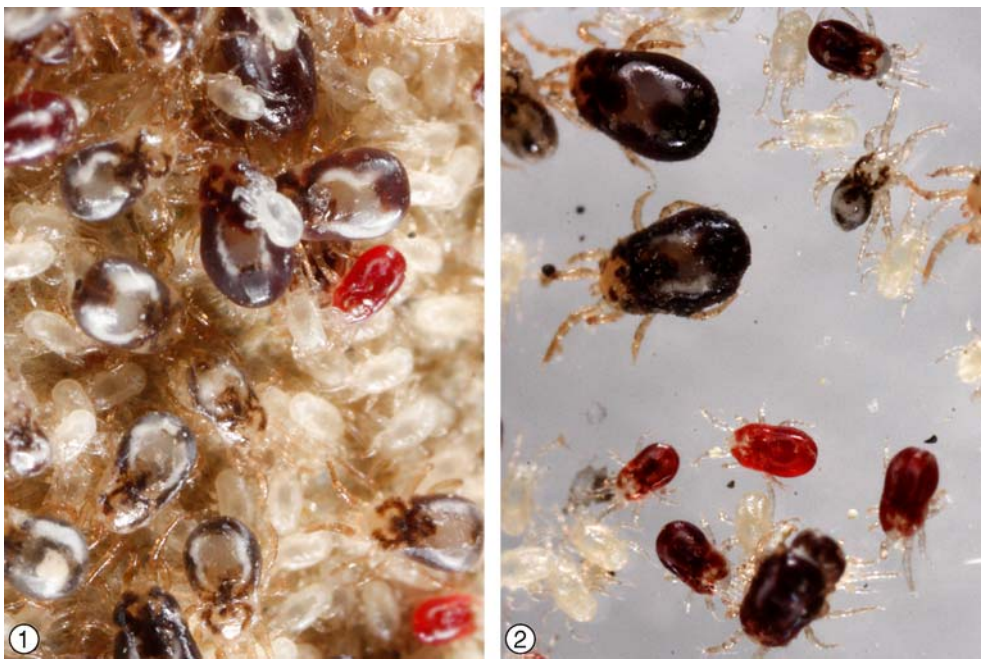

Fig. 1 *1* and *2* Light micrographs of developmental stages of poultry red mite *D. gallinae* from the stables in Nancy/France and Rommerskirchen/Germany including fed and unfed individuals. Some are brilliant white (just after molt)

is 100 ml concentrate diluted to 25 L water (2,000 ppm phoxim). Mite-Stop® (extract) was diluted 1:33 with water, the dilution recommended by Alpha-Biocare. Controls were carried out applying the same volumes of water instead of the test biocides.

The experiments delivered the following results:

1. Permanent exposure: 100% of the mites originating from Rommerskirchen and being exposed to either ByeMite® (2,000 ppm) or Mite-Stop® (1:33) were killed within 24 h. The French mites showed different reactions. The exposition to Mite-Stop® killed 100% of the mites, while ByeMite® left survivors after 24 h of exposure, killing only 96.2%. This might be due to resistance.
2. Brief contact: After brief contact of only 4 s, Mite-Stop® (1:33) killed 84.5% of the Rommerskirchen mites and 100% of the Nancy mites. ByeMite® killed only 27.8% of the Rommerskirchen mites and 30% of the Nancy mites. This shows that ByeMite® needs longer contacts.

In the second series of experiments, only the half dose of the compounds was used. Permanent contact to 1,000 ppm ByeMite® led to a killing effect of 93.8% in the Rommerskirchen mites and 90.6% in the Nancy mites. On the other hand, Mite-Stop® killed 100% of the Rommerskirchen mites and only 98.2% of the Nancy mites. This shows that Mite-Stop® is highly efficient even in reduced dosage, which may occur at hidden crevices. After brief contact (4 s) with both diluted products, the following results were obtained: ByeMite® killed only 18.8% of the Rommerskirchen mites and 28.3% of the Nancy mites, while Mite-Stop® killed 22.1% of the Rommerskirchen mites and 59.3% of the Nancy mites.

The third series of experiments tested the fumigant activity of both products. It was shown that ByeMite® (2,000 ppm) killed about 4% of the Rommerskirchen mites and about 5% of the Nancy mites (corresponding to the numbers of dead mites in the controls). The results with Mite-Stop® were different from those with ByeMite®. The Rommerskirchen mites were all killed, while the Nancy mites survived as those that had been exposed to ByeMite® at both farms.

This result shows again that there are apparently different sensibilities in *D. gallinae* strains. While Mite-Stop® has apparently fumigant activities in the Rommerskirchen strain, both Mite-Stop® and ByeMite® do not affect the Nancy mites at hidden places by fumigant = gaseous transportation.

The present in vitro studies in principle confirm the results of that in vivo treatments (Meyer-Kuehling et al. 2007; Abdel-Ghaffar et al. 2008a, b; Locher 2009) However, it is also clearly shown that the biological product Mite-Stop® has some advantages in efficacy. Furthermore, Hamscher et al. (2007) showed that phoxim (in ByeMite®) treatment of chicken houses led to residues in eggs, so that they have to be removed after each spraying and chicken have then a withdrawal time of 25 days.

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