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Effect of different *Beauveria bassiana* strains against *Alphitobius diaperinus* from poultry farms in Colima state

Efecto de diferentes cepas de *Beauveria bassiana* contra *Alphitobius diaperinus* de granjas avícolas en el estado de Colima

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ABSTRACT

The aim of this study was to evaluate the effect of different *Beauveria bassiana* strains (*B. bassiana*) against *Alphitobius diaperinus* (*A. diaperinus*). A total of 360 beetles *A. diaperinus* (adults) were collected from poultry farms in Colima state. After verifying its viability (90% of germinated conidia), five of *B. bassiana* strains (Bb3, Bb4, Bb5, Bb6 and Bb17) were used. The five treatments of *B. bassiana*, presented a concentration of 1×10^8 conidia/mL. Fifteen beetles were used for the control and for each of *B. bassiana* strain, with four replicates/treatment. The dead *A. diaperinus* were incubated for 21 d, to promote mycelial growth and thus determine whether the death was caused by *B. bassiana*. The resulting data set was analyzed by descriptive statistics. The comparison between groups was assessed by Analysis of Variance and frequency histogram. The results showed that all fungal isolates were pathogenic against *A. diaperinus* beetles. The Bb4 strain showed a difference between groups, with respect to mortality (5.25 ± 0.95 dead *A. diaperinus* beetles). In dead beetles, mycosis due to mycelium growth of *B. bassiana*, presented the best results in Bb4 strain (2.50 ± 1.29 beetles *A. diaperinus* with mycosis) equivalent to 16.66% with the lowest value in Bb3 strain and Bb6 strain (3.33 and 1.66% of mycosis) respectively. The results found could be used in future trials to evaluate the Bb4 strain under normal conditions within the bird facilities.

Keywords: *Beauveria bassiana*, *Alphitobius diaperinus*, conidia, mycosis.

RESUMEN

El objetivo del presente trabajo fue evaluar el efecto de diferentes cepas de *Beauveria bassiana* (*B. bassiana*) contra *Alphitobius diaperinus* (*A. diaperinus*). Un total de 360 escarabajos *A. diaperinus* (adultos), fueron colectados de granjas avícolas en el estado de Colima. Después de comprobar su viabilidad (90% de conidios germinados), cinco cepas de *B. bassiana* (Bb3, Bb4, Bb5, Bb6 y Bb17) fueron utilizadas. Los cinco tratamientos de *B. bassiana*, presentaron una concentración de 1×10^8 conidios/mL. Se emplearon 15 escarabajos para el testigo y por cada cepa de *B. bassiana*, con cuatro réplicas/tratamiento. Los *A. diaperinus* muertos se incubaron por 21 d, para promover el crecimiento del micelio y con ello determinar si la muerte fue causada por *B. bassiana*. El conjunto de datos resultante se analizó mediante estadística descriptiva. La comparación entre grupos se llevó a cabo por Análisis de Varianza e histograma de frecuencia. Los resultados mostraron que todos los aislamientos fúngicos eran patógenos contra escarabajos *A. diaperinus*. La cepa Bb4 presentó diferencia entre grupos, con respecto a la mortalidad (5.25 ± 0.95 escarabajos *A. diaperinus* muertos). En los escarabajos muertos, la micosis por crecimiento del micelio de *B. bassiana*, presentó los mejores resultados en la cepa Bb4 (2.50 ± 1.29 escarabajos *A. diaperinus* con micosis) equivalente al 16.66%. Con el valor más bajo en las cepas Bb3 y Bb6 (3.33 y 1.66 % de micosis) respectivamente. Los resultados encontrados, se podrían utilizar en próximos ensayos para evaluar la cepa Bb4 en condiciones normales dentro de instalaciones para aves.

Palabras clave: *Beauveria bassiana*, *Alphitobius diaperinus*, conidios, micosis.

INTRODUCTION

Among the main challenges facing the poultry industry, the control of *Alphitobius diaperinus* (*A. diaperinus*) (Crippen *et al.*, 2018) stands out, since this beetle is a reservoir of numerous pathogens in birds and humans (do Carmo *et al.*, 2019). In addition, although the main habitat of *A. diaperinus*, is manure or garbage (Govorushko, 2019), its larvae can climb walls (Santoro *et al.*, 2008) and support posts in bird facilities (Rice and Lambkin, 2009). Causing damage to insulation materials during their pupation processes (Hazeleger *et al.*, 2008; Wales *et al.*, 2010; Crippen *et al.*, 2018). The control of this insect is generally carried out with insecticides (Arena *et al.*, 2019). Highlighting the i) fenitrothion, ii) cyfluthrin and iii) spinosad (Tomberlin *et al.*, 2014; Hickmann *et al.*, 2018). However, many of these chemicals have no effect on the adult stage of the beetle (do Prado *et al.*, 2013).

In addition, Lambkin *et al.* (2010) reported a greater resistance of *A. diaperinus* to fenitrothion, in 11 poultry farms for fattening. Especially southeast of Queensland, where numerous applications of fenitrothion have been carried out constantly. A control alternative for *A. diaperinus* is the entomopathogenic fungus *Beauveria bassiana* (*B. bassiana*) (Martins *et al.*, 2016).

This fungus is used as a biological insecticide or biopesticide controlling a large number of parasites such as caterpillars, termites, whiteflies, aphids and beetles (Daniel *et al.*, 2019), causing them mycosis or white muscardine disease (Santoro *et al.*, 2008; Mascarín and Jaronski, 2016). In Mexico, as a result of a previous investigation carried out by (Prado *et al.*, 2014), the pathogenicity of five strains of the *Beauveria bassiana* fungus in adults of *Alphitobius diaperinus* beetle was evaluated under laboratory conditions, obtaining good results. Therefore, from this study, the need arose to carry out research with New Mexican strains of the *Beauveria bassiana* fungus. Therefore, the objective of the present study was to evaluate the effect of different strains of *B. bassiana* (Deuteromycotina: Hyphomycetes) against *A. diaperinus* (Coleoptera: Tenebrionidae) of poultry farms in Colima state.

MATERIAL AND METHODS

Experimental location.

The bioassays were performed at the DES Biological Control Laboratory: Agricultural Sciences of the University of Colima. Laboratory conditions were 25±1 °C temperature, 80±4% of relative humidity (HR), and 12:12 h light/dark (L/O).

Origin of *A. diaperinus*.

A total of 360 *A. diaperinus* beetles (adults) were collected from poultry farms in Colima state, during the 7th week of production (September and October 2016). The capture was carried out in various sites of the bird's bed, with the use of entomological clamps. *A. diaperinus* were transported in bottles 6 cm in diameter x 7 cm high, to the laboratory for taxonomic identification according to (Chernaki and Almeida, 2001; Dunford and Kaufman, 2006).

Origin of *B. bassiana* strains.

Five strains of *B. bassiana* (Bb3, Bb4, Bb5, Bb6 and Bb17) from the entomopathogenic fungi collection of the DES Biological Control Laboratory: Agricultural Sciences of the University of Colima and the Laboratory of Parasitology and Biological Control of the Department of Veterinary and Zootechnics, Life Sciences Division of the University of Guanajuato.

Experimental stage (Phase 1). Multiplication, viability and concentration of *B. bassiana* strains.

The five strains of *B. bassiana* were multiplied in solid medium solid dextrose sabouraud agar (**ADS**) and incubated for 21 d. One day before performing the bioassay, the conidia were harvested with a bacteriological handle and placed in a 2x8 cm glass jar with a capacity of 25 mL, containing 10 mL of sterile distilled water with *Tween* 80 (00102; Sigma Chemical CO., Saint Louis, MO) at 0.01%. The mixture was homogenized (stirring) for 5 min and the viability of the conidia of each strain was determined by microculture ([Santoro et al., 2008](#); [Mascarin and Jaronski, 2016](#)). It was considered as a viable strain, one where more than 90% of the germinated conidia were observed ([Geden and Steinkraus, 2003](#); [Daniel et al., 2019](#)). To determine the concentration of conidia, a Neubauer chamber was used. The five treatments of *B. bassiana* (Bb3, Bb4, Bb5, Bb6 and Bb17) had a concentration of 1×10^8 conidia/mL.

Experiment (Phase 2). Pathogenicity.

[Rezende et al. \(2009\)](#) and [Alves et al. \(2015\)](#) suggested using between 10 and 20 beetles. Therefore, 15 *A. diaperinus* beetles (adults) were used for the control and for each strain of *B. bassiana* (Bb3, Bb4, Bb5, Bb6 and Bb17), with four replicates/treatment. Each *A. diaperinus* was transferred to a sterile 250 mL beaker containing 10 mL of suspension (1×10^8 conidia/mL) of each treatment and (only sterile distilled water with 0.01% *Tween* 80) for witness. After inoculation, *A. diaperinus* were placed in Petri dishes, with wet filter paper with sterile distilled water. Mortality was recorded every 12 h after inoculation for a period of 10 d. The dead beetles were individually placed in Petri dishes with wet filter paper with sterile distilled water and incubated for 21 d, to promote mycelial growth and thereby determine if the death was caused by *B. bassiana*.

Statistical analysis.

The statistical program (SAS, System, v. 9.0.2, Cary, NC) was used to process the data obtained. To evaluate the design completely randomly, the following model was tested:

$$\gamma_{ij} = \mu_i + \epsilon_{ij}$$

where:

- γ_{ij} = observation of the j-th u.e. of i-th treatment;
- μ_i = mean of the ith treatment; and
- ϵ_{ij} = experimental error of the unit *ij*.

The resulting data set was analyzed for descriptive statistics by (PROC UNIVARIATE; SAS, 2010). The comparison between treatments (control, Bb3, Bb4, Bb5, Bb6 and Bb17) was carried out by (PROC ANOVA; [SAS, 2010](#)). When significant effect was found by

group ($P < 0.05$), the Tukey multiple comparison test was performed. The data obtained for mycosis were transformed to the arcsine ($\sqrt{\bar{x}/100} * \sin^{-1}$), expressed as a percentage and plotted.

RESULTS

Descriptive statistics for total deaths and total deaths from mycosis, caused by the five strains of *B. bassiana* on *A. diaperinus* beetles (adults) of poultry farms in Colima state. It can be seen that the strains tested in the total of dead beetles and the total of beetles killed by mycosis showed higher values than the control, where the Bb4 strain was the one that obtained the highest values, as shown in table 1.

Table 1. Descriptive statistics for total deaths and total deaths from mycosis of *Beauveria bassiana* on *Alphitobius diaperinus*, $n = 360$ beetles

	$\bar{x} \pm DE^a$	IC ^b	$P_{10}-P_{90}$	$P_{25}-P_{75}$
Total dead				
Control	1.00 ± 0.81	0.00 – 2.19	0.00 – 2.00	0.25 – 1.75
Strain of <i>B. bassiana</i>				
Bb3	1.50 ± 1.73	0.00 – 4.25	0.00 – 4.00	0.25 – 3.25
Bb4	5.25 ± 0.95	3.72 – 6.77	4.00 – 6.00	4.25 – 6.00
Bb5	2.50 ± 1.00	0.90 – 4.09	2.00 – 4.00	2.00 – 3.50
Bb6	2.00 ± 0.81	0.70 – 3.29	1.00 – 3.00	1.25 – 2.75
Bb17	2.00 ± 1.41	0.00 – 4.25	0.00 – 3.00	0.50 – 3.00
Total deaths by mycosis				
Control	0.00 ± 0.00	0.00 – 0.00	0.00 – 0.00	0.00 – 0.00
Strain of <i>B. bassiana</i>				
Bb3	0.50 ± 0.57	0.00 – 1.41	0.00 – 1.00	0.00 – 1.00
Bb4	2.50 ± 1.29	0.44 – 4.55	1.00 – 4.00	1.25 – 3.75
Bb5	1.00 ± 1.15	0.00 – 2.83	0.00 – 2.00	0.00 – 2.00
Bb6	0.25 ± 0.50	0.00 – 1.04	0.00 – 1.00	0.00 – 0.75
Bb17	0.75 ± 0.95	0.00 – 2.27	0.00 – 2.00	0.00 – 1.75

^aMean $\pm$ standard deviation; ^b95% confidence interval; ^cConfirmation by mycelium growth of *B. bassiana*.

The Bb4 strain of *B. bassiana* showed a difference between groups, with respect to mortality (5.25 ± 0.95 dead *A. diaperinus* beetles). The lowest mortality among strains of *B. bassiana* was quantified in Bb3 (table 2). In dead beetles, mycosis due to mycelium growth of *B. bassiana*, presented the best results in strain Bb4 (2.50 ± 1.29 beetles *A. diaperinus* with mycosis) equivalent to 16.66% (figure 1).

Table 2. Comparison of pathogenicity of five strains of *Beauveria bassiana* against *Alphitobius diaperinus* from poultry farms in Colima state

	Strains of <i>B. bassiana</i>					
	Control	Bb3	Bb4	Bb5	Bb6	Bb17
Mortality	1.00 ± 0.81^a	1.50 ± 1.73^a	5.25 ± 0.95^b	2.50 ± 1.00^a	2.00 ± 0.81^a	2.00 ± 1.41^a
Mycosis	0.00 ± 0.00^a	0.50 ± 0.57^a	2.50 ± 1.29^b	$1.00 \pm 1.15^{a,b}$	0.25 ± 0.50^a	0.75 ± 0.95^a

Within lines, values with different letters differ significantly from each other ($P < 0.05$). All data are presented by mean $\pm$ DE.

The Bb5 strain recorded (1.00 ± 1.15 *A. diaperinus* beetles with mycosis) equivalent to 6.66% (figure 1). With the lowest value in strains Bb3 and Bb6 (3.33 and 1.66% of mycosis) respectively.

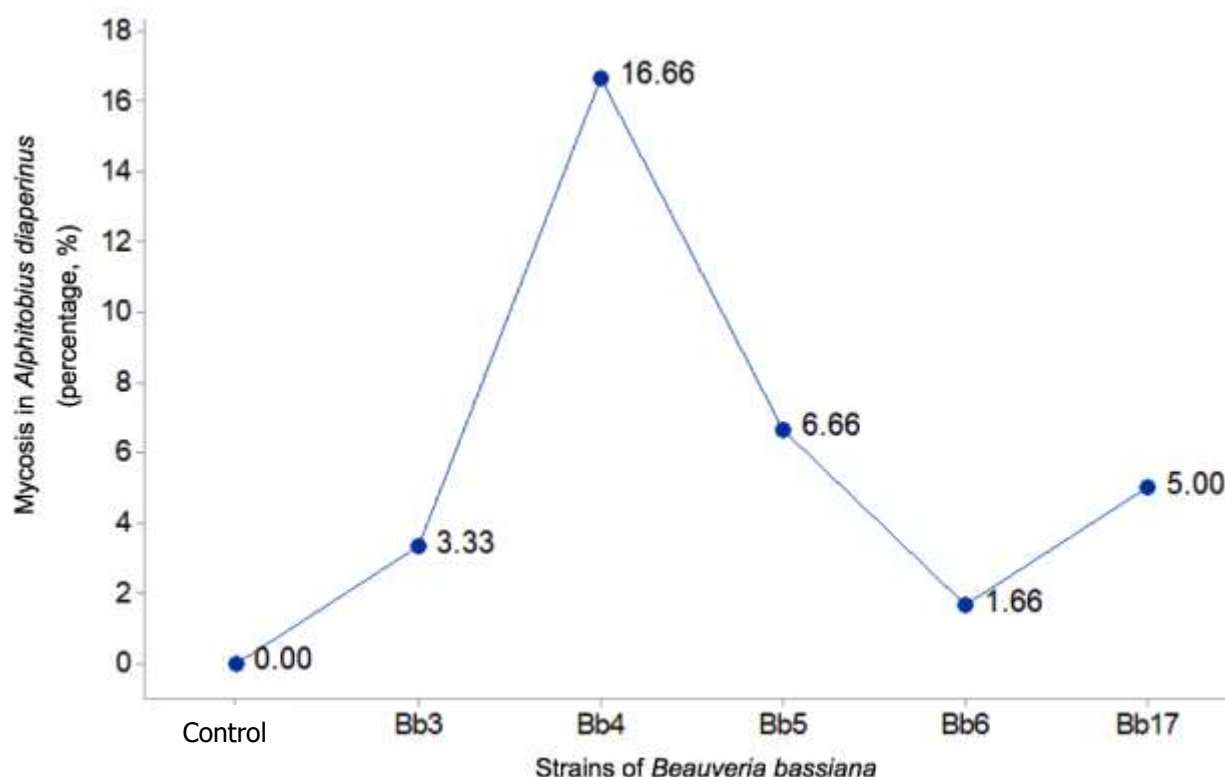


Figure 1. Mycosis by *Beauveria bassiana* in *Alphitobius diaperinus* of poultry farms in the state of Colima, $n = 360$ beetles.

DISCUSSION

The results showed that all fungal isolates were pathogenic against *A. diaperinus* beetles, with a mycosis-induced mortality of 16.66% maximum and with significant differences between strains (Bb3, Bb4, Bb5, Bb6 and Bb17). These results are similar to the reports of (Rohde *et al.*, 2006; Rezende *et al.*, 2009), who worked with the same beetle. It has been described that the first contact between *B. bassiana* and *A. diaperinus*, occurs when the spore (conidium) is deposited on the surface of the insect, hence the importance of the pathogenic dose (1×10^8 conidia/mL) (Santoro *et al.*, 2008).

The conidium begins the development of its germination tube and its appressorium to fix itself to the beetle. For adequate germination, a HR of 92% and a temperature of between 23 and 25 °C (Vertyporokh *et al.*, 2019) are required. Then through enzymes such as proteases, lipases and chitinases, the entomopathogenic fungus enters *A. diaperinus* through its soft parts (Mascarin and Jaronski, 2016).

Within the beetle, *B. bassiana* branches its structures and colonizes the host's cavities (Daniel *et al.*, 2019). With toxins p. eg, beauvericin, beauverolide, bassianolide and oxalic breaks the immune system of *A. diaperinus*, invading all its tissues and killing the beetle

(Geden and Steinkraus, 2003). Thus beginning the saprophytic phase of *B. bassiana* with its multiplication and growth (Mascarin and Jaronski, 2016).

However, the highest expected mortality did not reach significant levels. Daniel et al. (2019) found that *B. bassiana* isolates are highly virulent and also indicated a wide variability in virulence among strains (from 2 to 70%). This indicates that, as in the present study, it is mandatory to evaluate as many strains as possible and thus be able to select the one with the greatest pathogenicity against *A. diaperinus*.

CONCLUSION

Beauveria bassiana proved useful as a biological control measure for *Alphitobius diaperinus* from poultry farms in Colima state. The potential to cause mycosis of strain Bb4, on strains Bb3, Bb5, Bb6 and Bb17 under laboratory conditions, is highlighted. The results found could be used in future trials to evaluate the Bb4 strain under normal conditions in poultry facilities.

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