

Light and scanning electron microscopic investigations on MiteStop®-treated poultry red mites

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Abstract Recent studies of the neem seed product Mite-Stop® showed that it has a good acaricidal effect against all developmental stages of the poultry red mite, *Dermanyssus gallinae*. In vitro tests proved an efficacy at direct contact, as well as by fumigant toxicity. Light and scanning electron microscopic (SEM) investigations showed no clear, morphologically visible signs of an effect caused by fumigant toxicity. Direct contact with the neem product, however, seemed to be of great impact. Chicken mites turned dark brown or even black after being treated with the neem product. SEM analysis showed damages along the body surface of the mites.

Introduction

The poultry red mite *Dermanyssus gallinae* De Geer 1778 is the most important ectoparasite of layers in Europe (Chauve 1998). The chicken mite is a blood-sucking parasite that can lead to high economic losses. Control and production losses in Europe have been estimated at €130 million per annum (van Emous 2006; Mul et al. 2009). Heavy infestations can lead to severe stress among the laying hens resulting in a decrease of egg production, egg quality, weight gain in young birds, and it can even cause death (Chauve 1998; Kirkwood 1967; Pospischil 2001). Furthermore, *D. gallinae* can act as a potential vector and reservoir for several bacterial and viral pathogens such as *Salmonella* spp., *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Borrelia anserina*, *Coxiella burnetii*, chicken pox virus, Newcastle disease virus, and St. Louis encephalitis virus (Chirico et al. 2003; Lundh et al. 2005; Moro et al. 2007; Smith et al. 1945).

Control of the poultry red mite is still difficult today, especially for food producing poultry, because there is currently no registered compound available on the German market. Chicken mites are usually controlled by treating poultry houses with synthetic acaricides. Because of the repeated use of these acaricides, some have become less effective (Beugnet et al. 1997; Chauve 1998; Nordenfors et al. 2001; Mul et al. 2009).

Due to the life habits of *D. gallinae*, less intensive farming systems like barns, free range, and organic farming show much higher prevalence rates than cage systems. The ban of traditional cage systems for poultry in Europe by 2012, as well as the removal of acaricides from national markets due to the increase in acaricide resistance and welfare concerns, will probably increase the problems caused by the poultry red mite (Sparangano et al. 2009).

Parts of this publication are included in the Ph.D. thesis of Doctor Nina Locher. This thesis has been accepted at the Free University of Berlin (Germany) to obtain the Doctor of Veterinary Medicine degree.

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The present situation calls for studies on the efficacy of alternative control methods such as i.e., plant-derived acaricides. The most important pesticides appear to be those derived from the seeds of the neem tree *Azadirachta indica* A. Juss. Several neem products proved to be efficacious against viruses, bacteria, endoparasites, and arthropods (Schmutterer 2002). Moreover, the complex mixture of several different bioactive analogues in neem is thought to restrict the development of resistance (Mulla and Su 1999).

Recent studies on the acaricidal activity of the neem product MiteStop® have shown its acaricidal effect against all developmental stages of *D. gallinae* (Abdel-Ghaffar et al. 2008, 2009; Locher 2009; Schmahl et al. 2010; Locher et al. 2010). These studies proved not only an efficacy at direct contact, but also showed a fumigant toxicity of the extract.

In order to investigate morphologically visible effects caused by the neem product, light and scanning electron microscopic analyses of treated chicken mites had been carried out.

Material and methods

MiteStop®

The acaricide MiteStop® is a patented product of Alpha-Biocare GmbH, Düsseldorf (Germany). It is a special patented formulation of the extract of the seeds of the neem tree *Azadirachta indica* A. Juss. It is sold as a concentrated product, which has to be diluted with tap water prior to use.

Mites

The mites were collected in different infested poultry houses in Germany. All investigations were carried out within 3 days after collection, mostly at the same day.

Treatment of mites

Four different methods of application were used. *Method A* (wetting of individual mites) was used to evaluate the contact toxicity of very small doses of the extract. Mites were placed on a filter paper (Rundfilter MN 615, Machery-Nagel) in a petri dish (92 × 16 mm, Sarstedt) and were treated individually with 0.5 µl of a selected water dilution (undiluted, 1:20, 1:40, 1:60, 1:80). The control group was treated exclusively with water. *Method B1* (contact with wet acaricide) and *method B2* (contact with dried acaricide) were used to evaluate the toxicity of a treated area. 0.3 ml of undiluted and diluted (1:20, 1:40, 1:60, 1:80) acaricide was applied to filter papers. Control filter papers were treated with just water.

In method B1, the wet filter paper was placed in a petri dish, and groups of about 20–30 mites were placed on the filter paper. In method B2, the filter paper was left to dry at room temperature before putting it into a petri dish. In order to prevent any mites from escaping, the petri dishes were sealed with Parafilm®.

Method C was conducted to investigate the vapour phase toxicity. Groups of 20–30 mites were placed into an Eppendorf cup (2 ml). The lid of the cups had been cut off to seal the opening with a special foil allowing the entrance of vapours. The sealed cups were then placed in a glass container (20 ml). A folded filter paper treated like in method B1 and B2 was added, and the container was sealed with a plastic lid. The petri dishes and the glass containers were stored at room temperature.

Light microscope analysis

For light microscope (LM) analysis, mites were examined unfixed or were fixed in 4% formaldehyde for 1–2 h. The fixed mites were then stored in a mixture of 70% ethanol and 4% glycerin. Specimens were either embedded in pure glycerin or in Hoyer's medium (Pritchard and Kruse 1982). The examination was done by using a light microscope (Olympus BX50) with a digital camera (Olympus E-410).

Scanning electron microscope analysis

For scanning electron microscope (SEM) analysis, mites were fixed in 5% glutaraldehyde in soda-cacodylate buffer (0.1 M, pH 7.3) for 24 h, then rinsed with soda-cacodylate buffer (four times, 10 min/wash). A dehydration in a graded acetone series (50, 60, 70, 80, 90, 96, and 100%, 5 min each) followed. Mites were then stored in 100% acetone at 4°C. Critical point drying with CO₂ as the transition fluid

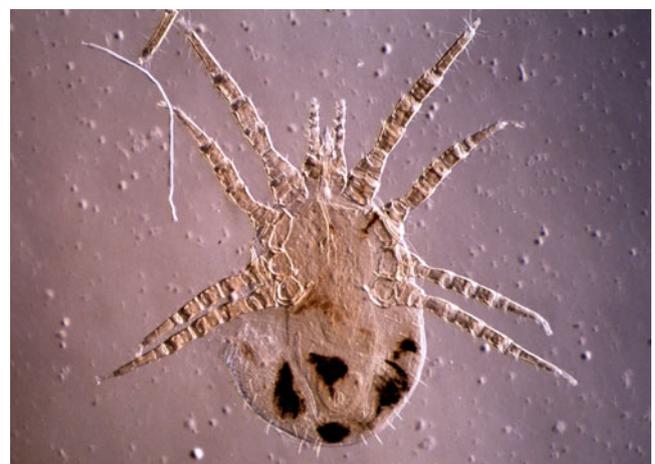


Fig. 1 Light micrograph of an untreated *D. gallinae* that had sucked blood (black dots), and is in the phase of digestion



Fig. 2 Light micrograph of an adult stage of *D. gallinae*. Since it is fully engorged with blood, the body appears (name giving) completely red

was done with the critical-point apparatus CP II 120 (Balzers Union, Witten, Germany). Dried mites were sputtered with gold using the Sputtering Device (Balzers Union, Witten, Germany) and then examined using the scanning electron microscopes AMR 1000 (Leitz) and 1430 VP (LEO).

Results

Light microscope analysis

The LM analysis showed that untreated stages (larvae, nymphs, adults) sucked or nonfed ones showed the typical structure even after incubation in water (Figs. 1, 2). However, most of the treated mites (method A and B1) turned brown or even black and got a shiny surface (Fig. 3). Many mites were covered in MiteStop® completely although they only came into contact with the neem



Fig. 3 a, b LM micrographs of two MiteStop®-treated (method B1) mites embedded in Hoyer's medium. Most of the body is coloured like amber. a Ventral view. The opisthosoma is coloured greyish white. b Caudolateral view. c, d LM micrographs of two MiteStop®-treated

mites (method B1) on a filter paper. c Cranioventral view. The colour of the body is brown and black with a shiny surface. d Lateral view of a very dark coloured mite. The body surface is shiny

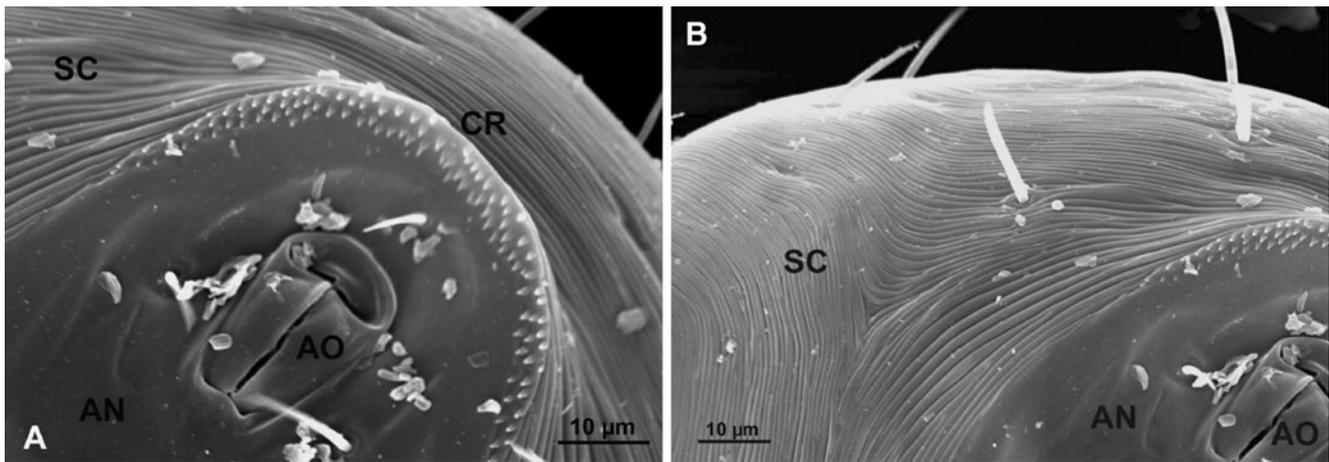


Fig. 4 a, b SEM micrographs of an untreated mite. Ventral view of the opisthosoma. The soft cuticle (SC) is folded. The anale (AN) is made of hard cuticle. The cribrum (CR) has its position at the caudal end of the anale. Its tiny teeth are clearly visible. AO anal opening

product through their legs or the ventral part of the body. Mites treated according to methods B2 and C showed no or only slight changes in appearance. A few mites appeared dry.

Scanning electron microscopic analysis

Mites treated according to method B2 (contact with dried acaricide) showed no or only slight changes in morphology. Some of the treated mites shrivelled slightly. Method C (fumigant toxicity) introduced no visible effect.

Many mites treated with the acaricide after method A (wetting of individual mites) and B1 (contact with wet acaricide) had a dry appearance and had shrunk dorsoventrally. The whole body surface was covered with particles of different sizes. Clear structures of the integument, like the parallel arrangement of the soft cuticle or the teeth of the cribrum, were lost (Figs. 4, 5).

Discussion

The aim of the LM and SEM analyses was to detect changes in the morphology in the treated mites that could explain the effect caused by the neem product. Many of the treated mites turned into a dark brown or even black colour. Maurer (1985) described the same effect in moths of the species *Ephestia kühniella* which were also treated with an extract of the seeds of the neem tree. He assumed that the effects resemble the development of the so-called black bodies, which are caused by azadirachtin and leading to an accumulation of melanized haemocytes.

The shiny surface is likely to show that the neem product covers the mite completely, even though the mite came only in contact with the treated filter paper by its legs. SEM micrographs showed particles of the neem product all over the body. The particles were glueing to the surface of the hard and soft regions of the cuticle or had even sunk into

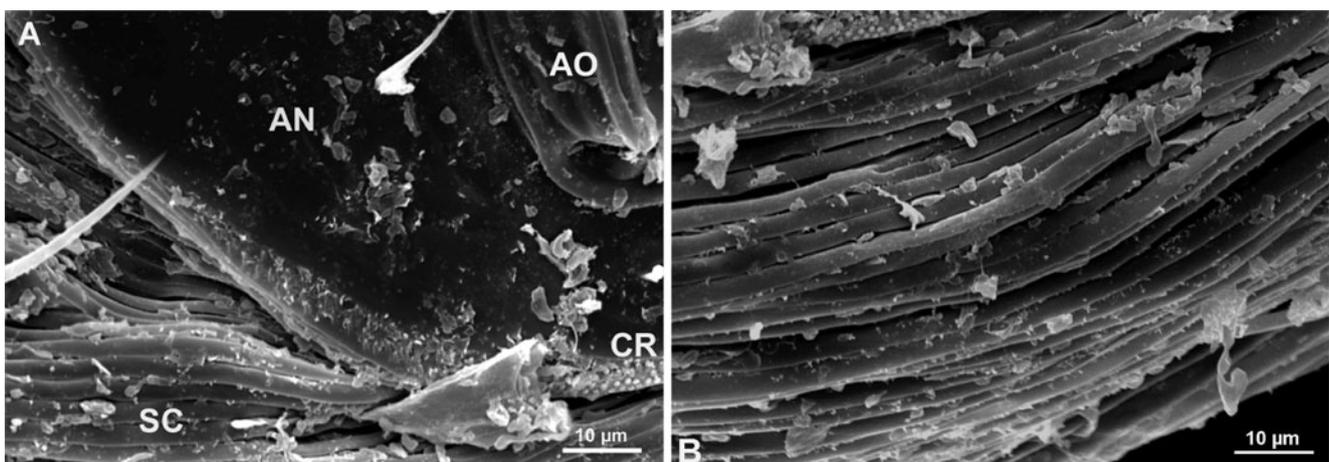


Fig. 5 a, b SEM micrographs of a treated mite. Ventral view of the opisthosoma. The soft cuticle (SC) and the anale (AN) are covered with particles of different sizes. The parallel arrangement of the soft cuticle (SC) is lost. The teeth of the cribrum (CR) have lost their clear form

the soft cuticle, destroying the parallel arrangement of foldings. It might be possible that these particles could have an effect on the respiratory system of the mites by blocking the stigmata and pores. Further investigations are necessary.

The neem product seems to interact with the cuticle resulting in severe damages. An effect similar to this was also described for amorphous diatomaceous earth (Mewis and Ulrichs 1999). Diatomaceous earth breaks the water barrier of the cuticle and increases the transpiration rate of water across the cuticle. As a result, the mites die from dehydration.

Considering that the fumigant toxicity does not lead to an effect visible by LM and SEM, further investigations should be carried out by using a transmission electron microscope (TEM). TEM investigations could also draw some conclusions about the effect on the cuticle of the chicken mites. However, from our pilot examinations, it is already known that, e.g., in lice, the product covers the fine aqueous layer at the end of the tracheoles thus, blocking mechanically the transport of oxygen into the cells at the end of the tracheoles. The results described here were confirmed in mite stages treated *in vivo* in Germany and Egypt (Abdel-Ghaffar et al. 2008, 2009; Schmahl et al. 2010).

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