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#### ■ Author(s)

Kasburg CR<sup>I</sup>  
Alves LFA<sup>II</sup>  
Oliveira DGP<sup>III</sup>  
Rohde C<sup>IV</sup>

<sup>I</sup> Laboratório de Biotecnologia Agrícola – UNIOESTE, Cascavel

<sup>II</sup> Professor/Bolsista Produtividade em Pesquisa, CNPq – UNIOESTE, Cascavel

<sup>III</sup> Professor – UTFPR, Santa Helena

<sup>IV</sup> Professor – UTFPR, Medianeira

#### ■ Mail Address

Corresponding author e-mail address  
Luis Francisco Angeli Alves  
Laboratório de Biotecnologia Agrícola – UNIOESTE, Rua Universitária, 2069  
85819-110. Cascavel, PR, Brasil  
Tel: (005545) 32203288  
Email: [luis.alves@unioeste.br](mailto:luis.alves@unioeste.br)

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Hematophagous mites, microbial control, ectoparasites, laying hens.

## Activity of Some Brazilian Isolates of Entomopathogenic Fungi Against the Poultry Red Mite *Dermanyssus Gallinae* De Geer (Acari: Dermanyssidae)

### ABSTRACT

Poultry red mite *Dermanyssus gallinae* is a cosmopolitan and hematophagous species commonly found in layer houses around the world. Poultry mite infestations may cause anemia, stress, low body weight and egg production, and mortality. Mite control is typically based on chemical products, but they are not effective and leave residues in eggs; therefore, alternative control methods, such as entomopathogenic fungi, need to be researched. This study aimed at evaluating, in the laboratory, the activity of Brazilian isolates of entomopathogenic fungi against *D. gallinae*. The mites were collected from a commercial layer house and were sprayed with conidial suspensions ( $1 \times 10^8$  conidia/mL) of five isolates of *Beauveria bassiana* and *Metarhizium anisopliae*. All tested isolates were pathogenic for the red mite, with confirmed mortality ranging from 22.9 to 52.4%. This demonstrates the potential of the tested entomopathogenic fungi isolates for mite control, and reinforces the need for further studies with other isolates, application strategies, and with fungal formulations.

### INTRODUCTION

Brazilian egg production is based on intensive farming systems, which ensure greater yield in a smaller physical space. However, it favors the development of arthropod pests, such as the red mite *Dermanyssus gallinae* (De Geer) (Acari, Dermanyssidae). This mite feeds on poultry, causing weight loss, reduced egg production, blood spoliation anemia and, in more severe cases, death. Studies have shown that the red mite can carry *Escherichia coli*, *Salmonella* and *Coxiella burnetii*, as well as viruses (Saint Louis encephalitis virus, avian poxvirus, avian paramyxovirus type I and Newcastle disease virus). In all these cases, the mite may acquire these microorganisms by feeding on the blood of infected birds (Moro *et al.*, 2007; Harrington *et al.*, 2011; Pereira, 2009; 2011; Sparagano *et al.*, 2014).

Mite control in poultry houses is based on chemical acaricides that are apparently effective because they cause momentary reduction of the pest population. Misuse of these products can lead to residues in the eggs, poisoning of workers and poultry in layer houses, environmental contamination; and can also select individuals resistant to the active ingredients (Chauve, 1998; Pereira, 2009; Liebisch, 2011).

As an alternative to chemical control, entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok isolates from Europe were evaluated against red mites (Steenberg & Kilpinen 2003). In addition, studies conducted in the laboratory and in the field in Iran and Egypt confirmed the action of fungal isolates from Europe and Asia against *D. gallinae* (Steenberg *et al.*, 2006; Kaoud, 2010; Tavassoli *et al.*, 2008; Steenberg & Kilpinen, 2014).



However, in Brazil, studies on alternative pest control are focused only in the *in-vitro* evaluation of plant extracts (Morrone *et al.*, 2001; Soares, 2012; Oliveira *et al.*, 2014). Therefore, the objective of the present study was to evaluate for the first time the pathogenicity of Brazilian isolates of *B. bassiana* and *M. anisopliae* against *D. gallinae*.

## MATERIALS AND METHODS

Mites were collected from a commercial layer house. Engorged mite females were selected based on their description (Flechtman, 1973), placed in glass tubes closed with gauze, and kept at 26°C, 70% RH and 12 h photoperiod for acclimatization until use in the experiment (Soares, 2012). Isolates of the fungi *B. bassiana* (Unioeste 01, Unioeste 02, Unioeste 04 and Unioeste 05) and *Metarhizium anisopliae* (Unioeste 22) were evaluated (Table 1).

The fungi were grown in culture medium (sporulation medium) in Petri dishes (10 days at 26°C; 12 h photoperiod) (Alves *et al.*, 1998). After incubation, conidia were collected by scrapping the surface of the culture medium. A suspension of conidia was prepared ( $1 \times 10^8$  conidia/mL) in distilled water + Tween 80® at 0.01%. Fifteen adult female mites were transferred to a Petri dish and sprayed with 1 mL of the conidial suspension using a Potter spray tower ( $0.7 \text{ kgf/cm}^2$ ). As control treatment, only distilled water + Tween 80® to 0.01% was applied. After spraying, mites were transferred to glass tubes closed with cotton gauze plug, and observed daily for seven days. The dead mites were removed using a fine-tipped brush and placed in a humid chamber and incubated under the above-mentioned conditions for the confirmation of mortality by the fungus. All treatments included seven replicates of 15 mites each. Experiments were repeated twice.

A completely randomized experimental design was applied. Total and confirmed mortality data were transformed into  $\arcsin \sqrt{100}$  and analyzed for normality and variance homogeneity by the Shapiro-Wilk and Levene's tests, respectively. Means were compared by Tukey's test ( $p \leq 0.05$ ) using Sisvar statistical software (Ferreira, 2011).

## RESULTS AND DISCUSSION

All *B. bassiana* isolates were pathogenic for the poultry red mite *D. gallinae* (confirmed mortality between 22.9 and 52.4%). Significant differences were detected only between Unioeste 2 and Unioeste 4 isolates. The *M. anisopliae* Unioeste 22 isolate caused 52.4% total mortality and only 38.1 % confirmed mortality (Table 1).

Steenberg & Kilpinen (2003) also found 60% mortality of the poultry red mite by *B. bassiana* and 30% by *M. anisopliae* in the laboratory. The acaricidal activity of a commercial product based on *B. bassiana* conidia in a powder formulation was also demonstrated under laboratory conditions, with 65% mortality after five days of inoculation (Kaoud, 2010).

Isolates of *M. anisopliae* were also pathogenic against mite *D. gallinae* in the laboratory, with mortality ranging between 40 and 70% depending on the tested isolates, and, under field conditions, they were shown to be efficient in commercial poultry houses treated with a conidia suspension at a high concentration ( $1 \times 10^9$  conidia/mL) (Tavassoli *et al.*, 2008, 2011).

The results of the present study demonstrate the potential of the tested entomopathogenic fungi isolates for the control of the poultry red mite. However, it should be noted that this is a preliminary test with Brazilian isolates, and further research is suggested to find more virulent isolates and to test

**Table 1** – Total and confirmed mortality of *D. gallinae* by different isolates of entomopathogenic fungi.

Treatment	Host <sup>1</sup>	Total mortality	Confirmed mortality
Control		25.7 ± 1.29 b	0.0 ± 0.00 c
<i>Beauveria bassiana</i>			
Unioeste 01	<i>Astyus variegatus</i> (adult)	49.5 ± 1.75 ab	43.8 ± 1.70 ab
Unioeste 02	<i>Alphitobius diaperinus</i> (larvae)	71.4 ± 1.22 a	65.7 ± 1.14 a
Unioeste 04	<i>Alphitobius diaperinus</i> (larvae)	37.2 ± 1.71 ab	22.9 ± 1.28 b
Unioeste 05	<i>Alphitobius diaperinus</i> (adult)	59.1 ± 1.10 ab	52.4 ± 1.07 ab
<i>Metarhizium anisopliae</i>			
Unioeste 22	Soil, <i>Ilex paraguayensis</i> plantation	52.4 ± 0.59 ab	38.1 ± 0.56 ab
CV (%)		36.3	42.5

<sup>1</sup>Entomopathogenic Fungi Collection - Laboratory of Agricultural Biotechnology, Unioeste-Cascavel, PR, Brazil. *Astyus variegatus* and *Alphitobius diaperinus* - Coleoptera: Melyridae and Tenebrionidae, respectively.

Means followed by the same letter incolumn do not differ by Tukey's test ( $p \leq 0.05$ )



application strategies and fungal formulations, both in the laboratory and in the field.

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