Veterinary Parasitology 178 (2011) 374–378

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Field bioassay of *Metarhizium anisopliae* strains to control the poultry red mite *Dermanyssus gallinae*

M. Tavassoli[a,*], M. Allymehr[b], S.H. Pourseyed[a], A. Ownag[c], I. Bernousi[d], K. Mardani[e], M. Ghorbanzadegan[a], S. Shokrpoor[a]

[a] *Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, West Azarbaijan, Iran*
[b] *Poultry Diseases Division, Department of Clinical Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*
[c] *Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, West Azarbaijan, Iran*
[d] *Department of Genetic and Biometry, Faculty of Agriculture Science, Urmia University, Urmia, West Azarbaijan, Iran*
[e] *Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, West Azarbaijan, Iran*

ARTICLE INFO

Article history:
Received 11 July 2010
Received in revised form
21 December 2010
Accepted 17 January 2011

Keywords:
Poultry red mite
Dermanyssus gallinae
Metarhizium anisopliae
Biological control

ABSTRACT

The poultry red mite, *Dermanyssus gallinae* is one of the most economically deleterious ectoparasite of laying hens worldwide. To evaluate the efficacy of three strains (V245, 3247 and 715C) of entomopathogenic fungus *Metarhizium anisopliae* with potential as acaricides against *D. gallinae*, this investigation was carried out in a commercial caged laying poultry farm in Naghedeh, West Azarbaijan of Iran. The parasite infestation already existed in the farm. Sunflower oil suspension of all fungal strains, each in two concentrations (1×10^7 and 1×10^9 conidia/ml) were used separately as spray on hens and cages, and in the control group the cages were only sprayed with sunflower oil and sterile distilled water. For estimating the population rate of mites before and after treatment, special cardboard traps were fixed to cages during a 1-month period. The traps were placed on weeks –1, 0, 1, 2 and 3 and always removed after 1 w. The results showed that the population rates post fungal treatment with the lower concentration were not significantly different compared to the control group. However, the reduction in mite numbers induced by all three strains at the concentration of 1×10^9 conidia/ml was significantly higher than the control ($P < 0.05$). The results revealed that under field conditions, higher concentrations of *M. anisopliae* will be required for controlling *D. gallinae*.

1. Introduction

Dermanyssus gallinae, known as poultry red mite (De Geer, 1778) (Acari: Dermanyssidae), is one of the most important ectoparasites affecting laying hens in many countries (Chauve, 1998; Sparagano et al., 2009). It may become a serious pest, causing high feed conversion, decrease in egg production, irritation, feather pecking and anaemia and in some cases even death of its host (Wojcik et al., 2000; Kilpinen et al., 2005). The mite also may temporarily attack humans and cause a nuisance for personnel working at heavily infested poultry houses (Brockis, 1980; Rosen et al., 2002; Bellanger et al., 2008; Cafiero et al., 2008).

Apart from production losses in heavily infested flocks due to blood-feeding and short breeding cycle (7–10 days) of *D. gallinae*, the mite is also known to be a possible vector of various poultry pathogens, including *Salmonella* spp., avian spirochetes, chicken pox virus, Newcastle disease virus and agents of erysipelas, fowl typhoid and cholera

* Corresponding author at: Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, P.O. Box 1177, Urmia, West Azarbaijan, Iran. Tel.: +98 441 2972616; fax: +98 441 2771926.
E-mail address: mtavassoli2000@yahoo.com (M. Tavassoli).

0304-4017/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2011.01.031

as well as disease agents of other livestock species such as eastern equine encephalitis virus (Zeman et al., 1982; Durden et al., 1993; Chauve, 1998; Chirico et al., 2003; Valiente Moro et al., 2007, 2009; De Luna et al., 2008; Brännstrom et al., 2010).

In addition to physical cleaning of poultry houses, other conventional control methods mostly rely on the use of synthetic chemical products to kill the mite population. The most frequently used acaricides are organophosphorous compounds, carbamates and pyrethroids. The use of these products, however, has become increasingly hampered by more strict legislation (Fiddes et al., 2005), development of mite resistance (Kim et al., 2004; Marangi et al., 2009), human health hazards due to chemical residues in food products and environmental contamination (Dalton and Mulcahy, 2001). Therefore, the present situation has prompted researchers to study on alternative control methods which they are more environmentally friendly such as the use of, e.g. predatory mites (Lesna et al., 2009), plant-derived acaricides (George et al., 2010; Locher et al., 2010) and inert dusts (Kilpinen and Steenberg, 2009; Maurer et al., 2009). Recently, particular attention has been focused on the development of entomopathogenic fungi (Hajek and Delalibera, 2010), such as *Metarhizium anisopliae*, for controlling of a range of mites and ticks of veterinary importance (Brooks and Wall, 2001; Gindin et al., 2002; Pourseyed et al., 2010). The safety of these fungi towards humans and the environment and less problems with mite resistance are clearly important criterions to prefer them for use in controlling parasites than chemical acaricides (Pell et al., 2001; Thomas and Read, 2007).

In our previous study, we investigated the potential of *M. anisopliae* as a biocontrol agent against poultry red mite under laboratory conditions (Tavassoli et al., 2008). The aim of the present study was to evaluate the acaricidal effect of three different strains of *M. anisopliae* against *D. gallinae* in a heavily infested Iranian caged laying poultry farm.

2. Materials and methods

2.1. Farm details

The study was performed at a commercial layer farm with a history of heavy red mite infestation in Naghedeh, West Azarbaijan of Iran in October 2008. The farm had a cage system containing 15,000 laying birds (70 weeks old) of HY–Line W36, with 3750 cages (three to five hens per cage) in two floors and 16:8 h (L:D) photoperiod. The egg collection and feeding systems were manual. During the field bioassay, the temperature and relative humidity of the henhouse were recorded 16–20 °C and 50–60%, respectively.

2.2. Mite traps

There is no established guideline for the estimation of mite population density in a stocked poultry house. In our study, sixteen corrugated cardboard traps (two traps per cage) measuring 100 mm × 70 mm × 3 mm were used. The traps were fixed to distinct parts of cages (Nordenfors and Chirico, 2001) using metal wire. Each trap was used only once, collected weekly, and placed immediately into labelled self-sealing plastic bags. Collected traps containing mites of all stages were then transferred to the Department of Parasitology, Faculty of Veterinary Medicine, Urmia University for storage until counting of red mite.

2.3. Fungus strains and preparation

Three strains of *M. anisopliae* including V245, 3247 and 715C were used in this study. The first two strains were a part of the fungal culture collection at the University of Wales Swansea, UK while the Iranian strain was held at the University of Tehran, Iran. In our previous in vitro study, V245 and 715C were found to be the most effective strains for controlling red mites (Tavassoli et al., 2008). Fungal virulence was maintained by passaging twice through fowl tick *Argas persicus* before being cultured on PDA (potato dextrose agar; E. Merck, Germany) in Petri dishes (90 mm × 15 mm) in a dark incubator at 25 °C. Conidia were harvested 14 days post inoculation (DPI) by adding sterile distilled water (dH_2O). After that, suspensions were poured into sterile glass tubes and homogenized on a vortex mixer. The number of conidia were determined by direct count using a Neubauer haemocytometer and adjusted to final concentrations of 1×10^7 and 1×10^9 conidia/ml by dilution with sterile aqueous 0.05% Tween 80 solution as described by Butt and Goettel (2000). Conidia viability for each strain was determined by placing three droplets of the sunflower oil suspension of fungal conidia on PDA and counting conidia with protruding germ tubes under a light microscope at 40× magnification 48 h later (Goettel and Inglis, 1997).

2.4. Field bioassay

Heavy natural infestation with *D. gallinae* already existed in the farm. Three strains of *M. anisopliae* each in two concentrations (1×10^7 and 1×10^9 conidia/ml) were prepared. Trials for each concentration and control group were performed in four rows selected randomly as replicates (two cages per replicate). As oil suspension of fungal concentrations has higher affinity to feather and cage surfaces (Sewify and Habib, 2001), 40 ml sunflower oil was added per 1.5 l fungal spore suspension. Portable sprayers with coarse spray droplets were used for spraying the above suspension on hens and cages (180 ml per cage). The feed troughs were free of feed, and laid eggs were collected before spraying. For the control group the cages were only sprayed with sterile distilled water containing sunflower oil. The first monitoring was done by collecting one set of traps prior to the spraying (week 0). Collected traps were kept in a freezer (−20 °C) overnight to immobilize the mites (Nordenfors and Höglund, 2000), and finally, mites were transferred to a Petri dish and counted individually with the help of a binocular magnifier, but if they were abundant, the total number of the population was estimated by volume (Nordenfors and Höglund, 2000). All treatment and control groups were monitored at 1–4 weeks post spraying to calculate the mite population.