

Performance of *Metarhizium rileyi* applied on *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)

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ABSTRACT

This study aimed to evaluate the efficiency of different strains of the entomopathogenic fungus *Metarhizium rileyi* in the control of *Helicoverpa armigera* caterpillars in laboratory conditions. Caterpillars between the 2nd and 3rd instar were used, ranging in size from 0.7 to 1.2 cm length. The experimental design used was completely randomized, composed of five treatments and five replications, each one consisting of 50 insects. The treatments T1 - Control (sterile distilled water), T2 - *M. rileyi* UFMS 02 strain, T3 - *M. rileyi* UFMS 03 strain, T4 - *M. rileyi* UFMS 06 strain, and T5 - *M. rileyi* UFMS 07 strain were evaluated. All treatments were applied (2 mL/insect) in suspensions of the order of 1.0×10^9 conidia mL⁻¹, and Tween 80[®] was added in all treatments. Evaluations were performed daily to verify mortality and sublethal effects. For emerging adults, Filial Generation (FG), the biological cycle was evaluated. The data referring to larval mortality for the Parental Generation (PG) and GF and pupal for GF were submitted to analysis of variance, and the Scott-Knott test grouped the averages at 5% probability. The strains tested did not provide pathogenicity in the larval phase of *H. armigera* for PG and FG. However, there was a reduction in oviposition in all treatments regarding the control. There was an effect on the reproductive phase of GF caterpillars exposed to *M. rileyi*.

Keywords: Lepidoptera pests, Entomopathogens, Integrated Pest Management.

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RESUMO

Objetivou-se avaliar a eficiência de diferentes cepas do fungo entomopatogênico *Metarhizium rileyi* no controle de lagartas *Helicoverpa armigera* em condições de laboratório. Para condução do bioensaio, foram utilizadas lagartas entre 2^o e 3^o instar, com tamanho variando de 0.7 a 1.2 cm de comprimento. O delineamento experimental utilizado foi o inteiramente casualizado, composto por 5 tratamentos e 5 repetições, cada uma composta por 50 insetos. Foram aplicados 2 mL/inseto nos tratamentos: T1 - Testemunha (água destilada esterilizada), T2 - *M. rileyi* strain UFMS 02; T3 - *M. rileyi* strain UFMS 03; T4 - *M. rileyi* strain UFMS 06; T5 - *M. rileyi* strain UFMS 07, em suspensões da ordem de 1.0×10^9 conídios mL⁻¹; para todos os tratamentos foram adicionados Tween 80[®]. As avaliações foram realizadas diariamente para verificação da mortalidade e efeitos subletais. Para os adultos emergentes (Geração Filial – GF) foi avaliado o ciclo biológico. Os dados referentes à mortalidade larval para a Geração Parental (GP) e GF e pupal para GF foram submetidos à análise de variância e, as médias de tratamentos, comparadas pelo teste de Scott-Knott a 5% de probabilidade. As cepas testadas não proporcionaram patogenicidade na fase larval de *H. armigera* para GP e GF, porém houve redução quanto à oviposição em todos os tratamentos em relação à testemunha. Ocorreu efeito sobre a fase reprodutiva das lagartas da GF expostas a *M. rileyi*.

Palavras-chave: Lepidóptero-praga, Entomopatógenos, Manejo integrado de Pragas.

1. Introduction

The rapid establishment and population growth of *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) and consequent damage to production systems were caused by a cumulative process of inadequate management practices, characterized by the successive planting of host plant species (corn, soybeans, and cotton) in extensive and contiguous areas, associated with inappropriate handling of pesticides (EMBRAPA, 2013).

According to Dias et al. (2019), the primary method of management of this species has been with pesticides, most of the time, recurrently and ineffectively. For Wyckhuys et al. (2013), the lack of rationalization in the use of pesticides, besides causing the population reduction of natural enemies and biological imbalances in agricultural systems, causes contamination and public health problems, derived from the toxic effects on humans. The author also emphasizes that the use of a mixture of non-selective pesticides has become predominant, with the same site of action on target and non-target organisms (natural enemies). The control of this noctuid has become, in many cases, inefficient, due to the selection of resistant populations, with reports of resistance to 49 active ingredients in several countries (IRAC, 2016). Given the above, *H. armigera* is seen as the species with the highest number of resistance cases (640) registered worldwide (Wyckhuys et al., 2013).

Integrated Pest Management (MIP) proposes the use of a set of management tactics, such as the application of chemical and biological insecticides to reduce the population of the pest and the selection pressure due to the indiscriminate use of synthetic products. This combination of strategies and use of technologies allows a more efficient action, more beneficial for the environment and more economical for the producer (EMBRAPA, 2013).

Biological control is an alternative to chemical products, with the set use of parasitoids, predators, and entomopathogenic microorganisms to pest insects (Tiago et al., 2014). According to Lopes et al. (2018), fungi have high genetic variability, infect different stages of the host's development, acting by contact, adhering to the insect's integument, and propagules have a high capacity for dissemination.

The entomopathogenic fungus *Metarhizium rileyi* (Ascomycota: Clavicipitaceae) (Kepler et al., 2014) is known as a biological control agent for pest Lepidoptera, with about 30 species of lepidopterans registered as susceptible to this fungus, with epizootics occurring in caterpillars that attack several crops and weeds (Ignoffo et al., 1976). According to Dias et al. (2019), the caterpillars of the Noctuidae family are among the most susceptible to this pathogen; and under favorable

environmental conditions, this fungus can drastically reduce populations of these insects.

Some reports, such as those by Manjula and Murthy (2005), demonstrate high efficiency in the laboratory regarding the mortality of *H. armigera* using *M. rileyi*, in a concentration of 10^7 conidia mL⁻¹. Costa et al. (2015) report mortality of 33.1% of *H. armigera* larvae in Bahia, occurring naturally in the field. According to Nunes et al. (2010), the virulence of *M. rileyi* strains on *H. armigera* caterpillars may vary depending on the edaphoclimatic conditions of each region and the susceptibility of the populations of this pest to strains.

Based on the above, this study aimed to evaluate the performance of different strains of the entomopathogenic fungus *M. rileyi* in the control of *H. armigera* caterpillars under laboratory conditions, and, later, to verify the effect of this entomopathogen on the biological cycle of the pest.

2. Material and Methods

Four strains of the entomopathogenic fungus *Metarhizium rileyi* (Table 1), belonging to the entomopathogen bank of the Federal University of Mato Grosso do Sul, Campus Chapadão do Sul, were used. The fungi were multiplied in 9 cm diameter Petri dishes containing Sabouraud culture medium, according to Dias et al. (2019). This study consisted of two bioassays. The first bioassay used second-generation caterpillars between the 2nd and 3rd instars (ranging from 0.7 to 1.2 cm length), each exposed to 2 mL of fungal suspension containing the strains UFMS 02, UFMS 03, UFMS 06, and UFMS 07. In the control treatment was applied distilled water. Treatments were applied with the aid of the adapted Potter spray tower, with a pressure of 1.0 MPa. The suspensions were standardized in a Neubauer® chamber at a concentration of 1×10^9 conidia mL⁻¹, with the addition of Tween 80® in all treatments. After application, the caterpillars were placed inside Petri dishes and stored in B.O.D. (Biological oxygen demand) at a temperature of 30 ± 1 °C, $70 \pm 10\%$ RH (Relative Humidity) and a photophase of 12h, fed with an artificial diet based on white beans adapted from Greene et al. (1976).

The evaluations were carried out daily, quantifying mortality in the larval phase, sublethal effects in the pupal and adult stage, observing the oviposition resulting from the treatments. The percentage of reduction in oviposition treatments was also evaluated concerning the control. The dishes were reviewed daily, considering the duration of the larval period until pupation. Pupae up to 48 h were removed with the aid of entomological forceps, weighed on an analytical scale, and separated by sex (Panizzi and Parra, 2009).

Table 1: Origin of the strains of the entomopathogenic fungus *Metarhizium rileyi*.

Strain	Host	Crop	Collect location
UFMS 02	<i>Anticarsia gemmatalis</i>	Soybean	Chapadão do Sul - MS
UFMS 03	<i>Alabama argilacea</i>	Cotton	Chapadão do Sul - MS
UFMS 06	<i>Spodoptera frugiperda</i>	Corn	Chapadão do Sul - MS
UFMS 07	<i>Helicoverpa armigera</i>	Soybean	Chapadão do Sul - MS

For the parental generation (PG), emergence, and adult deformity pattern were evaluated. Adults from PG, classified without deformities, were grouped according to the treatments. The second bioassay, the filial generation (FG), originated from the treatments, was carried out evaluating the biological cycle, quantifying larval, pupal, and adult mortality and the number of oviposition, comparing them with the control (progeny). The bioassay was repeated three times.

The experimental design was completely randomized, consisting of five treatments and five replications, each one composed of 50 caterpillars. Mortality data were submitted to the analysis of variance, and the Scott-Knott test grouped the treatment averages at a 5% probability.

3. Results and Discussion

There was no significant difference between treatments regarding the mortality of *H. armigera* caterpillars for the parental generation (GP) and filial generation (FG) (Table 2).

Table 2. Percentage of average mortality (\pm SD) of *Helicoverpa armigera* of parental generation (PG) caterpillars, treated with different strains of *Metarhizium rileyi* and filial generation (FG).

Treatments	% Mortality (PG) ¹	% Mortality (FG) ¹
Control	1.0 \pm 0.007 a	2.6 \pm 0.009 a
Strain UFMS 2	0.8 \pm 0.006 a	4.0 \pm 0.010 a
Strain UFMS 3	1.0 \pm 0.005 a	2.4 \pm 0.009 a
Strain UFMS 6	1.0 \pm 0.005 a	4.2 \pm 0.010 a
Strain UFMS 7	0.8 \pm 0.005 a	1.2 \pm 0.007 a
CV %	32.20	28.65

Averages followed by the same letter in the column belong to the same group by the Scott-Knott test at 5% probability.

¹ For analysis of transformed data $(x+0,5)^{0,5}$

Table 4. Average oviposition (\pm SD) and percentage of reduction in the oviposition of *Helicoverpa armigera* parental generation (PG) treated with the fungus *Metarhizium rileyi* and filial generation (FG).

Treatments	Number of eggs (PG)	Oviposition reduction (%) (PG)	Number of eggs (FG)	Oviposition reduction (%) (FG)
Control	384.07 \pm 29.15 a	-	1764.00 \pm 19,82 a	-
Strain UFMS 02	15.61 \pm 9.50 b	60.53	0.00 \pm 0,00 c	100.00
Strain UFMS 03	120.54 \pm 11.80 b	68.62	0.00 \pm 0,00 c	100.00
Strain UFMS 06	149.96 \pm 8.08 b	60.96	1032.00 \pm 10,80 b	41.50
Strain UFMS 07	105.89 \pm 7.05 b	72.43	65.00 \pm 2,50 c	96.32
CV%	25.86	—	30.79	—

Averages followed by the same letter in the column belong to the same group by the Scott-Knott test at 5% probability.

There was no significant difference between treatments regarding the average weight of *H. armigera* pupae (Table 3).

Table 3. Average pupal weight (\pm SD) of *Helicoverpa armigera* of parental generation (PG) treated with different strains of *Metarhizium rileyi*.

Treatments	Pupae weight (PG) (g)
Control	0.394 \pm 0.0025 a
Strain UFMS 02	0.371 \pm 0.0015 a
Strain UFMS 03	0.366 \pm 0.0009 a
Strain UFMS 06	0.361 \pm 0.0008 a
Strain UFMS 07	0.376 \pm 0.0016 a
CV%	4.63

Averages followed by the same letter in the column belong to the same group by the Scott-Knott test at 5% probability.

For the reproductive phase, the average number of oviposition was evaluated, where the control obtained a higher amount of eggs, differing from the other treatments for both the parental and the filial generation (Table 4). The reduction in the number of eggs varied from 60.53 to 72.43% in the parental generation and from 41.50 to 100% in the filial generation concerning the control (Table 4).

The results were positive regarding the interference in the reproductive system of *H. armigera*, which demonstrates the ability of the fungus *M. rileyi* to promote the population reduction of the pest, even if it does not cause mortality.

The reduction of oviposition is a secondary or sublethal effect caused by the activity of entomopathogenic fungi, in which the observed effects are higher sex ratio, abnormal imago, infertile eggs, and reduced oviposition (Lopes et al., 2018). All these effects were observed in the present study, and most of the treatment eggs were infertile.

The effects of the reduction in the number of eggs in the filial generation suggest the vertical or transovarial transmission capacity of *M. rileyi*, which corroborates the increase in the ability to spread the disease. It is believed that the contagion of eggs in the present study was due to contact with the female's infected ovipositor at the time of laying, since larvae, pupae and young adults of filial generation emerge infected (Vega, 2018). Another hypothesis is that vertical transmission may have occurred, which consists of contamination of the egg still inside the reproductive system, due to the presence of the pathogen in the accessory glands and ovary (Shapiro-Ilan et al., 2012).

According to Vega (2018), also vegetative growth being essential for the colonization of the host, the production of conidia is responsible for the vertical transmission of the fungus to the progeny. However, for *H. armigera*, there are no reports on infection transmission between stages, sublethal or secondary effects, and regarding the generation after the one that occurred the infection. For other insects such as dipterans, Albernaz et al. (2009) showed, for the first time, that females of *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) treated with conidia of *M. anisopliae* can contaminate eggs during oviposition and thus transmit the mycosis to the eggs, reducing the number of new larvae. The transmission of mycosis from infected females to the eggs laid by them depends on the strain virulence and the quantitative contamination of the females with conidia. Bukhari et al. (2011), studying varying doses of *Metarhizium anisopliae* conidia in *Anopheles gambiae* (Meigan, 1818) (Diptera: Culicidae), observed that treated female contaminated healthy males during the mating flight and the female transmitted the fungus to the progeny.

Entomopathogenic fungi propagate in natural conditions by spreading conidia formed mainly on insects killed by infection, contaminating the environment, and new insects. Transmission occurs indirectly or also through direct contact between healthy insects and contaminated and/or infected live insects or dead insects with fungus (Zhang et al., 2016). Thus, there are few studies on the transmission of mycoses among live insects or at different stages. The increase in research on the dynamics of mycosis in a target population related to vertical transmission will contribute to more effective control of *H. armigera*.

Results obtained in the present research regarding low mortality suggest the action of the insect defense system. This system is composed of structural barriers, the rigid exoskeleton and active responses against foreign elements that reach their hemocoel (Dubovskiy et al., 2016), as well as the digestive system and respiratory barriers, which constitute the first line of defense for insects (Dunn, 1986; Bullet et al., 1999).

Another hypothesis to be considered is humoral activities, which may have promoted the insect's survival, selecting resistant individuals. This resistance may occur due to the high innate immunity of caterpillars, with the action of plasmatocytes, capable of forming a mass of capsules around foreign bodies, and of granular hemocytes, which phagocytize foreign pathogens, making it difficult for pathogens to act on caterpillars (Wang et al., 2010). According to Lopes et al. (2018), conidia and mycelia of the fungus *M. rileyi* are recognized by the insect's immune system, thus hindering their colonization.

The absence of observed mortality may also be related to the low ability of the strains of the fungus to use the nutrients available on the surface of the insect's cuticle for their development or the inability to recognize the susceptible host or penetrable infection site (Vega, 2018). *H. armigera* caterpillars have a coriaceous tegument (Czepak et al., 2013), a morphological characteristic that possibly directly interfered with conidial penetration.

Devi et al. (2003) reported, through bioassays, a lower susceptibility of *H. armigera* to the fungus *M. rileyi* than *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). However, this variation in susceptibility may be linked to the virulence of the strains tested (Ignoffo and Garcia, 1991; Vega, 2018).

According to Costa et al. (2015), the entomopathogenic fungus *M. rileyi* caused mortality in *H. armigera* first instar caterpillars; however, low control efficiency. This author concluded that one of the main factors that determine mortality is the developmental stage of caterpillars, which was significantly higher for neonates than other instars.

However, it must be considered that there was an energy cost for the insect to produce enzymes that could interrupt the colonization by the fungus, which can have secondary effects during its development, both in the young and adult stages (Lopes et al., 2018). As can be seen, the average pupal weight of the parental generation was higher in control, but it did not differ significantly from the other treatments (Table 3).

The lower weight found in pupae is probably part of an immune response of caterpillars infected by fungi. Which can consume nutrients, suppress defense or affect the vital function of insects (Clarkson and Charnley, 1996), with reduced feeding in some fungi-infected insects (Hornbostel et al., 2004; Vega, 2018).

These results indicate a possibility of using the fungus *M. rileyi* as a method of prophylactic control, that is, associated with other tools of Integrated Pest Management. In this way, it would be possible to keep the population of *H. armigera* below the control level without using chemicals, corroborating the results described by Ignoffo and Garcia (1991).

The strains of the fungus *M. rileyi* used in this work were not efficient regarding the mortality of *H. armigera*. However, it is promising, due to the reduction of oviposition and non-viability of eggs. Thus, it is of great importance to carry out new studies, using new strains and different concentrations to assess pathogenicity and possible secondary effects, intensifying the knowledge of the mechanisms involved in the vertical transmission of conidia from this fungus to the offspring.

Figure 1 represents a summary scheme of the methodology and results obtained in the present work. In summary, the strains tested did not provide pathogenicity in the *H. armigera* larval phase for parental and filial (F1) generation. However, there was a reduction in oviposition in all treatments regarding the control. Also, there was an effect on the reproductive phase of caterpillars of the filial generation (F1) exposed to *M. rileyi*.

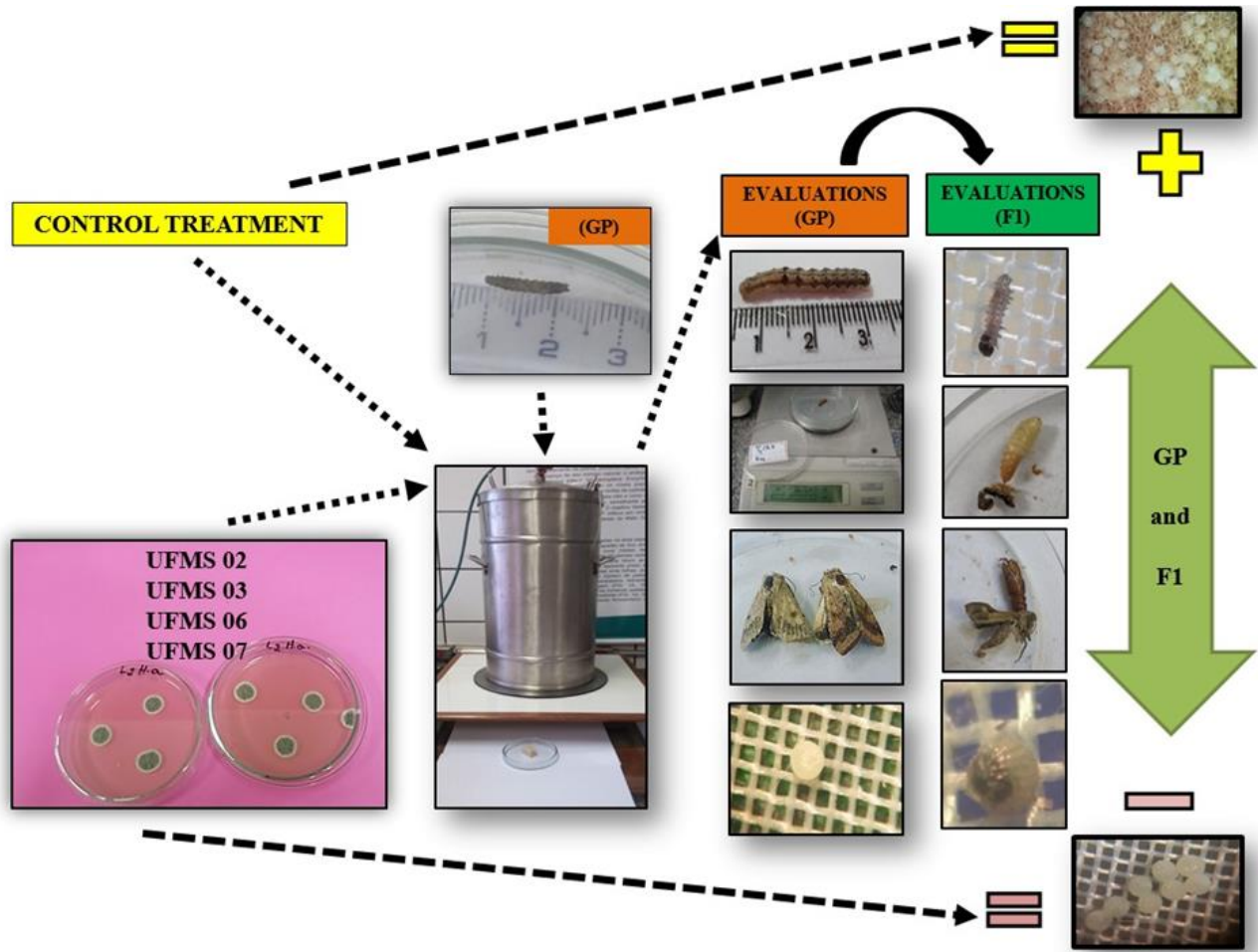


Figure 1. Summary scheme of the procedures adopted and results obtained in the present work.

4. Conclusions

The UFMS 02, 03, 06, and 07 strains did not provide pathogenicity in the larval phase of *H. armigera*. There was a reduction in oviposition in all treatments compared to the control for Parental and Filial Generations. The effect occurred on the reproductive phase of caterpillars of the Filial Generation exposed to *M. rileyi*, with a 40.5 to 100% reduction in egg production. The UFMS 02 and 03 strains stood out, both with a 100% reduction in the number of eggs in the Filial Generation.

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