

Bioactivity of fungi isolated from coconut growing areas against *Rhynchophorus palmarum*







Abstract – The objective of this work was to evaluate the chitinolytic activity and bioactivity of fungi isolated from the palm weevil (*Rhynchophorus palmarum*) and from soil samples from coconut (*Cocos nucifera*) crops against the insect itself. Initially, to determine the chitinolytic properties of the isolated fungi, their ability to hydrolyze chitin in a liquid culture medium was evaluated. Then, preliminary pathogenicity assays were carried out, using the bean weevil (*Acanthoscelides obtectus*) as a reference, to select the fungal isolates to be used in the experiments with the palm weevil. Finally, the bioactivity of two selected entomopathogenic fungi on palm weevil larvae and adults was assessed. There was no direct correlation between chitinolytic activity and pathogenicity capacity on the bean weevil nor between the isolates and bioactivity on the palm weevil. *Beauveria bassiana* CSU9 shows the highest activity on palm weevil larvae and adults, with a median lethal time of 0.8 and 14.4 days, respectively.

Index terms: *Beauveria bassiana*, *Trichoderma virens*, biological control agent, chitinolytic activity, entomopathogenic fungi.

Bioatividade de fungos isolados de áreas de cultivo de coco contra *Rhynchophorus palmarum*

Resumo – O objetivo deste trabalho foi avaliar a atividade quitinolítica e a bioatividade de fungos isolados do gorgulho-da-palmeira (*Rhynchophorus palmarum*) e de amostras de solos de áreas de cultivo de coco (*Cocos nucifera*) contra o próprio inseto. Inicialmente, para determinar as propriedades quitinolíticas dos fungos isolados, foi avaliada a sua capacidade de hidrolisar quitina em meio de cultura líquido. Em seguida, foram realizados ensaios preliminares de patogenicidade, tendo-se utilizado o gorgulho-do-feijão (*Acanthoscelides obtectus*) como modelo, para selecionar os isolados fúngicos a serem usados nos experimentos com o gorgulho-da-palmeira. Por fim, avaliou-se a bioatividade de dois fungos entomopatogênicos selecionados em larvas e adultos do gorgulho-da-palmeira. Não houve correlação direta entre a atividade quitinolítica e a capacidade de patogenicidade sobre o gorgulho-do-feijão nem entre os isolados e a bioatividade sobre o gorgulho-da-palmeira. *Beauveria bassiana* CSU9 apresenta a maior atividade sobre larvas e adultos do gorgulho-da-palmeira, com tempo letal médio de 0,8 e 14,4 dias, respectivamente.


Termos para indexação: *Beauveria bassiana*, *Trichoderma virens*, agente de controle biológico, atividade quitinolítica, fungos entomopatogênicos.

Lorena María López-Luján⁽¹⁾ ,
Sara Ramírez-Restrepo⁽²⁾ ,
Juan Carlos Bedoya-Pérez⁽²⁾ ,
Mauricio Salazar-Yepes⁽³⁾ ,
Natalia Arbeláez-Agudelo⁽¹⁾ ,
David Granada-García⁽¹⁾ 

⁽¹⁾ Corporación para Investigaciones Biológicas, Carrera 72A, nº 78B-141, Medellín, Colombia.
E-mail: lorena23062007@gmail.com, narbelaez@cib.org.co, davidgranada888@gmail.com

⁽²⁾ Institución Universitaria Colegio Mayor de Antioquia, Carrera 78, nº 65-46, Medellín, Colombia.
E-mail: sara.ramirez@colmayor.edu.co, juan.bedoya@colmayor.edu.co

⁽³⁾ Universidad Nacional de Colombia, Facultad de Ciencias, Museo Micológico MMUNM, Calle 59A, nº 63-20, Núcleo El Volador, Medellín, Colombia.
E-mail: masalazay@unal.edu.co

 Corresponding author

Received
February 22, 2022

Accepted
March 29, 2022

How to cite
LÓPEZ-LUJÁN, L.M.; RAMÍREZ-RESTREPO, S.; BEDOYA-PÉREZ, J.C.; SALAZAR-YEPES, M.; ARBELÁEZ-AGUDELO, N.; GRANADA-GARCÍA, D. Bioactivity of fungi isolated from coconut growing areas against *Rhynchophorus palmarum*. *Pesquisa Agropecuária Brasileira*, v.57, e02882, 2022. DOI: <https://doi.org/10.1590/S1678-3921.pab2022.v57.02882>.

Introduction

Coconut (*Cocos nucifera* L.) is one of the most popular crops around the world. In 2020, the coconut production worldwide almost reached 61 million tons, i.e., 12.3 million tons per hectare, with Indonesia, Philippines, India, Sri Lanka, and Brazil standing out as the largest producers, representing 31, 23, 18, 4.2, and 3.8%, respectively, of the total produced (Statista, 2020). Although Colombia only produces 0.145 million tons per year of coconut fruit – destined for domestic consumption and corresponding to 0.24% of the world market –, the country has a high potential for the growth of this crop due to its specific climatic conditions, which favor yields of 7.2 Mg ha⁻¹, above the world average of 5.0 Mg ha⁻¹ (Statista, 2020). However, as in other countries, Colombian coconut production is affected by several phytosanitary problems, among which the palm weevil (*Rhynchophorus palmarum* L.) is one of the most important (León-Martínez et al., 2019).

The palm weevil causes mechanical damage to palms, especially in the early stages of plant development, and is also the main vector of the *Bursaphelenchus cocophilus* (Cobb) Baujard nematode, the causal agent of the red ring disease (RRD) (Calvache et al., 1995). This nematode has a wide range of hosts, affecting several crops such as oil palm (*Elaeis guineensis* Jacq.), peach palm (*Bactris gasipaes* Kunth), and date palm (*Phoenix dactylifera* L.). The RRD causes yellowing and the progressive death of older leaves and shortening of emerging leaves, which affect photosynthetic processes and lead to palm death (Kambiré et al., 2021). The presence of *B. cocophilus* has been reported in Central and South America, as well as in the Caribbean islands. In Colombia, the RRD and palm weevil have affected more than 70,000 planted hectares, reducing yield by more than 50% in the main coconut growing areas in Cauca, Nariño, and Antioquia (Chinchilla, 2010); annual losses have been estimated at USD \$5.5 billion. Therefore, the RRD and palm weevil are considered the most threatening phytosanitary problems of palm crops (León-Martínez et al., 2019).

For the control of the palm weevil, insecticides based on cypermethrin, benzoylureas, and carbamates have been used (El-Bokl et al., 2010; El-Shafie et al., 2011). However, these types of insecticides have no effect on larval instars of palm weevil with cryptic

habits. Therefore, biotechnological applications and biological control agents have emerged as potential alternatives (Mazza et al., 2014; Santana et al., 2019). In this scenario, the use of entomopathogenic fungi (EPF) has proven to be effective in pest control (Awan et al., 2021; Poveda, 2021); however, few known studies have been conducted to find natural enemies of the palm weevil. EPF can penetrate the cuticle (chitin) of the insects and release enzymes and toxins that reduce immune responses in hosts, preventing them from expressing resistance mechanisms (Hussain et al., 2016). In this case, the chitinolytic activity is a relevant factor for the selection of fungi with a high infectious capacity and pathogenic potential.

The objective of this work was to evaluate the chitinolytic activity and bioactivity of fungi isolated from the palm weevil and from soil samples from coconut crops against the insect itself.

Materials and Methods

Soil samples and palm weevil adults were collected from four of the main coconut-producing areas in Colombia: Antioquia (8°45'0"N, 76°31'1"W), Cordoba (9°14'46"N, 76°07'43"W), Cauca (2°34'1"N, 77°52'59"W), and Nariño (1°48'0"N, 78°45'0"W). In each sampling area, 24 soil samples of 100 g were taken at six different points, mixed, and homogenized. In addition, eight cylindrical capture traps made of polyvinyl chloride, with 7.6 cm in diameter and 50 cm in length, were randomly distributed in each sampling area to collect naturally infected palm weevil adults. Each trap contained 0.5 mL Rhynchophorol C pheromone, whose active ingredient is 2-methyl-5-hepten-4-ol, and fresh sugarcane slices, with a diameter of 5.0 cm and a length of 10 cm.

Ten grams of homogenized soil were mixed to 90 mL saline solution (0.85% NaCl) and diluted at a ratio of 1:10. Then, 100 µL of the 10⁻² and 10⁻³ dilutions were added to sterile Petri dishes containing 15 mL potato dextrose agar (PDA, 39 g L⁻¹) supplemented with antibiotics specific for bacteria, i.e., with 3.4 mg L⁻¹ ampicillin and 0.5 g L⁻¹ streptomycin. The plates were incubated at 26°C, in the dark, for at least four days. The fungi isolates were purified and re-cultured in fresh PDA under the same conditions to obtain monosporic cultures (El Kichaoui et al., 2017). Conidia were mixed in 10 mL of 0.1% Tween 80 in sterile distilled

water, and the suspensions were diluted and adjusted to 50–100 spores per millimeter. A volume of 100 μL conidia suspension was added to sterile Petri dishes containing 15 mL PDA and incubated at 26°C, in the dark, for at least four days.

Thirty palm weevils naturally infected were washed with 1.0% hand soap and placed in Petri dishes containing a moistened piece of cotton. After ten days, a portion of the mycelium developed on the insects was added to 10 mL saline solution (0.85% NaCl) and vortexed for 2 min. A final suspension of 1.0 mL was used to isolate the fungi following the procedure previously described. Then, 20 palm weevils were placed individually in plastic boxes (30×16×8.0 cm), whose lids had several perforations. Sugarcane slices (diameter of 3.0 cm and length of 7.0 cm) placed on wet absorbent paper were used to feed the insects and as an oviposition site for the females. The sugarcane and wet absorbent paper were renewed once a week, and emergent larvae were collected weekly.

The palm weevil larvae for the bioactivity assays were placed individually in 21 mL cups and fed with red apple slices. Afterwards, the palm weevil larvae that would be used for rearing were placed individually in 21 mL cups with 5.0 g meridic diet, containing 18.5 agar, 50 g L⁻¹ Levapan bread yeast, 45 g L⁻¹ wheat flour, 45 g L⁻¹ corn flour, 1.6 g L⁻¹ sorbic acid, 4.0 g L⁻¹ ascorbic acid, 3.1 g L⁻¹ Pharmaton dietary supplement, and 0.5 mg L⁻¹ tetracycline (El-Shafie et al., 2013). Recipients and food were renewed weekly. After 60 days, the larvae were transferred to 50 mL falcon tubes containing coconut fiber to favor pupal development. The conditions established for all procedures were: photoperiod of 12 hours dark/light, relative humidity (RH) of 80%, and temperature of 30°C.

Three 5.0 cm colonized agar plugs of each fungal isolate grown in PDA, as described before, were added to 125 mL baffled Erlenmeyer flasks, containing 25 mL liquid culture medium with 4.2, 6.9, 2.0, 0.3, 0.5, 0.3, 1.0, and 10 g L⁻¹ (NH₄)₂SO₄, NaH₂PO₄, KH₂PO₄, MgSO₄·7H₂O, citric acid monohydrate, urea, tryptone, and glucose, respectively. The flasks were incubated on a rotatory shaker, at 30°C and 180 rpm, for 72 hours. The chitinolytic properties of all isolated fungi were evaluated by testing their ability to hydrolyze chitin in a liquid culture medium, containing 4.2, 6.9, 2.0, 0.2, 0.3, 0.005, 0.0016, 0.0014, 0.002, and 4.2 g L⁻¹ (NH₄)₂SO₄,

NaH₂PO₄, KH₂PO₄, Tween 80, MgSO₄·7H₂O, FeSO₄·7H₂O, MnSO₄, ZnSO₄, CaCl₂·2H₂O, and tryptone, respectively, and supplemented with 15 g L⁻¹ colloidal chitin (Sandhya et al., 2004). The colloidal chitin was prepared according to the protocol in Percot et al. (2003). A 6.0 mL aliquot of the inoculum was added to 125 mL baffled Erlenmeyer flasks containing 24 mL of the medium. The flasks were incubated on a rotatory shaker, at 30 and 180 rpm, for 120 hours. Aliquots of 2.0 mL were taken every 48 hours and centrifuged at 8,000×g, for 5 min, at 4°C. The cell-free supernatant was stored at 4°C and then used to evaluate the chitinolytic activity. All submerged fermentations were done in triplicate.

The chitinolytic activity was determined by the dinitro salicylic acid (DNS) method (Sandhya et al., 2004). Aliquots of 0.5 mL cell-free supernatant were added to the test tubes containing 0.5 mL of 0.5% colloidal chitin in phosphate buffer (pH 5.5) and 1.0 mL sterile distilled water. The used controls were: substrate, with 0.5 mL of 0.5% colloidal chitin in phosphate buffer and 1.5 mL sterile distilled water; and enzyme, with 0.5 mL cell-free supernatant and 1.5 mL sterile distilled water. Absorbance was measured in aliquots of 200 μL , at 540 nm, in the 680XR microplate reader (Bio-Rad, Hercules, CA, USA). One unit (1 U) of chitinolytic activity was defined as the amount of enzyme required to release 1.0 μg reducing sugars per millimeter per minute from a 0.5% colloidal chitin solution under the reaction conditions (Sandhya et al., 2004).

Conidial suspensions were prepared from fungal isolates grown on PDA, at 26°C, for ten days. The conidia were collected and placed in sterile falcon tubes containing 20 mL of 0.05% Tween 80 (v/v) in sterile distilled water. Spore concentration was adjusted to 10⁸ mL⁻¹ conidia. The viability of the conidia and the purity of the suspension were estimated by the percentage of germination of the conidia in PDA. The bean weevil [*Acanthoscelides obtectus* (Say)] was used in preliminary pathogenicity assays to select the fungal isolates that would be used in the experiments with the palm weevil. The bean weevil has the typical body structures of coleopterans and a short life cycle, besides being easily multiplied (Silva, 2017); in the present study, the bean weevil was reared from insects collected from commercial white bean (*Phaseolus vulgaris* L.) stocks. The insects were immersed in 0.5% sodium

hypochlorite for 10 s, and five insects were placed inside sterile glass jars containing 5 U of beans and then sprayed with 700 μL (10^8 mL^{-1} conidia) of conidial suspension. The control was sprayed with 700 μL of 0.01% Tween 80. The sterile glass jars were covered with a mesh cloth and incubated for 16 days at 12 hours dark/light photoperiod, 75% RH, and 29°C. Mortality was recorded every day to determine median lethal time (LT_{50}). For this, a nonlinear probability model was obtained with the options of discrete choice models (Probit) and a completely random statistical model was analyzed by the method of repeated measures over time ($\alpha=0.05$). The treatments consisted of the fungal isolates that showed the highest chitinolytic activity by the DNS method, evaluated in triplicate (Rodríguez-González et al., 2018b). The species *Beauveria bassiana* CSU9 was included in the present study since it has been widely reported as an EPF against coleopteran insects (Rodríguez-González et al., 2018a). Afterwards, palm weevil larvae were immersed in 0.5% sodium hypochlorite for 10 s, dried, and dipped in the conidia suspension for 20 s. The inoculated larvae were individually placed on a moistened and sterilized napkin inside plastic boxes (2.0×6.0×3.0 cm) containing a cotton swab impregnated with a sterile 10% sucrose solution. The plastic boxes were kept for 30 days at a 12 hour dark/light photoperiod, 30°C, and 90% RH. Larval mortality was recorded every two days, and the LT_{50} value was determined. Two of the fungal isolates that showed the lowest LT_{50} values on the bean weevil were selected and used as treatments, which were evaluated on three different dates, with three replicates of five individuals each. For the control, five larvae were dipped in 0.01% Tween 80 only. Finally, palm weevil adults – captured and quarantined in the laboratory under a 12 hour dark/light photoperiod, 29°C, and 90% RH for 15 days – were disinfected by immersion in 0.05% sodium hypochlorite for 30 s, rinsed three times with sterile water, dried, and dipped in the conidia suspension for 20 s. The insects were individually placed in plastic boxes (16×12×7.0 cm) containing portions of sugarcane as food, and the LT_{50} values were estimated. In all experiments, to verify if they had been inoculated in dead larvae or insects, the fungi were re-isolated in PDA.

The initial identification of the isolates was done by microscopic observation using morphological keys (Seifert et al., 2011). The fungal isolates selected

through the preliminary pathogenicity tests were identified. Their DNA was quantified using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Polymerase chain reactions (PCRs) were performed in the iCycler (Bio-Rad, Hercules, CA, USA) in a 30 μL reaction mix containing 1.0 X PCR buffer, 1.5 mmol L^{-1} MgCl_2 , 0.2 mmol L^{-1} dNTP, 1 U Taq DNA polymerase, 50–100 $\mu\text{g L}^{-1}$ template DNA, and 0.2 $\mu\text{mol L}^{-1}$ of each ITS1/ITS4 or EF1/EF2 primer. All PCRs were performed for 30 cycles, which consisted of denaturation for 1 min at 94°C, hybridization for 1 min at 50°C, extension for 1 min at 72°C, and final extension for 5 min at 72°C. The amplified products were analyzed by 1.0% (w/v) agarose gel electrophoresis and visualized with the GelRed Nucleic Acid Gel Stain (Biotium: Growing Products for Science, Fremont, CA, USA). The purified PCR amplicons were further sequenced by Macrogen (Seoul, Korea) and compared with those reported by BLAST (National Library of Medicine, Bethesda, MD, USA) to confirm the genetic identity of the isolates.

The analysis of variance (ANOVA), at a significance level of 5%, and multiple comparison tests were performed to determine the differences among treatments. Tests for normality of residuals and homogeneity of variances were performed before the ANOVA. All statistical analyzes were carried out with the Statgraphics Centurion XVI software (Statgraphics Technologies, Inc., Plains, VA, USA).

Results and Discussion

A total of 33 fungi belonging to five genera were isolated: 17 of *Trichoderma* spp., 12 of *Purpureocillium* spp., 2 of *Paecilomyces* spp., 1 of *Beauveria* sp., and 1 of *Metacordyceps* sp. (Table 1). Of these, based on the morphological differences between isolates of the same genera, 15 were selected to be used in the chitinolytic activity assays. The *Trichoderma harzianum* T12 isolate was also included in the present study as a reference since it has been reported as a chitinase-producing fungus with an enzymatic activity greater than 9 U (Sandhya et al., 2004; Abu-Tahon & Isaac, 2020). By comparing the sequences of the selected isolates with those of the isolates stored in the GenBank database (National Library of Medicine, Bethesda, MD, USA), it was possible to confirm the

identity of the following species: *Trichoderma virens* (JH Mill., Giddens & AA Foster) Arx; *Beauveria bassiana* (Bals.-Criv.) Vuill.; and *Metacordyceps chlamydosporia* (H.C. Evans) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, formerly called *Pochonia chlamydosporia* (Goddard) Zare & W. Gams.

At 48 hours, the highest values for the chitinolytic activities were: 24.9±5.9 U, with *M. chlamydosporia* CSU10; 7.6±1.1 U, with *Purpureocillium* sp. CSC1; 7.5±0.2 U, with *Trichoderma* sp. CU1; 6.5±0.7 U, with *T. virens* CSC11; and 6.3±0.1 U, with *Purpureocillium* sp. CSU2 (Table 1). These values were 4.0, 1.2, 1.2, 1.0, and 1.0 times, respectively, that obtained with *T. harzianum* T12, which was of 6.3±0.1 U. Consequently, the four listed isolates were considered to have great potential for insect control and were used in the pathogenicity assays with the bean weevil.

The lowest LT₅₀ values for the bean weevil were: 3.2±0.8 days, obtained with *T. virens* CSC11; 5.1±1.0 days, with *B. bassiana* CSU9; 5.8±0.8 days, with *Purpureocillium* sp. CSU2; and 6.1±1.0 days, with *Purpureocillium* sp. CSC1. Both *B. bassiana* CSU9 and *T. virens* CSC11 showed LT₅₀ values 51 and 69% lower than those of the control, respectively (Figure 1). Surprisingly, *M. chlamydosporia* CSU10 and *Trichoderma* sp. CU1 caused the lowest pathogenicity on the bean weevil despite exhibiting the highest

chitinolytic activities (Table 1). This could be explained by the fact that the pathogenicity of the EPF is not only defined by its ability to penetrate the insect cuticle but also by other factors such as its capacity to release different enzymes and toxins necessary to weaken the insect's immune system (Mondal et al., 2016; Boguś et al., 2017). In addition, although Silva (2017) concluded that the cuticle of adult coleopteran insects is mainly composed of chitin, Mondal et al. (2016) found that the external cuticle of palm weevil larvae is composed of other major compounds, as lipids and proteins, which was not considered in the present study. Both *T. virens* CSC11 and *B. bassiana* CSU9 were selected for further experiments since the virulence of *Trichoderma* sp. and *B. bassiana* isolates has been widely reported against insects, including beetles (Ricaño et al., 2013; Berini et al., 2016; Rodríguez-González et al., 2018a).

Beauveria bassiana CSU9 showed a better entomopathogenic activity than *T. virens* CSC11 in the palm weevil larvae, with LT₅₀ values of 0.8 and 29.6 days, respectively (Figure 2). However, in adults palm weevils, there were no differences between the LT₅₀ of *B. bassiana* CSU9 and *T. virens* CSC11, with 14.4 and 16.7 days, respectively. Considering the development of the mycelium in the legs, spittle, and elytra of the treated insects, *B. bassiana* CSU9

Table 1. Chitinolytic activity of selected entomopathogenic fungi.

| Code | Isolate | Enzymatic activity (units) | | |
|-------|-------------------------------------|----------------------------|----------|-----------|
| | | 48 hours | 96 hours | 144 hours |
| T12 | <i>Trichoderma harzianum</i> | 6.3±0.1 | 4.4±0.7 | 3.1±0.5 |
| CG1 | <i>Paecilomyces</i> sp. | 0.9±1.0 | 3.2±0.7 | 2.5±0.3 |
| CU1 | <i>Trichoderma</i> sp. | 7.5±0.2 | 8.6±0.4 | 6.5±0.2 |
| CSU2 | <i>Purpureocillium</i> sp. | 6.3±0.1 | 8.3±1.4 | 4.7±0.6 |
| CU6 | <i>Trichoderma asperellum</i> | 2.3±1.3 | 2.8±1.1 | 2.4±0.2 |
| CU7 | <i>Purpureocillium</i> sp. | 4.7±1.0 | 5.0±0.9 | 4.0±0.4 |
| CSC1 | <i>Purpureocillium</i> sp. | 7.6±1.1 | 5.2±0.8 | 4.9±2.2 |
| CSC4 | <i>Purpureocillium</i> sp. | 4.6±0.5 | 5.2±0.7 | 4.8±0.5 |
| CSC7 | <i>Trichoderma</i> sp. | 4.2±0.2 | 3.6±0.7 | 3.2±0.6 |
| CSC11 | <i>Trichoderma virens</i> | 6.5±0.7 | 6.1±0.3 | 5.6±1.0 |
| CSC13 | <i>Paecilomyces</i> sp. | 2.4±1.2 | 3.8±1.0 | 4.4±0.7 |
| CU3 | <i>Purpureocillium</i> sp. | 2.5±1.1 | 5.6±2.0 | 1.5±1.1 |
| CSU5 | <i>Trichoderma</i> sp. | 5.8±0.5 | 5.8±1.6 | 5.3±1.1 |
| CSU6 | <i>Trichoderma</i> sp. | 5.3±0.4 | 4.2±0.1 | 3.3±1.5 |
| CSU9 | <i>Beauveria bassiana</i> | 1.7±0.2 | 4.8±1.5 | 2.3±0.9 |
| CSU10 | <i>Metacordyceps chlamydosporia</i> | 24.9±5.9 | - | 33.5±4.5 |

proved to be effective against palm weevil larvae and adults, with a respective LT_{50} of 0.8 and 14.4 days. The mode of action of *B. bassiana* involves cuticle penetration through the integument due to the carbon- and nitrogen-rich resources present in the insect bodies (Islam et al., 2021). Moreover, this species has

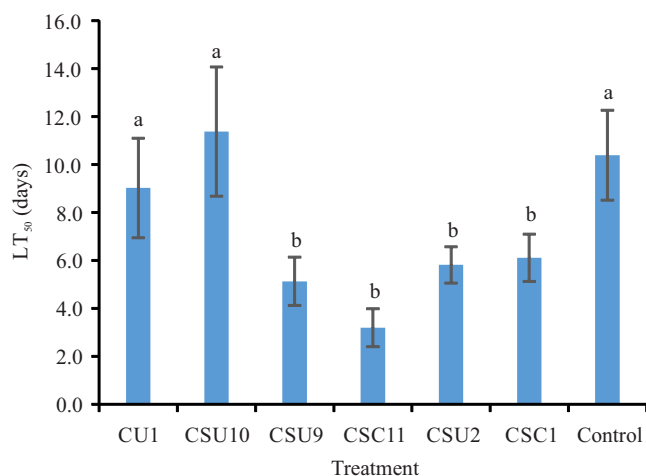


Figure 1. Values for median lethal time (LT_{50}) of selected fungi isolates on the bean weevil (*Acanthoscelides obtectus*). Bars with different letters show significant differences by Tukey's test, at 5% probability. CUI, *Trichoderma* sp.; CSU10, *Metacordyceps chlamydosporia*; CSU9, *Beauveria bassiana*; CSC11, *Trichoderma virens*; CSU2, *Purpureocillium* sp.; CSC1, *Purpureocillium* sp.; and Control, 0.01% Tween 80.

the ability to evade the immune response of insects (Lovera et al., 2020), a process that is mediated by the physical pressure exerted by the hyphae on the cuticle and by the enzymatic activity on its components. Xiao et al. (2012) observed that *B. bassiana* has an advanced adhesion mechanism involving hydrophobins that mediate cell surface hydrophobicity and virulence. Furthermore, two nonribosomal peptide synthetase genes encoding bbBeas and bbBsls, responsible for the synthesis of the insecticidal virulence factors beauvericin and bassianolide, respectively, have been identified recently (Batool et al., 2020); both may be effective strategies to facilitate pathogenic interactions with insects. *Trichoderma* sp., however, presents mycoparasitism as a mechanism of action, produces insecticidal secondary metabolites, and competes with host insects through more complex and slow processes (Poveda, 2021).

León-Marinez et al. (2019) collected isolates of *B. bassiana* from a commercial oil palm plantation and reported mortalities of palm weevil adults from 36 to 47% at 21 days after inoculation ($LT_{50} > 21$ days). Dembilio et al. (2012) used concentrations of *B. bassiana* higher than 10^9 mL^{-1} conidia to increase the mortality of *Rhynchophorus ferrugineus* (Olivier, 1790) adults after 21 days of treatment ($LT_{50} > 21$ days). Compared with the adult insects of these other species, *T. virens* CSC11 showed lower LT_{50} values (< 21 days) in the present study.

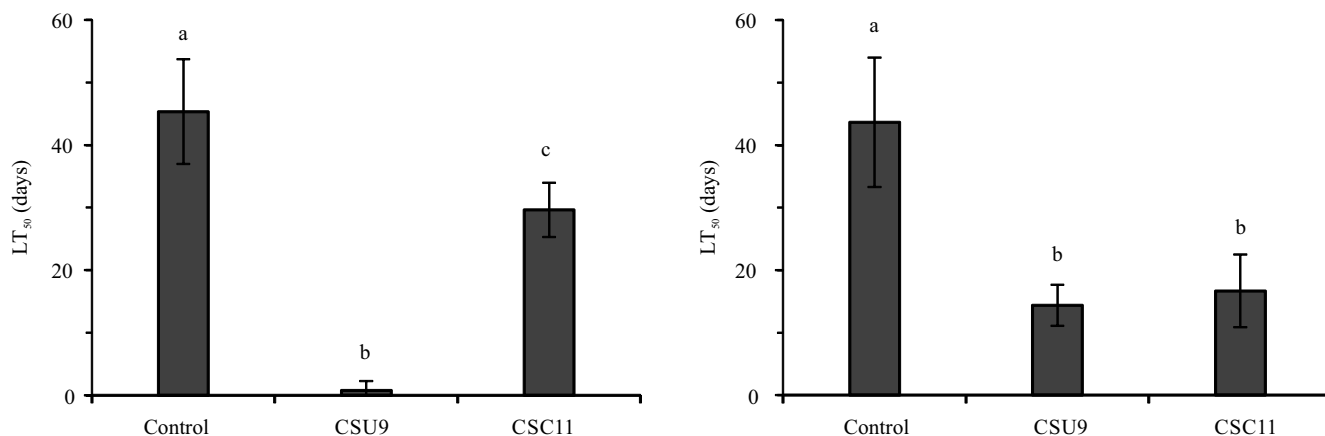


Figure 2. Median lethal time (LT_{50}) of *Beauveria bassiana* (CSU9) and *Trichoderma virens* (CSC11) on the palm weevil (*Rhynchophorus palmarum*): A, larvae; and B, adults. Bars with different letters show significant differences by Tukey's test, at 5% probability. Control, 0.01% Tween 80.

Conclusions

1. There is no direct correlation between the chitinolytic activity and the pathogenicity capacity of *Beauveria bassiana* CSU9 and *Trichoderma virens* CSC11 on the control of the palm weevil (*Rhynchophorus palmarum*).

2. Two entomopathogenic fungi – *T. virens* CSC11 and *B. bassiana* CSU9 – show potential to control palm weevil larvae and adults in the main coconut (*Cocos nucifera*) producing areas in Colombia.

Acknowledgments

To Sistema General de Regalías of Minciencias, to Corporación para Investigaciones Biológicas (CIB), and to Institución Universitaria Colegio Mayor de Antioquia (IUCMA), for funding.

References

- ABU-TAHON, M.A.; ISAAC, G.S. Anticancer and antifungal efficiencies of purified chitinase produced from *Trichoderma viride* under submerged fermentation. **The Journal of General and Applied Microbiology**, v.66, p.32-40, 2020. DOI: <https://doi.org/10.2323/jgam.2019.04.006>.
- AWAN, U.A.; XIA, S.; MENG, L.; RAZA, M.F.; ZHANG, Z.; ZHANG, H. Isolation, characterization, culturing, and formulation of a new *Beauveria bassiana* fungus against *Diaphorina citri*. **Biological Control**, v.158, art.104586, 2021. DOI: <https://doi.org/10.1016/j.biocontrol.2021.104586>.
- BATOOL, R.; UMER, M.J.; WANG, Y.; HE, K.; ZHANG, T.; BAI, S.; ZHI, Y.; CHEN, J.; WANG, Z. Synergistic effect of *Beauveria bassiana* and *Trichoderma asperellum* to induce maize (*Zea mays* L.) defense against the asian corn borer, *Ostrinia furnacalis* (Lepidoptera, Crambidae) and larval immune response. **International Journal of Molecular Sciences**, v.21, art.8215, 2020. DOI: <https://doi.org/10.3390/ijms21218215>.
- BERINI, F.; CACCIA, S.; FRANZETTI, E.; CONGIU, T.; MARINELLI, F.; CASARTELLI, M.; TETTAMANTI, G. Effects of *Trichoderma viride* chitinases on the peritrophic matrix of Lepidoptera. **Pest Management Science**, v.72, p.980-989, 2016. DOI: <https://doi.org/10.1002/ps.4078>.
- BOGUŚ, M.I.; WŁÓKA, E.; WRÓŃSKA, A.; KACZMAREK, A.; KAZEK, M.; ZALEWSKA, K.; LIGĘZA-ŻUBER, M.; GOŁĘBIEWSKI, M. Cuticle hydrolysis in four medically important fly species by enzymes of the entomopathogenic fungus *Conidiobolus coronatus*. **Medical and Veterinary Entomology**, v.31, p.23-35, 2017. DOI: <https://doi.org/10.1111/mve.12202>.
- CALVACHE G., H.; MORA TOQUICA, L.S.; GUEVARA ÁVILA, L.A. Anillo rojo – hoja corta en palma de aceite: tecnología disponible en Colombia. **Revista Palmas**, v.16, p.211-218, 1995.
- CHINCHILLA, C. **Anillo rojo en palma aceitera: una guía de manejo**. [2010]. Available at: <http://www.asd-cr.com/images/PDFs/Miscelaneos/Protocolo_Anillo_Rojo_Jun_10_final.pdf>. Accessed on: Feb. 10 2022.
- DEMBILIO, Ó.; QUESADA-MORAGA, E.; SANTIAGO-ÁLVAREZ, C.; JACAS, J.A. Potential of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* as a biological control agent against the red palm weevil, *Rhynchophorus ferrugineus*. **Journal of Invertebrate Pathology**, v.104, p.214-221, 2012. DOI: <https://doi.org/10.1016/j.jip.2010.04.006>.
- EL KICHAOUI, A.Y.; ABU ASAKER, B.A.; EL-HINDI, M.W. Isolation, molecular identification and under lab evaluation of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* against the red palm weevil *R. ferrugineus* in Gaza Strip. **Advances in Microbiology**, v.7, p.109-124, 2017. DOI: <https://doi.org/10.4236/aim.2017.71009>.
- EL-BOKL, M.M.; BAKER, R.F.A.; EL-GAMMAL, H.L.; MAHMOUD, M.Z. Biological and histopathological effects of some insecticidal agents against red palm weevil *Rhynchophorus ferrugineus*. **Egyptian Academic Journal of Biological Sciences**, v.1, p.7-22, 2010. DOI: <https://doi.org/10.21608/eajbsd.2010.14151>.
- EL-SHAFIE, H.A.F.; FALEIRO, J.R.; ABO-EL-SAAD, M.M.; ALEID, S.M. A meridic diet for laboratory rearing of red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). **Scientific Research and Essays**, v.8, p.1924-1932, 2013.
- EL-SHAFIE, H.A.F.; FALEIRO, J.R.; AL-ABBAD, A.H.; STOLTMAN, L.; MAFRA-NETO, A. Bait-free attract and kill technology (Hook™ RPW) to suppress red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in date palm. **Florida Entomologist**, v.94, p.774-778, 2011. DOI: <https://doi.org/10.1653/024.094.0407>.
- HUSSAIN, A.; RIZWAN-UL-HAQ, M.; AL-AYEDH, H.; ALJABR, A.M. Susceptibility and immune defence mechanisms of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) against entomopathogenic fungal infections. **International Journal of Molecular Sciences**, v.17, art. 1518, 2016. DOI: <https://doi.org/10.3390/ijms17091518>.
- ISLAM, W.; ADNAN, M.; SHABBIR, A.; NAVEED, H.; ABUBAKAR, Y.S.; QASIM, M.; TAYYAB, M.; NOMAN, L.; NISAR, M.S.; KHAN, K.A.; ALI, H. Insect-fungal-interactions: a detailed review on entomopathogenic fungi pathogenicity to combat insect pests. **Microbial Pathogenesis**, v.159, art.105122, 2021. DOI: <https://doi.org/10.1016/j.micpath.2021.105122>.
- KAMBIRÉ, M.S.; GNANWA, J.M.; BOA, D.; KOUADIO, E.J.P.; KOUAMÉ, L.P. Modeling of enzymatic activity of free β -glucosidase from palm weevil, *Rhynchophorus palmarum* Linn. (Coleoptera: Curculionidae) larvae: effects of pH and temperature. **Biophysical Chemistry**, v.276, art.106611, 2021. DOI: <https://doi.org/10.1016/j.bpc.2021.106611>.
- LEÓN-MARTÍNEZ, G.A.; CAMPOS-PINZÓN, J.C.; ARGUELLES-CÁRDENAS, J.H. Patogenicidad y autodiseminación de cepas promisorias de hongos entomopatógenos sobre *Rhynchophorus palmarum* L.(Coleoptera: Dryophthoridae). **Agronomía Mesoamericana**, v.30, p.631-646, 2019. DOI: <https://doi.org/10.15517/am.v30i3.36184>.

- LOVERA, A.; BELAICH, M.; VILLAMIZAR, L.; PATARROYO, M.; BARRERA, G. Enhanced virulence of *Beauveria bassiana* against *Diatraea saccharalis* using a soluble recombinant enzyme with endo- and exochitinase activity. **Biological Control**, v.144, art.104211, 2020. DOI: <https://doi.org/10.1016/j.biocontrol.2020.104211>.
- MAZZA, G.; FRANCARDI, V.; SIMONI, S.; BENVENUTI, C.; CERVO, R.; FALEIRO, J.R.; LLÁCER, E.; LONGO, S.; NANNELLI, R.; TARASCO, E.; ROVERSI, P.F. An overview on the natural enemies of *Rhynchophorus* palm weevils, with focus on *R. ferrugineus*. **Biological Control**, v.77, p.83-92, 2014. DOI: <https://doi.org/10.1016/j.biocontrol.2014.06.010>.
- MONDAL, S.; BAKSI, S.; KORIS, A.; VATAI, G. Journey of enzymes in entomopathogenic fungi. **Pacific Science Review A: Natural Science and Engineering**, v.18, p.85-99, 2016. DOI: <https://doi.org/10.1016/j.pusra.2016.10.001>.
- PERCOT, A.; VITON, C.; DOMARD, A. Optimization of chitin extraction from shrimps shells. **Biomacromolecules**, v.4, p.12-18, 2003. DOI: <https://doi.org/10.1021/bm025602k>.
- POVEDA, J. *Trichoderma* as biocontrol agent against pests: new uses for a mycoparasite. **Biological Control**, v.159, art.104634, 2021. DOI: <https://doi.org/10.1016/j.biocontrol.2021.104634>.
- RICAÑO, J.; GÜERRI-AGULLÓ, B.; SERNA-SARRIÁS, M.J.; RUBIO-LLORCA, G.; ASENSIO, L.; BARRANCO, P.; LOPEZ-LLORCA, L.V. Evaluation of the pathogenicity of multiple isolates of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) on *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) for the assessment of a solid formulation under simulated field conditions. **Florida Entomologist**, v.96, p.1311-1324, 2013. DOI: <https://doi.org/10.1653/024.096.0410>.
- RODRÍGUEZ-GONZÁLEZ, Á.; CARRO-HUERGA, G.; MAYO-PRIETO, S.; LORENZANA, A.; GUTIÉRREZ, S.; PELÁEZ, H.J.; CASQUERO, P.A. Investigations of *Trichoderma* spp. and *Beauveria bassiana* as biological control agent for *Xylotrechus arvicola*, a major insect pest in Spanish vineyards. **Journal of Economic Entomology**, v.111, p.2585-2591, 2018a. DOI: <https://doi.org/10.1093/jee/toy256>.
- RODRÍGUEZ-GONZÁLEZ, Á.; CASQUERO, P.A.; SUÁREZ-VILLANUEVA, V.; CARRO-HUERGA, G.; ÁLVAREZ-GARCÍA, S.; MAYO-PRIETO, S.; LORENZANA, A.; CARDOZA, R.E.; GUTIÉRREZ, S. Effect of trichodiene production by *Trichoderma harzianum* on *Acanthoscelides obtectus*. **Journal of Stored Products Research**, v.77, p.231-239, 2018b. DOI: <https://doi.org/10.1016/j.jspr.2018.05.001>.
- SANDHYA, C.; ADAPA, L.K.; MADHAVAN NAMPOOTHIRI, K.; BINOD, P.; SZAKACS, G.; PANDEY, A. Extracellular chitinase production by *Trichoderma harzianum* in submerged fermentation. **Journal of Basic Microbiology**, v.44, p.49-58, 2004. DOI: <https://doi.org/10.1002/jobm.200310284>.
- SANTANA, C.C.; SILVA-JÚNIOR, E.F.; SANTOS, J.C.N.; RODRIGUES, É.E. da S.; SILVA, I.M. da; ARAÚJO-JÚNIOR, J.X.; NASCIMENTO, T.G. do; BARBOSA, L.A.O.; DORNELAS, C.B.; FIGUEIREDO, I.M.; SANTOS, J.C.C.; GRILLO, L.A.M. Evaluation of guanlylhydrazone derivatives as inhibitors of *Candida rugosa* digestive lipase: biological, biophysical, theoretical studies and biotechnological application. **Bioorganic Chemistry**, v.87, p.169-180, 2019. DOI: <https://doi.org/10.1016/j.bioorg.2019.03.030>.
- SEIFERT, K.; MORGAN-JONES, G.; GAMS, W.; KENDRICK, B. **The genera of hyphomycetes**. Utrecht: CBS-KNAW Fungal Biodiversity Centre, 2011.
- SILVA, P.H. da. **Control biológico del gorgojo de la judía *Acanthoscelides obtectus* (Say) (Coleoptera: chrysomelidae: Bruchinae) en la región de Castilla y León - España**. 2017. Tesis (Doctorado) – Universidad de León, León.
- STATISTA. Available at: <https://www.statista.com/statistics/1040499/world-coconut-production-by-leading-producers/>. Accessed on: June 20 2020.
- XIAO, G.; YING, S.-H.; ZHENG, P.; WANG, Z.-L.; ZHANG, S.; XIE, X.-Q.; SHANG, Y.; ST. LEGER, R.J.; ZHAO, G.-P.; WANG, C.; FENG, M.-G. Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. **Scientific Reports**, v.2, art.483, 2012. DOI: <https://doi.org/10.1038/srep00483>.