Veterinary Parasitology 262 (2018) 20–25

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Research paper

Activity of native and commercial strains of *Metarhizium* spp. against the poultry red mite *Dermanyssus gallinae* under different environmental conditions

Hadas Tomer[a], Tal Blum[a], Ilan Arye[c], Adi Faigenboim[b], Yuval Gottlieb[d], Dana Ment[a,*]

[a] Department of Entomology and Nematology, Plant Protection Institute, ARO, The Volcani Center, HaMaccabim Road 68, Rishon LeZion, 7528809, Israel
[b] Plant Science Institute, ARO, The Volcani Center, 68 HaMaccabim Road, Rishon LeZion, 7528809, Israel
[c] Department of Poultry, Ministry of Agriculture and Rural Development Extension Service, 68 HaMaccabim Road, Rishon LeZion, 7528809, Israel
[d] Koret School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

ARTICLE INFO

Keywords:
Microbial control
Entomopathogenic fungus
Poultry house
Abiotic conditions

ABSTRACT

The poultry red mite (PRM), *Dermanyssus gallinae*, is a major pest of laying hens with extremely limited control means. To evaluate the potential of natural and commercial entomopathogenic fungi (EPF) for use against *D. gallinae*, we tested four wild populations of *D. gallinae* from Israeli farms. The genus *Aspergillus* was identified as the most abundant isolates from the mites. Additionally, eight new isolates of *Metarhizium* belonging to the specie *M. brunneum* were identified. At all sites from which mites were collected in both seasons, the abundance of fungi on *D. gallinae* was greater during the winter season than during the summer season. Through indirect inoculations of adult *D. gallinae*, we examined the virulence of the native *Metarhizium* species, the commercial strain Ma-43 and a previously described acaropathogenic strain (Ma-7). All of the *Metarhizium* strains caused 56–95% mortality of adult mites by seven days after inoculation at a concentration of 5×10^5 conidia per cm^2. The efficacies of Ma-43, Ma-7 and the most promising native strain were tested under optimal abiotic conditions (28°C; 85–100% RH) and abiotic conditions similar to those typically found in a poultry house (30 °C; 60% RH). Under optimal conditions, the efficacy of all three stains ranged between 85 and 92%. In contrast, under poultry-house conditions, the efficacy of control ranged between 30 and 40%. The incidence of mycoses on mite cadavers was significantly decreased under poultry-house conditions. These results demonstrate the potential of native and commercial *Metarhizium* strains for use as biopesticides. Future research should address suitable delivery methods and formulations for the effective control of *D. gallinae* under poultry-house conditions.

1. Introduction

The red poultry mite, *Dermanyssus gallinae*, poses a significant threat to egg-laying hens worldwide (Sparagano et al., 2014). In Israel, high levels of *D. gallinae* are consistently found in poultry houses throughout in the country and it is anticipated that *D. gallinae* will become even more abundant with the reform in laying-hen production practices in Israel following changes in EU regulations (Arye I., unpublished).

Worldwide, the current strategy for *D. gallinae* control relies solely on synthetic acaricides of the organophosphate, abamectin and pyrethroid families (Sparagano et al., 2014) and recently on the systemic insecticide fluralaner, from the isoxazoline family (Brauneis et al., 2017; Thomas et al., 2017). As such, only a very limited number of chemical treatments are available as many conventional mite products have been withdrawn from European markets, banned or are no longer effective due to resistance of the mites. The increased need for a sustainable method for controlling poultry-mite infestations has been thoroughly described in recent reviews (Sparagano et al., 2014; Sigognault Flochlay et al., 2017).

There are several natural enemies of *D. gallinae* such as predatory flies and mites, as well as pathogens including nematodes, fungi and bacteria. But, none of the above organisms has been routinely integrated in the management of *D. gallinae*. In recent years, entomopathogenic fungi (EPF) have been shown to be pathogenic toward both *D. gallinae* and the northern fowl mite, *Ornithonyssus sylviarum*, and their efficacy in poultry houses has been demonstrated (Tavassoli et al., 2008; Rassette et al., 2011; Steenberg and Kilpinen, 2014; Immediato et al., 2015). The most outstanding advantages of EPF are their ability to directly penetrate the cuticle of arthropods (Tanada and Kaya, 1993) and the fact that a single EPF strain can have a broad

* Corresponding author.
E-mail addresses: gottlieb.yuval@mail.huji.ac.il (Y. Gottlieb), danam@agri.gov.il (D. Ment).

https://doi.org/10.1016/j.vetpar.2018.09.010
Received 9 June 2018; Received in revised form 7 August 2018; Accepted 19 September 2018

spectrum of hosts. However, EPF kill mites more slowly than chemical acaricides and, in the absence of favorable abiotic environmental conditions, their efficacy is low. There are more than 700 known EPF, mainly from the divisions Ascomycota and Zygomycota (Roy et al., 2010). In 2006, 129 myco-insecticide products were commercially available worldwide. Most of those products were based on Ascomycetes of the specie complex *Metarhizium* and *Beauveria* (de Faria and Wraight, 2007).

In this study, we isolated native EPF including *Metarhizium* strains from natural populations of *D. gallinae* and examined the activity of each *Metarhizium* strain against wild populations of *D. gallinae*, relative to that of a commercial strain and a previously described strain known to possess acaricidal activity (Gindin et al., 2002). We also investigated the virulence potential of the different strains, first by indirect application of conidial suspensions and then by evaluating the efficacy of the most virulent strains under optimal abiotic conditions and abiotic conditions resembling those typically found in a poultry house.

2. Materials and methods

2.1. Mites

Wild *Dermanyssus gallinae* mites were collected in advance from four open-shed egg-producing poultry farms in the center of Israel after confirming with the farmers that no acaricides had been applied. Collections were carried out during the winter (January–February) and summer (August–September) of 2015 (Table 1). At each farm, brushes were used to collect mites into 50-mL tubes and the mites were then transferred immediately to the lab for fungal isolation and bioassays.

2.2. Fungal isolation and culturing

Fungi from wild populations of *D. gallinae* were isolated by placing the adult mites on Sabouraud dextrose agar (SDA)-based selective media containing 10 μg/mL dodine (Sigma) and 500 μg/mL chloramphenicol (Sigma). Plates were incubated at 28 °C and observed daily for signs of fungal growth. Once hyphae were visible, they were aseptically harvested with a sterile loop, plated onto a fresh selective media and incubated at 28 °C. Single-spore subcultures were established from a single colony per mite sample. If a sample contained a mite infected with two or more fungi that were morphologically different from one another, a random single spore isolate of each was selected. *Metarhizium* was identified under a microscope to detect conidiophores and conidia morphology (Humber, 2012).

2.3. Molecular phylogenetic identification

The isolated *Metarhizium* strains were taxonomically classified through comparative analysis of the 5′ region of elongation factor-1 alpha (5′-*TEF1*), as described in Bischoff et al. (2009). Five EPF-strain sequences representing species in the *Metarhizium* complex were retrieved from the ARS Microbial Genomic Sequence Database (http://199.133.98.43/cgi-bin/bigsdb/bigsdb.pl?page=plugin&name=PhyloTree&db=public_ars_entomopathogen_isolates). Fifteen 5′-*TEF1* sequences were aligned using the MAFFT program (Version 7, http://mafft.cbrc.jp/alignment/server/) with default parameters. The Gblocks server was used for the selection of conserved blocks in the multiple alignment (Talavera and Castresana, 2007). A maximum-likelihood tree with 100 bootstrap replicates created using PhyML 3.0 software (Guindon et al., 2010) was constructed based on the automatic nucleotide model selection of AIC (Akaike Information Criterion; Lefort et al., 2017). The tree was graphically designed using FigTree Version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

2.4. Fungal virulence bioassay

The fungi were grown on SDA for 2 weeks at 28 °C and conidia were harvested by scraping the agar and suspending the scrapings in sterile, distilled water containing 0.01% Triton X-100, in glass tubes. The suspension was vortexed and filtered through Miracloth (Calbiochem; La Jolla, CA) and spore concentrations were determined with a hemocytometer. Suspensions were adjusted to the required conidia concentrations in 0.01% Triton X-100 and the percentage of viable conidia was determined on SDA prior to each bioassay. Only conidial suspensions with at least 95% germination were used. Sterile filter paper was placed in 55-mm-diam. Petri dishes and impregnated with 0.5 mL of sterile water containing 0.01% Triton X-100 without (control) or with fungal conidia at 1×10^7 conidia per mL (i.e., 5×10^5 conidia per cm^2). Adult mites (20–30 per dish) were distributed on the impregnated filter paper with a camel-hair brush. The mites were counted and incubated at 28 °C and ~85% RH. The numbers of infected mites (i.e., mites on which mycelia developed) were counted daily under a stereomicroscope. Each treatment was replicated five times for each isolate and the whole bioassay was repeated at least twice.

2.5. Humidity and temperature assays

We examined the effect of abiotic environmental conditions on the mortality of *D. gallinae* treated with indirectly applied *M. brunneum* conidia as described above. The tested regimes were 28 °C and ~85% RH for optimal conditions (defined in a previous study; Ment et al., 2010) and 30 °C and 60% RH for non-optimal conditions. The parameters for non-optimal conditions were defined after measuring the temperature and relative humidity during the visits in the four different poultry houses. Different temperature regimes were achieved by incubating the experimental systems at 28 and 30 °C. Humidity regimes were achieved by inserting a plastic vessel into the experimental system of the 55-mm Petri dishes. To achieve 60% RH and mimic the environmental conditions found in poultry houses, the vessels contained 0.25 mL of a saturated solution of ammonium nitrate (NH_4NO_3; Winston and Bates, 1960). To achieve 85–100% RH for optimal conditions, the vessels contained distilled water. To confirm the humidity conditions in each setup, a surrogate system (without mites) was monitored using a hygrometer.

Table 1
Mite collection sites and relative abundance of fungi based on morphological identification.

Mite collection site code	Mite collection site coordinates	Sampling month	% Abundance based on morphological identification			
			Aspergillus	*Metarhizium*	*Penicillium*	Unidentified
A	31°56′4.19″N 34°46′22.07″E	January	42.8	21.4	14.3	21.4
		September	3.1	0	0	0
B	31°48′56″N 34°47′47″E	February	74.3	17.1	2.8	5.7
		September	4.3	0	0	0
C	32°11′45.59″N 34°54′28.42″E	August	1.6	0	0	0
D	31°42′35.28″N 34°56′42.35″E	September	8.3	0	0	0