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ORIGINAL PAPER

Field study on the efficacy of an extract of neem seed (Mite -Stop®) against the red mite *Dermanyssus gallinae* naturally infecting poultry in Egypt

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Abstract Infestations with the poultry red mite *Dermanyssus gallinae* represent a major ectoparasite problem in poultry and affects egg and meat production worldwide. The effects of the neem seed product Mite-Stop® against the red poultry mite were investigated. Five primitive poultry farms in two small villages in the Nile Delta and Giza district were selected for the study. The neem extract was diluted 1:40 and 1:50 with tap water just prior to use. Application of the two dilutions of the provided product was performed to soil, cracks and crevices of the examined area as well as to mite-infested birds on day 0 and day 7. Two hours after treatment soil dust was collected from sprayed regions of the stable and from unsprayed control regions of the same stable. The treated chickens were also checked for mites 2 h after each treatment. The examination of the chickens 2 h after spraying showed that they were free of mites. The examination of treated soil with the Tullgren funnel apparatus 2 h after the first spraying on day 0 already showed a considerable reduction of living mites compared to controls. Seven days after the first treatment of the soil the number of living mites was reduced for 80% in the treated soil and decreased even more after the second spraying, since those larvae that had hatched from eggs in the meantime were killed. The 1:40 dilution of the neem seed extract with tap water was superior to the 1:50 dilution. These results clearly show a very high killing rate of the extract, if the mites come in direct contact with the compound. However, in order to obtain extinction also of hidden and freshly hatched stages repeated spraying should be done three times within 8–10 days.

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Introduction

Mites have successfully invaded almost all regions of the biosphere under very different environmental conditions. Forty thousand different mite species have been described to date, (Kautz et al. 2006) feeding as predators, as plant parasites, or respectively as blood suckers. This remarkable diversity is reflected in a wide morphological and biological specialization. A great number of species are ecto- as well as endoparasites of vertebrates. Therefore the systematic unit Acari comprises a large and economically important group of arthropods. Some members of this group may infest farm animals and poultry causing great losses in meat, milk, wool, and egg production (Behan-Pelletier 1999; Meyer-Kühling et al. 2007).

Furthermore, these parasites may transmit agents of harmful diseases (bacteria, viruses, and Protozoa) to man and/or to his domestic animals (Vreeken-Buijs et al. 1998). The poultry mite *Dermanyssus gallinae* is one of the most dangerous blood-feeding ectoparasites affecting poultry production. This parasite may also attack many other game and wild birds causing irritation, anemia, and even death in some cases and may result, e.g., in production of blood-stained eggs (Chauve 1998). This mite also occasionally bites mammals and man, where it causes an itching dermatitis in people who come in contact to poultry stables,

nests, or cages of birds, while these blood feeders introduce in poultry loss of body weight, retardation of growth, and reduction of egg production (Nordenfors 2000).

Furthermore, different other poultry pathogens were reported, such as those of the fowl spirochaetosis, chicken pox virosis, Newcastle virosis, pullorum disease, fowl typhoid fever, and fowl cholera (Zeman et al. 1982; Valiente-Moro et al. 2007). Besides this possible vectorship the red poultry mite *D. gallinae* is in general most important as a direct pest, because all developmental stages suck rather large quantities of blood and reproduce very rapidly. The life cycle can be completed under optimum conditions within 7 days, so that chicken stables may become overcrowded with mites within a very short time.

Massive infestations were recorded all over the world reaching especial high rates in Europe. In France, poultry becomes attacked in almost all regions of the country (Beugnet et al. 1997; Reynaud et al. 1997). The same situation was recorded in Sweden (Hoglund et al. 1995; Nordenfors et al. 1996) and Switzerland (Maurer et al. 1993; Maurer and Baumgartner 1994). Therefore, it seems that the mite problem will increase in several countries in the near future due to the increasing number of huge stables. Therefore control methods and biocidal products have to be become ameliorated. Several control methods including chemical control agents were recorded. More than 35 compounds including organochlorines, organophosphates, pyrethroids, and carbamates have been applied against the poultry red mite (Nordenfors et al. 2001; Thind and Ford 2007). At the same time some alternative control methods such as the biological control using the bacterium *Bacillus thuringiensis* were also applied. The latter method was proven to be also effective against a number of dust mites (McKeen et al. 1988; Hassanain et al. 1996).

The aim of the present study was to evaluate the antimite efficacy of the neem seed product Mite-Stop® and its effects under field conditions in poultry farms naturally infested with *D. gallinae.*

Materials and methods

Mite-Stop® is a special formulation of an extract of the seeds of the neem tree (*Azadirachta indica)* being produced by Alpha-Biocare GmbH, Düsseldorf (Germany). This product was used against the blood sucking poultry red mite *D. gallinae* (Acari: Dermanyssidae) in Egypt.

Site of the study

To improve health of chicken during production of poultry meat and/or eggs, nearly all large scale poultry farms in Egypt use rather dangerous chemical compounds (such as formalin and others) for fumigation of their farms to control pests. The present study with a rather harmless compound was performed at five chicken farms in two small villages in the Nile delta and in the Giza province in Egypt. There all farmers harbor in their houses small primitive poultry facilities either for production of meat and/or eggs. The rearing is done mostly in small rooms with floors being covered with dust and straw. Prior to the application of the product to the soil and/or onto chickens, five heavily infested locations were identified by personal communication with the farmers who came previously in direct contact with infested areas and subsequently had suffered from severe itching due to bites of the mites. Soil samples were also collected from each site for two successive weeks in order to determine the quantity of the *D. gallinae* mites (Figs. 1, and 2).

Application

The provided product Mite-Stop® (an extract) was freshly diluted by tap water 1:40 or 1:50 and was thoroughly mixed by shaking before use. The spray solution was applied on day 0 and day 7 onto the floor that directly surrounded the birds. During the spraying (done at 10 o'clock a.m., at 30°C) the birds remained in the rooms and were thus exposed to the spray, too. Before spraying the chicken food and the eggs were removed from the stables. Soil samples and birds had been examined for the occurrence of mites before application of the product to the floor. Similar amounts of chickens—kept in separate rooms—were not treated and were used as controls.

Birds

The chickens used during the experiments belonged to Egyptian local breeds (Fayoumi and Baldy), had a weight of 1.3 kg and were about 21 weeks in age. There were kept always 30–40 female chickens in such rooms. Among the females one to two males were reared.

Detection of the parasite in the soil

Soil samples and wastes from cracks and crevices were randomly collected from each chicken facility. Each sample of each of the five farms had a weight of about 1 kg. The samples were placed in tightly closed plastic bags and transported to the laboratory for extraction of mite within 1 day after sampling using a modified Tullgren funnel apparatus (Sutherland 1998). This apparatus consisted of the following components:

1. A zinc metal funnel of 33 cm in depth and of 18 cm diameter.
2. Four mesh sieve that was fixed at about 3 cm below the funnel rim.

Fig. 1 Chicken shamble at test sites
Fig. 2 When investigating the chickens, many bloody mite-biting sites are seen

Fig. 3 Dead adult *D. gallinae* from ventral
Fig. 4 Killed mallophaga from feathers of treated chicken

3. A wooden box with a 60-W lamp was inverted on the funnel. The distance between the lamp and the sieve was about 10 cm.
4. A clear plastic tube (5.7 cm deep and 3.2 cm in diameter) was placed at the end of the funnel stem to collect the mites. It was covered with a thin layer of Vaseline at its upper margin to prevent the escape of mites.

Extraction procedure

1. The soil sample was placed onto the sieve (1 kg soil/funnel) and was covered with the wooden box.
2. The lamp was switched on to provide light and heat. The mites are hygrotactic, thermotactic, and phototactic. This introduced their movement downwards—away from the heat of the lamp.

Table 1 Example for mean numbers of counts of living mites found in soil of stables (mites/g soil) 2 h after spraying of two dilutions of the neem seed extract (1:40, 1:50) on day 0

Mites	Day 0			Day 7		
D. gallinae (number/g)	Control	1:40	1:50	Control	1:40	1:50
	0.300	0.180	0.240	0.350	0.070	0.140
	0.380	0.228	0.304	0.410	0.082	0.164
	0.420	0.252	0.336	0.481	0.096	0.192
	0.510	0.306	0.408	0.390	0.078	0.156
Total per 4 g	1.610	0.966	1.288	1.631	0.326	0.652

The dust was taken 2 h after spraying on day 0 and on day 7 before spraying. The soil of controls originated from unsprayed regions of the same stables. Dead mites did not move to collection tubes. The results show that death of most mites is introduced several hours after the first spraying reducing the number until day 7 after treatment

3. Thus finally the mites fall through the sieve into the clear tubes.

At 6-h intervals starting from placing the soil samples in the apparatus, the contents of the tubes were examined. The specimens were sorted and counted by means of a dissecting stereo microscope.

Detection of mites on infested birds

Mites from infested birds were collected by brushing them with a fine hair brush wetted with alcohol.

Identification of mites

Mite specimens were mounted on glass slides in Hoyer's medium consisting of 50 ml distilled water, 200 g chloral hydrate, 30 g gum arabic, and 20 ml glycerine. When covered by a glass slip, the slides were gently heated until bubbles appeared. This allowed stretching and clearing of the specimens. Preparations were then examined and the parasites became identified according to their morphologic species characteristics (Fig. 3).

Results and discussion

It was shown in the present study that the administration of the water-diluted neem seed extract was efficacious against *D. gallinae* at the selected mite-infested locations. A single application led to an efficacy of more than 80% after the first administration, the second treatment increased the dramatic reduction of the mite population. The 1:40 dilution was slightly more effective and is thus recommended for further applications (Table 1).

In contrast, the mite population in untreated sites was considerably larger and remained stable at a high level. This high infestation rate affected not only the birds but also the farm workers who came in contact with those blood suckers during feeding the chickens or collecting the eggs. Examination of infested birds (Fig. 2) before and after treatment showed a considerable decrease in the number of mites collected, almost zero living ones especially after the second application of the compound. Sprayed birds showed in the follow-up no clinical signs or any alterations of their skin or feathers indicating the absence of side effects of the applied material. Similar effects using neem extracts had been reported by Lundh et al. (2005). The neem seed extract also killed the mallophaga that fed on the feathers of poultry (Fig. 4).

With respect to the different chemical compounds used until now against the red chicken mite, phoxim is one of the most common antiparasitic agent against this mite (Meyer-Kühling et al. 2007). However, there arise questions onto the safety of this and similar chemical compounds.

Phoxim residues in eggs were shown by using high-performance liquid chromatography diode array analysis after treatment of stocked poultry housing facilities (Hamscher et al. 2007).

Moreover, propoxur residues in eggs were determined when applying the same analytical method indicating an amount of residues above the allowed limits for eggs in the European Union (Hamscher et al. 2007).

Therefore a future ban of the use of chemical acaricides is realistic, especially in consideration of the forthcoming of a more restrictive animal welfare legislation for the production of more safe meat and eggs in a nonpolluted environment (Chauve 1998). Moreover, repeated long term chemical control may induce resistance in the mite strains. The application of the *B. thuringiensis* against the red mites infesting poultry is also not advisable, since the exotoxin produced by the bacteria is toxic also to vertebrates (McKeen et al. 1988).

Therefore shifting priority to control mites by effective and safe natural products is extremely recommended.

In conclusion the spraying of the watery solution neem seed extract on day 0 and 7 showed that the mite population was highly reduced after 1 week and remained at an acceptable low level. Since the nightly active mites may be hidden during spraying or hatch only after spraying procedure from their eggs, a three times spraying within 10 days may be reasonable for the first time. After this spraying should be done when stable is empty. However, a weekly monitoring of all poultry houses should be done in order to treat again areas where mites are found. This protects best the health of the animals.

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