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Treatment with a neem seed extract (MiteStop®) of beetle larvae parasitizing the plumage of poultry

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Abstract Beetles of the species *Alphitobius diaperinus*, *Dermestes bicolor*, and *Dermestes lardarius* may transmit severe agents of diseases on poultry and may in addition harm as larvae the skin and feathers thus leading to severe economic losses. The present study deals with a control measurement using a neem seed extract (MiteStop®) being diluted with tap water. It was shown that spraying of a 1:33 dilution kills both larvae and adults of these part-time parasites as was previously shown for other parasites such as mites, ticks, and blood sucking or biting insects.

Introduction

Beetles belong to the insect order Coleoptera, the members of which are characterized by stiff chitinous forewings and fine hind wings, the latter being used for their rather slow flights. The beetles belong in size and number to the most

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J. Mehlhorn C. and O. Vogt Institute for Brain Research, Heinrich Heine University Düsseldorf, Düsseldorf, Germany impressive and worldwide distributed insects reaching in some species a length of up to 20–25 cm, while others remain tiny below 1 mm. Their life cycle runs holometabolically including often up to 12 larvae, which grow up via molts until they form a pupa, within which the final sexually fertile female or male stage is formed in a temperature-dependent time. While the adults of many species do not feed anymore, the larvae have a huge need of food that they get—depending on the species—from plants, animal or human feces, detritus, dead bodies etc. Thus many species are important pests in plant and crop production, in food store system, in animal stables and/or in human households introducing yearly billions of economic losses in our overcrowded world.

In contrast to the typical ticks, mites, and mosquitoes, adult beetles and their larvae only seldom not attack living animals or humans and thus they are in their absolute majority no parasites. However, there are some exceptions such as some members of the beetle families Tenebrionidae (dark beetles) and Dermestidae (mould beetles, bacon beetles). While in general, these beetles are attracted by stored food (often being already contaminated with fungi like Aspergillus species), lay their eggs onto these materials, and as the hatched larvae feed there, they may come close to the bodies of feeding poultry and enter the plumage of these birds (Heimbucher and Kutzer 1978; Weidner 1982). In the case of young chicks, the predatory larvae of both groups of beetles may feed as true parasites on the skin and/ or at the base of tiny feathers, thus introducing skin damages and blood losses. Furthermore, these small wounds are "open gates" for the entrance of agents of diseases such as the herpes virus of the Marek's Disease (acute avian leucosis), Gumbora virus disease (infectious bursitis of chicken), salmonellosis, and various Escherichia coli infections which may harm considerably the health of

poultry birds or even kill them (Wiesner and Ribbeck 1978). Especially freshly hatched or very young chicks die very often from bacterial superinfections at the biting sites of the larvae, the anterior ends of which stick in the surface of the skin (Wiesner and Ribbeck 1978; Rommel 2000; Heimbucher and Kutzer 1978; Jacobs and Renner 1988; Weidner 1982). Furthermore, these beetles may become intermediate hosts of parasites when bearing the larvae, cysticercoids of numerous tapeworms (Mehlhorn 2008; Eckert et al. 2008). In addition, the feces and the larval exuvial remnants may introduce severe allergic reactions in the breathing system of humans and animals (Wiseman et al. 1959; Wittich 1940; Sheldon and Johnston 1941; Klaschka and Jung 1976). Thus control of such part-time parasites and/or food pests is highly desirable, since otherwise the economic win will disappear due to reduction of the egg-laying rate, slow body growth, or due to death as consequence of superinfections of wounds respectively due to transmission of agents of diseases. However, the parttime parasitic beetles fly in stables or are (mostly) enclosed in poultry food and therefore "strong chemical insecticides" cannot not be used. Otherwise, eggs and/or meat of the poultry cannot be used before a waiting time. Therefore, biologically active compounds have the advantage that they might be even used inside stables with chicken and without withdrawal of eggs during the treatment period.

The present paper deals with the efficacy of a neem seed extract (MiteStop[®]) on larvae and adults of the "brilliant black cereal fungus beetle" (lesser mealworm) *Alphitobius diaperinus* and the larvae and adults of the so-called dermestid "striped bacon beetle" *Dermestes bicolor* respectively on the closely related species *Dermestes lardarius* (common bacon beetle). All are common in poultry stables being attracted by light and smelling odors of the chicken food. *A. diaperinus* (Panzer 1797) is a beetle of warm countries and was imported in 1960 with cereals to Europe and became since then the most common beetle in poultry stables, a status which it always had in warm countries such as in the Near and Far East (Wiesner and Ribbeck 1978), while the *Dermestes* species are already endemic in Europe since long.

Materials and methods

Beetles

A. diaperinus (Panzer, 1797). Adults (Figs. 1, 2, and 3) and larvae (Fig. 4; looking similarly like those of the flour beetle *Tenebrio molitor*, however being somewhat less long) were obtained from a poultry farm close to Dubai (United Arabian Emirates).

Fig. 1 Micrograph of *A. diaperinus*. Note the dorsal fine depressions of the cuticle and the two claws at the feet as well as the typical antennae



D. bicolor (Fabricius, 1781) and *D. lardarius* (Fabricius, 1781; Fig. 5). Adults and larvae were sent to the institute by several chicken owners for species determination.

Materials

As means for the treatment, a neem seed extract (MiteStop[®]) was used, which was developed and is produced by the university spin-off company Alpha-Biocare GmbH, Düsseldorf, Germany. This product is a biocide of the class 18 according to the EU regulations.

Methods

The MiteStop[®] product is sold as a concentrate, which has to be diluted with tap water freshly prior to use. For the tests, dilutions of 1:20, 1:33, and 1:60 have been used by spraying the product on larvae and adult beetles being situated either uncovered in glass petri dishes or being mingled among debris from the floor of chicken stables. The behavior of the treated specimens was followed for the next hours. Untreated and just water-treated specimens were controlled for 1 week.

Results

The tested beetles had the following specifications:

1. A. diaperinus

Adults: These beetles (Figs. 1, 2, and 3) had a size of up to 5.5–7 mm in length and a width of 2.5–3 mm. The whole body appeared brilliant black. The forewings (elytrae) were characterized by longitudinally running seven to eight rows of fine dot-like depressions, while the back of the head, the shield covering the breast segments, and the scutum contained identical tiny depressions without being arranged in rows. The neck shield had its broadest extension at its terminal



Fig. 2 Micrograph of the dorsal side of the head and of the breast shield of *A. diaperinus*. Note that the *bean-shaped* compound eyes are hardly recognizable

end. The compound eyes were situated at the lateral sides of the head and showed a bean-like central depression (due to the outer rim of the head). The antennae had the typical appearance of the family Tenebrionidae, where the size of the single segments is slightly enlarged starting from the scapus to the terminal outmost one (Fig. 2). The terminal segment of the tarsus was rather large and was provided with two claws (Figs. 1 and 3).

The larvae of this beetle species reached a length of up to 15 mm, looked very similar (with brownish and yellow banding alterations) like the larvae of *T. molitor* beetles (Fig. 4). However, their diameter was smaller and the *Alphitobius* larvae had a dotted terminal end with a fine peak.

From the life cycle, it is known that the first larvae (2 mm) hatch after 5–6 days from the egg at 25°C and develop via seven molts and a pupal stage within 35–50 days at high temperatures of about 30°C or within more than 100 days at lower temperatures to the adult stage. The larval development proceeds in chicken food. However, the larvae also are able to enter wooden or other covers of the walls of stables, where pupation occurs and thus introduce damages and hollows, which can be used by mites, ants, fungi etc. The larvae are also found in the plumage of chicken feeding on skin and tiny feathers.

2. D. bicolor

This beetle may reach a length of up to 9 mm and has a basically black color. However, since its shield

Fig. 3 Micrograph of a leg of *A. diaperinus* showing the typical tibia and the tarsal segments (the also present second claw is not seen in this perspective)





Fig. 4 Micrograph of a carcass of a larva of *A. diaperinus*. Note the banding (*dark/light*)

and the "shoulders" bear fine yellowish hair, the beetle got the name "bicolor, two colors". The chitinous elytrae, forewings are characterized by a deep line of depressions, which are mostly even deeper at the terminal ends of the wings. This appearance of the "furrows" led to the second name "striped bacon beetle". The larvae have the typical appearance of the larvae of the family Dermestidae being provided with fine, long hair protruding from the segment surface and from the terminal end. These larvae are also found in the plumage of especially young chicks feeding on the tiny feathers and from skin.

3. D. lardarius

This so-called common bacon beetle reaches a length of up to 9 mm and is characterized by a grey band of hair stretching across the elytrae, forewings (Fig. 5). The larvae prefer meat and organic detritus and thus often feed dead chicks, but are also seen feeding at the skin of living chicken. Pupation occurs in hiding places, in wood openings of the wall etc.



Fig. 5 Micrograph of the dorsal view of *D. lardarius*. Note the *broad* grey appearing band on the elytrae

The treating trials were always done with freshly prepared tap water dilutions of MiteStop[®]: 1: 20, 1:33, and 1:60 being sprayed onto the developmental stages of both beetle species.

It was seen that in all dilutions, larvae and adults stopped all movements immediately after being sprayed wet. In the case of 1:20 dilution none of the stages (larvae, adults) started again any movement. In the case of the 1:33 dilution all larvae did not recover at any time, while some of the adults started slow movements after they had been dried again, but the movements stopped finally at 15 min after the treatment. In the case of the 1:60 dilution all larvae did not show any movement after the treatment, while all adult beetles started again rather normal movements just after they had dried. However after 2 h, about two thirds were dead and the rest followed after 24 h. The condition, however, was that the adult beetles had become completely wet during spraying otherwise there were survivors in the 1:60 dilution series. The untreated controls kept their motility and were still alive after weeks. The beetles treated with pure water stopped suddenly their movements after they had been sprayed, but became fully active after drying and kept their motility for weeks.

Discussion

Beetles are in general not parasites. However, they may serve as intermediate hosts for several parasites of humans and animals (Mehlhorn 2008; Eckert et al. 2008). Furthermore, they are able to transmit agents of diseases such as viruses, bacteria, and parasitic stages (e.g., oocysts of coccidia), if they contaminate the food of animals with their pathogencontaining feces. Some beetles, however, such as the three species tested here, may enter the plumage on hair of animals and feed there on the skin and or fine lamina of feathers or hair leading to damages, which might get superinfected. Both species tested here are known for this behavior and thus pose great problems (Weidner 1982; Heimbucher and Kutzer 1978), if they are once introduced into a stable via contaminated food. The occurrence of these beetles which may become extremely dangerous, in case they harbor and transmit viruses and bacteria (Wiesner and Ribbeck 1978), makes it necessary to establish methods to eliminate those beetles from rearing and/or breeding facilities of poultry. The difficulty is that the beetles develop in food, from where they may enter the plumage. Thus, one possibility is to empty the infested stables completely and to use strong chemical insecticides of any type. This is expensive and takes time, during which no chicken breeding can occur or no egg-laying is possible. Therefore it is desirable to have a product, which can be used in the stable and even on the poultry while animals are in the stable. The product MiteStop® offers such a possibility, since it is based

on a biological, non-toxic extract of the seeds of the neem tree (Azadirachta indica). Many tests have shown that this extract is able to control a large number of mites, ticks, and insects (blood sucking or not; Abdel-Ghaffar and Semmler 2007; Heukelbach et al. 2006; Abdel-Ghaffar et al. 2009, 2010; Schmahl et al. 2010; Semmler et al. 2010). Since this extract is also used in a 1:10 dilution in human anti-lice shampoos with a proven grade of "very good" skin compatibility (Pittermann et al. 2008) and is used, e.g., on the skin of many animals inclusive chickens (Al-Quraishy et al. 2011a, b), a use in case of an infestation of poultry houses with such beetles does not pose any health problem. The 1:33 tested dose turned out to be safe and very efficacious and would eliminate at the same time red poultry mites (Dermanyssus gallinae), flies, fleas (larvae, adults), mallophages, bugs, other beetles, cockroaches, etc. (Schmahl et al. 2010; Al-Quraishy et al. 2011a). Thus in total, a cost sharing control of a broad spectrum of pests and parasites would be possible. However during treatment, it must be made sure that the targets (larvae, adults) were fully covered with the product, since it acts mechanically by blocking the oxygen uptake at the ends of the fine tracheoles after it had entered the outer spiracles of the tracheal system.

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