Effect of native strains of *Paecilomyces* sp. (Bainier) and *Lecanicillium* sp. (Zimm) on the control of *Carmenta foraseminis* Eichlin (Lepidoptera: Sesiidae) on cocoa (*Theobroma cacao* L.) crops

Efecto de las cepas nativas *Paecilomyces* sp. (Bainier) y *Lecanicillium* sp. (Zimm) en el control de *Carmenta foraseminis* Eichlin (Lepidoptera: Sesiidae) en cultivos de cacao (*Theobroma cacao* L.)

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Abstract

The fruit borer, *Carmenta foraseminis* Eichlin, is an insect that has increased up its attack in cocoa (*Theobroma cacao* L.) in recent years in northern Santander, Colombia. The objective was to evaluate the pathogenicity of two native strains *Paecilomyces* sp. Giav-3 and *Lecanicillium* sp. Giav-4, on larvae of *C. foraseminis.* Isolations were made from soil samples collected in the municipality of Tibú, Northern Santander, Colombia. After obtaining pure cultures, macroscopic and microscopic characterization for gender identification of the strains using taxonomic keys was done. The evaluation was conducted by immersion of larvae in suspensions of the isolates at concentrations of 0, 10^6, 10^7 and 10^8 conidia/ml. For both strains there was a linear trend in mortality, which was directly proportional to the concentrations of the inoculum. The LC_{50} and LC_{90} for Giav-3 were $10^{6.95}$ and $10^{8.70}$ conidia/ ml, and for Giav-4 was $10^{6.6}$ and $10^{8.04}$ conidia/ml, respectively. This indicates that Giav-4 required the lowest concentration of inoculum to remove 50 to 90% of the treated population, indicating it is more effective against larvae.

Key words: Biological control, *Lecanicillium* sp., *Paecilomyces* sp., *Theobroma cacao.*

Resumen

El pasador del fruto, *Carmenta foraseminis* Eichlin, es un insecto que ha acentuado su ataque en cultivos de cacao (*Theobroma cacao* L.) en los últimos años en Norte de Santander (Colombia). El objetivo de este estudio fue evaluar la patogenicidad de las cepas nativas Giav-3 *Paecilomyces* sp. y Giav-4 *Lecanicillium* sp. sobre larvas de *C. foraseminis*. Para el efecto, se realizaron aislamientos a partir de muestras de suelo recolectadas en el municipio de Tibú, Norte de Santander. Después de obtenidos los cultivos puros se procedió a la caracterización macroscópica y microscópica para la identificación del género de los aislados mediante claves taxonómicas. La infección fue realizada mediante inmersión de larvas en las suspensiones de los aislados en concentraciones de 0, 10^6, 10^7 y 10^8 conidios/ml. En ambos aislados se observó una tendencia lineal respecto a la mortalidad, la cual fue directamente proporcional a las concentraciones del inóculo. La CL_{50} y CL_{90} para Giav-3 fue de $10^{6.95}$ y $10^{8.70}$ conidios/ml y para Giav-4 de $10^{6.6}$ y $10^{8.04}$ conidios/ml, respectivamente. Lo cual indica

que esta última requirió la menor concentración de inóculo para eliminar el 50% y 90% de la población tratada, lo que supone una mayor efectividad contra las larvas.

Palabras clave: Control ecológico, *Lecanicillium* sp., *Paecilomyces* sp., *Theobroma cacao.*

Introduction

In Colombia the crop of cocoa (*Theobroma cacao* L.) is the basis of the economy and livelihood of more than 35,000 families. Norte de Santander registers an average annual grain production of 1756 t (Fedecacao, 2008). In this region in recent years it has been accentuated the attack by the fruit borer *Carmenta foraseminis* Eichlin (Lepidoptera: Sesiidae) (Eichlin, 1995). The female insect lays eggs on the surface of the fruit; after hatching, the larvae bore into the bark along the placenta, stunts the grains and feeds on the seeds, while depositing their droppings and facilitating internal rot (Delgado., 2004; Navarro *et al.,* 2004).

Currently, to combat this insect, a cultural management practice is used, that consists in arranging the fruits affected in plastic bags for 3 months to ensure the death of larvae and adults, and avoid reinfestation of the crop (Montes, 2010). Ethological control using traps with the pheromone (Z, Z) -3, 13-octadecadienol acetate, specific to the Sessidae family, has not proved effective in attracting adults of *C. foraseminis* (Mirelles, 2005). Although there are no studies on ecological control of this pest, Navarro & Cabin (2006) recommend releasing between 100,000 and 500,000 adults of wasps *Trichogramma pretiosum* (Riley) per hectare.

Under the concept of integrated pest management (IPM), an alternative is the use of entomopathogenic fungi which can be propagated in the insect population, comprising a group of great interest for pest control (Vergara, 2004). Approximately 80% of the diseases that attack insects have a fungus as a causative agent (Badii and Abreu, 2006). These fungi present unique invasion mechanisms, which allow them to pass through the insect cuticle, acting as important agents of control in a similar way to contact insecticides (Tellez *et al.,* 2009). Approximately 100 genera and 700 species of entomopathogenic fungi are known, the most important are: *Metarhizium, Beauveria, Aschersonia, Entomophthora, Zoopthera, Erynia, Eryniopsis, Akanthomyces, Fusarium, Hirsutella, Hymenostilbe, Paecilomyces* and *Lecanicillium* (Monzón, 2001). Due to the importance of ecological control using entomopathogenic fungi, the aim of this study was to evaluate the pathogenicity of native isolates with potential for ecological control of *C. foraseminis,* or cocoa fruit borer.

Materials and Methods

Origin of the isolates

The isolates *Paecilomyces* sp. and *Lecanicillium* sp. - respectively named *Grupo de Investigación Ambiente y Vida* -Giav-3 and Giav-4 used in this study were obtained from soil samples collected in the municipality of Tibú (8° 39' N and 72° 59' W, 75 masl) Norte de Santander. 10 composite samples/ha were collected at a depth between 10 and 15 cm deep. From each of these samples subsamples of 500 g were taken, which were preserved in plastic bags with airtight seal, properly identified by date, place and collection farm, before being deposited at a temperature of 4 °C for transport and conservation in the laboratory.

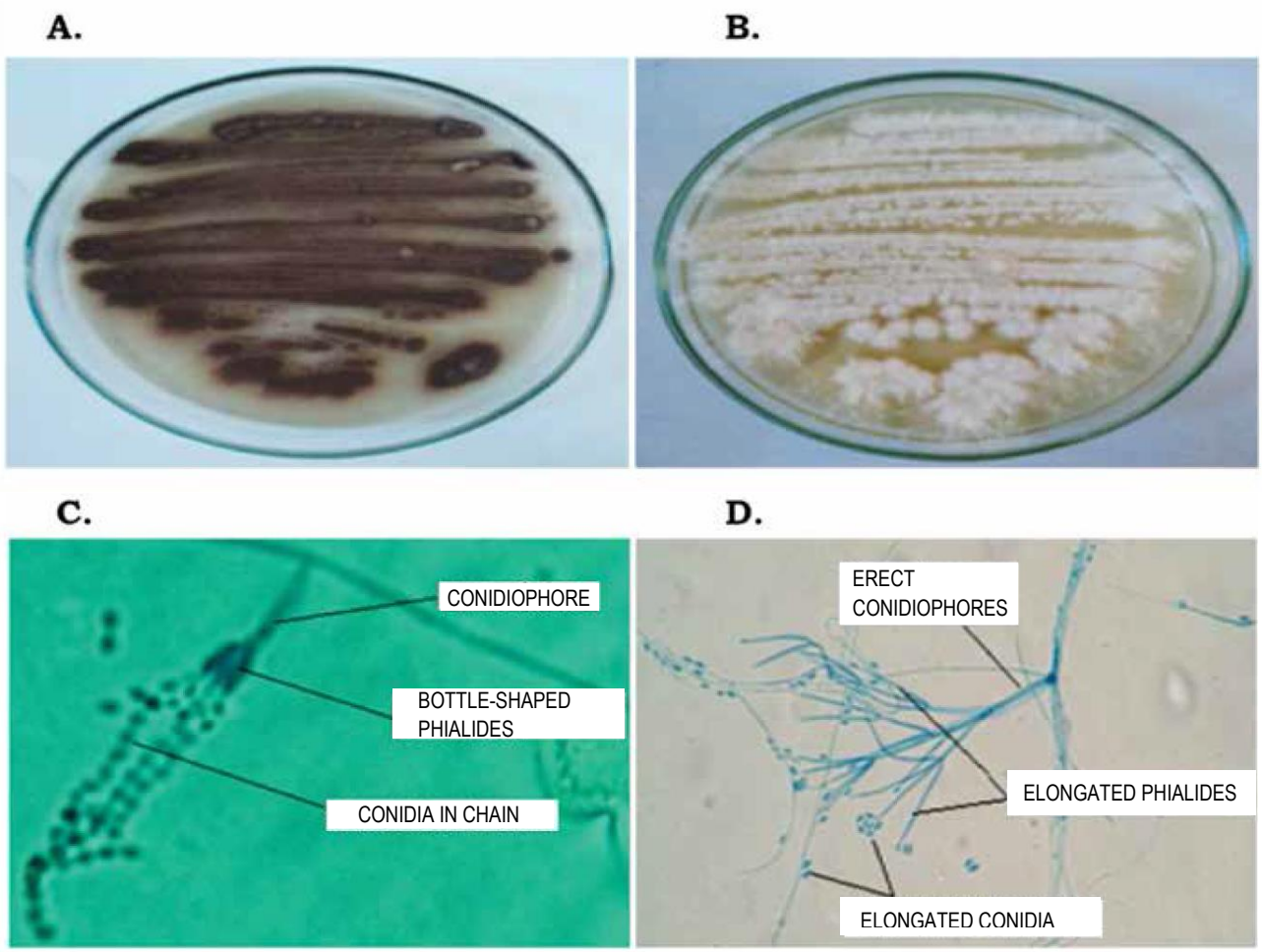


Photo 1. Morphological characteristics of the isolates obtained from soil. **A** = Colonies of *Paecilomyces* sp. **B** = Colonies of *Lecanicillium* sp. **C** = Micrograph of structures of *Paecilomyces* sp. **D** = Micrograph of structures of *Lecanicillium* sp.

Identification of the strains

The isolation and identification of fungi strains were performed at the Laboratory of Applied Microbiology, Faculty of Agricultural and Environmental Sciences of the University Francisco de Paula Santander. For this the protocol proposed by Murcia and Salamanca (2006) was used with incubation at 28 °C, followed by propagation in monosporic cultures to ensure authenticity and purity of the sample.

Once pure cultures were obtained the morphological characterization and identification of isolates was done, according to macroscopic and microscopic characteristics of growth using the taxonomic keys of Barnett and Hunter (1972), and Samson *et al.* (1981). For the macroscopic characterization, passes from the pure culture of the fungus on potato dextrose agar (PDA) plus chloramphenicol (1g/l) were performed and incubated at 28 °C. Eight days later the characteristics of the culture, color on both sides of the colony, border and texture were recorded. A similar technique to the above was used for microscopic characterization, however after 5 days, single staining was performed with lactophenol blue and with the aid of a binocular Olympus microscope (1000 X magnification) the morphology, arrangement of the conidiophores and

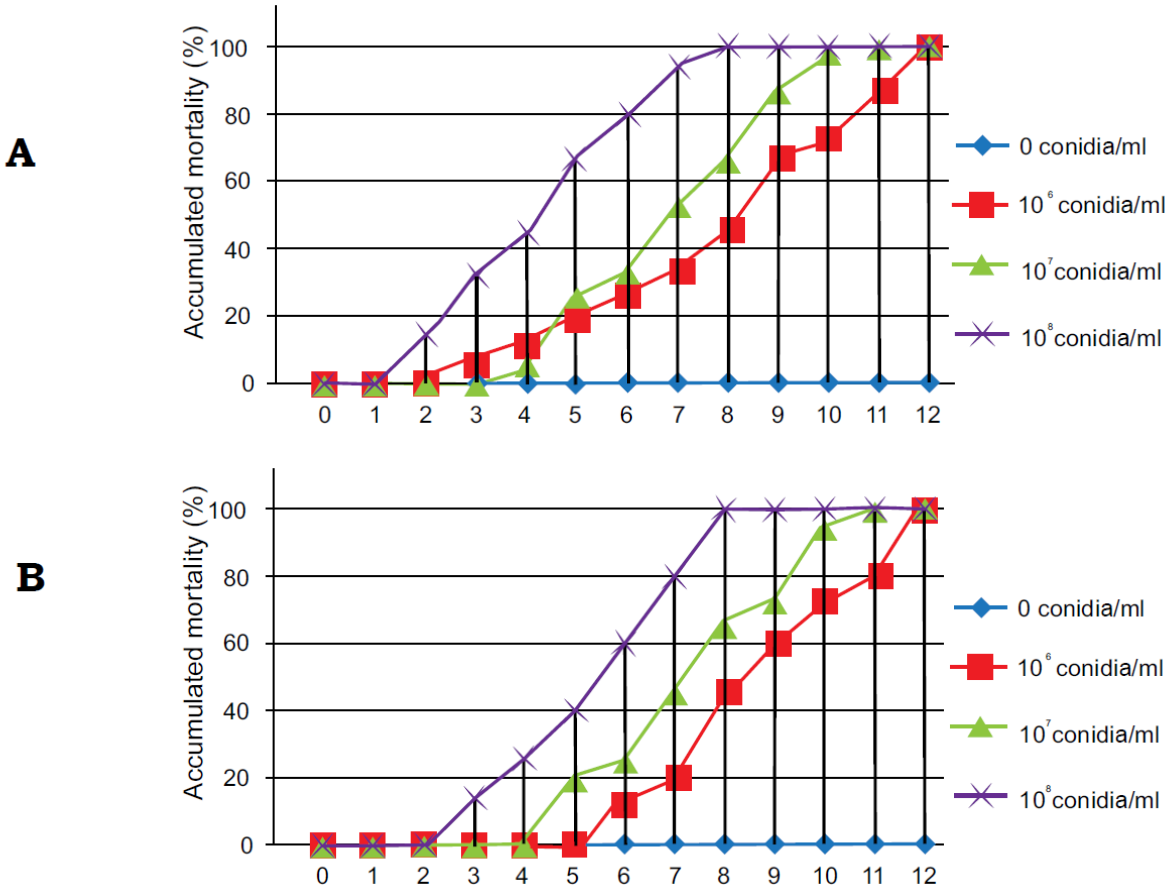


Figure 1. Mortality curves of *C. foraseminis*, inoculated with different concentrations of isolates (**A**) *Lecanicillium* sp. and (**B**) Giav-3 *Paecilomyces* sp.

Table 1. Tukey´s test for the average larvae mortality of *C. foraseminis*, 7 days after inoculation with different concentrations of isolate Giav-3 *Paecilomyces* sp.

Concentration	Cases (no.)	Mean[a]	S.E.	Homogenous groups s
0	3	0.00	7.45	a
10^6	3	26.66	7.45	ab
10^7	3	46.66	7.45	bc
10^8	3	80.00	7.45	c

[a] Means with same letters do not statistically differ (Tukey, 0.05).
S.E. = Standard error

conidia was observed (Rodriguez and Del Pozo, 2003).

Pathogenicity trials

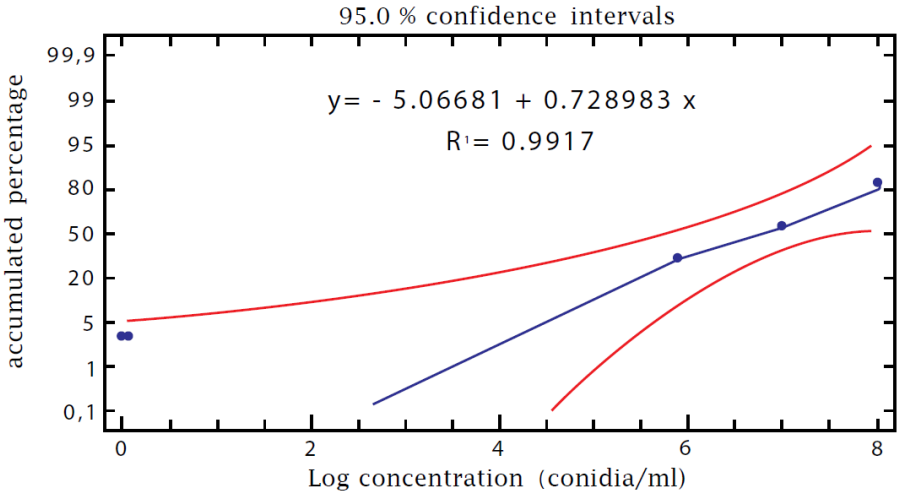
The inoculum was obtained from isolates grown on PDA and incubated at 28 °C until sporulation. Fungal suspensions were prepared with conidia collected from the culture surface, by adding sterile distilled water. Conidial concentrations were determined in a Neubauer chamber.

For the pathogenicity test late-stage larvae of *C. foraseminis* were used, obtained from infested cocoa fruits that were superficially disinfected according to the method used by Garcia *et al.* (2008), and inoculated by immersion in suspensions of Giav-3 and Giav-4 isolates at concentrations of 10^6, 10^7 and 10^8

Table 2. Tukey's test for the average larvae mortality of *C. foraseminis*, 7 days after inoculated with different concentrations of isolate Giav-4 *Lecanicillium* sp

Concentration	Cases (no.)	Mean[a]	S.E.	Homogenous groups
0	3	0.00	5.77	a
10^6	3	33.33	5.77	b
10^7	3	53.33	5.77	b
10^8	3	93.33	5.77	c

*Means with same letters donot statistically differ (Tukey, 0.05).
S.E. = Standard error.



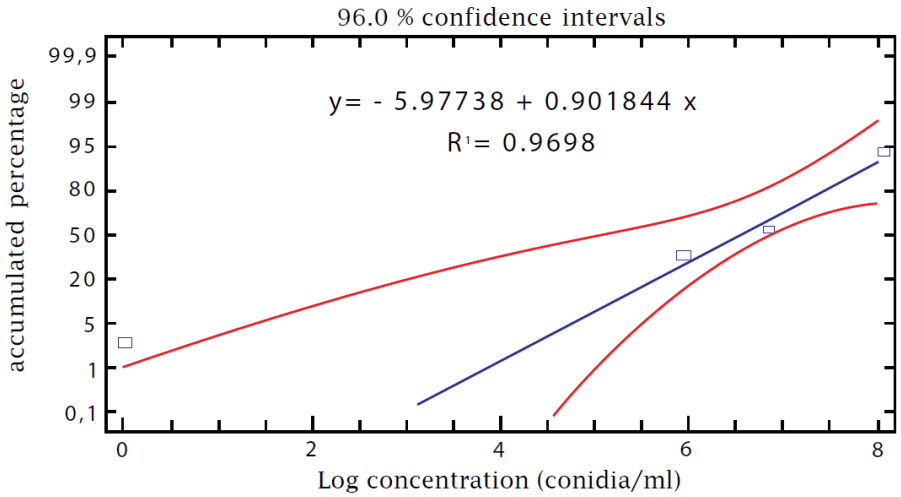


Table 2. LC50 y LC90 calculated by linearization of larvae mortality curves of *C. foraseminis*, at day 7 post-inoculation with different concentrations of isolate. (**A**) Giav-3 *Paecilomyces* sp., and (**B**) Giav-4 *Lecanicillium* sp.

conidia/ml. As a control, larvae treated in sterile distilled water were used. The treated larvae were transferred to plastic containers containing cocoa shells with thermal pretreatment in water at 100 °C for 30 min and fresh seeds for feeding. The observations for larval mortality were made daily for 12 days. To confirm the causative agent, dead insects were incubated in petri dishes with paper towels moistened with sterile distilled water.

A completely randomized design was used, with three repetitions per treatment, consisting of three concentrations of inoculum for each isolate, plus the control with sterile distilled water. The experimental unit consisted of five larvae, for a total of 15 experimental units per treatment. The number of dead larvae was recorded by daily observations expressed as percentages of mortality over time. The results were subjected to analysis of variance and comparison of means by the Tukey test, using the experimental design package Statgraphics Centurion XV professional registered trademark Stat Point, Inc. (2006). The sigmoid mortality curve was linearized by Probit transformation and the lethal concentration (LC) for the 50% (LC_{50}) and 90% (LC_{90}) of the population was calculated through regression equations (Rodriguez *et al.*, 2006).

Results and Discussion

Morphological characterization of the isolates

The isolate Giav-3 *Paecilomyces* sp. on PDA medium plus chloramphenicol (1g/l), incubated at 28 °C for 8 days presented pink-grayish colonies, powdery appearance, regular borders, (Photo 1A) and colorless or whitish reverse; while isolation Giav-4 *Lecanicillium* sp. presented whitish to cream color, irregular edges, cottony appearance (Photo 1B) and reverse with cream or pale yellow pigmentation. These characteristics are consistent with those described by Rodriguez and Del Pozo (2003) for the entomopathogenic fungi *P. fumosoroseus* (Wize) Brown and Smith and *Verticillium lecanii* (Zimm.) Viegas [= *Lecanicillium lecanii* (Zimm)] respectively. In the microscopic observations after 5 days of growth at 28 °C, it was observed that isolation Giav-3 had erect conidiophores, branched at the apex, widened phialides at the base tapering to the apex, conidia in chains (Photo 1C). Giav-4, for its part, presented erect, elongated and branched conidiophores, slender and elongated phialides, elongated conidia solitary or in pairs (Picture 1D). The above characteristics match those described by Barnett and Hunter (1972) and Samson *et al.* (1981) for the genera Lecanicillium and Paecilomyces.

Pathogenicity

The concentrations of the isolates were compared on day 7 after inoculation, which coincided with a mortality of larvae> 90% for the first strain. According to this, the isolate Giav-4 in day 7 post-inoculation showed mortalities of larvae of 93.33% with a concentration of 10^8 conidia ml and 53.33% when the concentration was 10^7 conidia/ml (Figure 1A). Meanwhile, the isolate Giav-3 for the same day post inoculation showed 80% mortality of larvae at a concentration of 10^8 conidia/ml and 46.66% at a concentration of 10^7 conidia/ml (Figure 1B). Diseased individuals inoculated presented weakness and disorientation, changes in color, reduction in feeding, and dark spots on the seed coat, which correspond with germinated spores of the fungus (Badii and Abreu, 2006).

For the isolate Giav-3 no differences ($P > 0.05$) were found between the concentration of 10^6 conidia/ml vs. control, however the treatments of 10^7 and 10^8 conidia/ml showed the highest rates of mortality - 46.66 and 80.00%, respectively, which were different in relation to the control (Table 1). For isolate Giav-4 ($P < 0.05$) differences were found among the control and other treatments; the treatment 10^8 conidia/ml with a mortality average of

93.33% was higher than the treatments 10^6 and 10^7 conidia/ml (Table 2).

With both isolates a linear trend was observed in mortality rates of larvae, which was directly proportional to the concentrations of the inoculum. After probit transformation, the linear equation for Giav-3 was: $Y = -5.06681 + 0.728983x$ (R2 = 0.9917) (Figure 2A) and for Giav-4 was: $Y = -5.97738 + 0.901844x$ (R2 = 0.9698) (Figure 2B). With these equations, values (conidia / ml) for LC_{50} ($1 \times 10^{6.95}$) and LC_{90} ($1 \times 10^{8.70}$) for Giav-3 and LC_{50} ($1 \times 10^{6.6}$) and LC_{90} ($1 \times 10^{8.04}$) for Giav-4 were obtained. This indicates that the isolate Giav-4 required the lowest concentration of inoculum to eliminate 50% and 90% of the treated population and therefore presented greater effectiveness against the larvae.

Nuñez *et al.* (2008) found that the mortality effect of *L. lecanii* on the second nymph stage of *Aleurodicus cocois* (Curtis) was higher than that of *P. fumosoroseus* and that the mixture of both fungi, which confirms the high effectiveness of entomopathogenic *V. lecanii*. Other species of entomopathogenic fungi such as *M. anisopliae* require a lower concentration of inoculum to remove 50% and 90% of larvae *of Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in tomato, with the LC_{50} and LC_{90} of $1 \times 10^{4.4}$ and $1 \times 10^{7.6}$ conidia/ml, respectively (Rodriguez *et al.*, 2006). The results of this study show that in vitro conditions the isolates Giav-3 Paecilomyces sp. and Giav-4 *Lecanicillium* sp. are pathogenic for *C. foraseminis*, being more effective Giav-4 strain.

These findings are important for an integrated pest management, as both strains were virulent in vitro, and could be potentially included as a biological control strategy within an integrated management of *C. foraseminis*; however the field effectiveness of this strategy should be evaluated in subsequent work.

Conclusions

- The native isolates Giav-3 *Paecilomyces* sp. and Giav-4 *Lecanicillium* sp. were pathogenic for last stage *C. foraseminis* larvae, in which it was observed that the greater the concentration of fungus, the higher the mortality. The isolate Giav-4 *Lecanicillium* sp. requires the lowest concentration of inoculum to remove 50% or 90% of the treated population, indicating greater effectiveness against larvae.

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