

Scientific Note

A Technique to Estimate the Conidial Viability of *Metarhizium flavoviride* Gams & Rozsypal (Hyphomycetes) Formulated in Vegetable Oil

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Técnica de Determinação da Viabilidade de *Metarhizium flavoviride* Gams & Rozsypal (Hyphomycetes) Formulado em Óleo Vegetal

RESUMO - Foi desenvolvida uma técnica simples para determinação da viabilidade de conídios do fungo patogênico a gafanhotos *Metarhizium flavoviride* Gams & Rozsypal (Hyphomycetes), formulado em óleo vegetal. A técnica consiste em espalhar a suspensão fúngica oleosa sobre meio de cultura e cobrir com uma lamínula. Assim, uma suspensão de 10 µl fúngica oleosa ou aquosa (10⁶ conídios/ml) foi espalhada sobre blocos (1.0 x 1.0 x 0.2 cm) do meio de cultura BDA (batata, dextrose e agar). Em seguida, a suspensão foi firmemente coberta com uma lamínula estéril. O conjunto foi então transferido para placas de Petri e mantido a 28°C, com observações realizadas 6, 9, 12, 15 e 18 h após inoculação. Foram registrados altos níveis de germinação (> 90%) de *M. flavoviride* formulado em óleo com ou sem querosene, a partir de 15 h. Após 18 h esses níveis e o crescimento vegetativo foram comparáveis àqueles observados em suspensão aquosa.

PALAVRAS-CHAVE: Insecta, gafanhoto, fungos entomopatogênicos, formulação oleosa, viabilidade.

The fungus *Metarhizium flavoviride* Gams & Rozsypal is under investigation in Brazil for the biological control of the grasshoppers *Rhammatocerus schistocercoides* (Rhen) and *Schistocerca pallens* (Thunberg) (Magalhães *et al.* 1996, Moreira *et al.* 1996, Vicentini & Magalhães 1996), and the walking stick *Stiphra robusta* Mello-Leitão (S. Vicentini, pers. commun.). The most promising formulation for *M. flavoviride* against grasshoppers is based on oils amended for ultra-low volume application (Lomer & Prior 1992, Bateman *et al.* 1993, Magalhães *et al.*

1996).

The viability estimate of *M. flavoviride* conidia formulated in vegetable oils using aqueous agar media is hindered by the coverage of conidia with oil. Due to polarity differences between oil and water, conidia do not have a direct contact with the aqueous medium when seeded. Attempts to remove the oil from the conidial surface using different concentrations (0-10%) of detergents (Tween 80, Triton) have led to inconsistent results. Stathers *et al.* (1993) developed a technique to remove oil from conidia involving vacuum

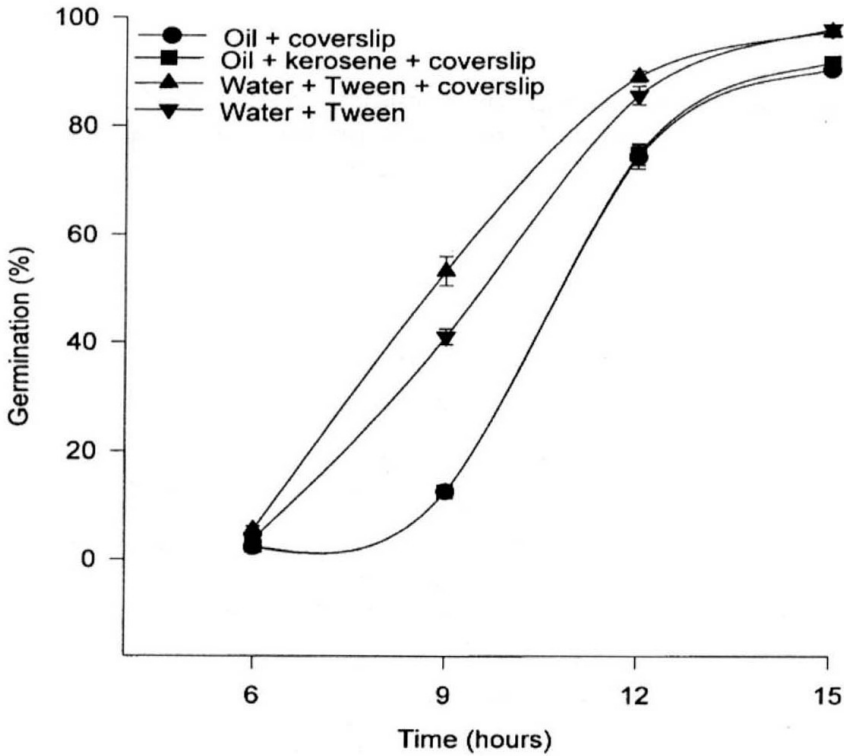


Figure 1. Germination of *Metarhizium flavoviride* (CG 423) at 28°C, during 15 h, using different techniques for sample preparation.

filtration through a cellulose nitrate membrane, followed by incubation in gelatin medium. Another method to assess fungal viability is based on the enumeration of colony-forming-units (CFU) (Inglis *et al.* 1993). However, it requires a selective medium, serial dilutions and a fine adjustment for each system added to the problem of high variation of CFU between replicates. In this note, we present a simpler technique to estimate the viability of *M. flavoviride*, in which conidia formulated in oil are spread on nutrient medium and covered with a coverslip.

Conidia of *M. flavoviride* (CG 423) were produced on parboiled rice as described by Magalhães & Frazão (1996), with some modifications as follows. After eight days incubation at 30°C, conidia were harvested using a

500 µm sieve and formulated in soybean oil, with and without kerosene (5%), and stored at 4°C. Kerosene was used to suspend the conidia more uniformly in the formulation. To estimate viability, blocks (1.0 x 1.0 x 0.2 cm) of potato dextrose agar medium (potato 170g, dextrose 20g, agar 20g, water 1 liter) were placed on sterile glass slides. Ten µl of the oil suspension (10⁶ conidia/ml) were transferred to the agar blocks, firmly covered with a sterile glass coverslip (22 x 22 mm), and the preparation incubated at 28°C. Germination and growth of *M. flavoviride* formulated in soybean oil, soybean oil + kerosene (5%), and water + Tween 80 (0.1%) were recorded at 6, 9, 12, 15 and 18 h. For comparison, conidia formulated in soybean oil + kerosene and in water + Tween 80 were also spread on agar

medium (conventional method, without coverslip). A conidium was considered viable when the germ tube length was at least equal to its width. The number of viable and non-viable conidia ($> 400/\text{slide}$ or Petri plate), and the germ tube length were assessed under an optical microscope (400x magnification). Germ tube length data were compared using the Student-Newman-Keuls test ($P = 0.05$).

M. flavoviride germinated at similar percentages when formulated in oil, with or without kerosene, plated on nutrient medium and covered with a coverslip (Fig. 1). At 18 h,

air bubbles. The growth rate of *M. flavoviride* formulated in oil or in oil + kerosene, under a coverslip, was comparable to the growth rate in aqueous suspension with no coverslip (conventional method). However, with a coverslip on top of the aqueous formulation the growth rate was significantly higher ($P < 0.001$) (Fig. 2). After 12 h, the germ tube growth in aqueous suspension, under the coverslip, was so profuse that measurements were not possible.

The use of the technique described herein prevents the formation of air bubbles and allows a more precise determination of the co-

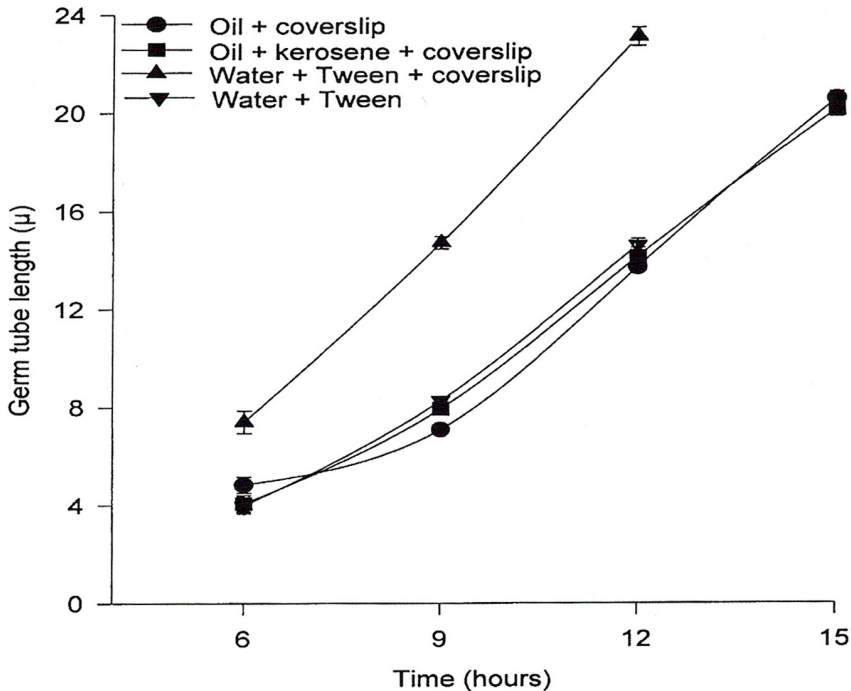


Figure 2. Germ tube growth of *Metarhizium flavoviride* (CG 423) at 28°C, during 15 h, using different techniques for sample preparation.

germination was very high ($> 97\%$) and comparable to that estimated by the conventional aqueous suspension method. However, when the oil formulation was plated and no coverslip used, germination was poor and very difficult to quantify, since non-germinated conidia were frequently confounded with small

nidial viability of *M. flavoviride*. In addition, this method is suitable for viability estimation of other species of fungi, such as *M. anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin (B. Magalhães & H. Frazão, unpublished). Its simplicity may make this technique a very useful tool in pro-

grams involving mycopesticide applications in the field.

Acknowledgments

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