REVISTA de la FACULTAD de AGRONOMIA

Biocontrol of *Cosmopolites sordidus* using entomopathogenic fungi under laboratory conditions, Ecuador

Biocontrol de *Cosmopolites sordidus* mediante el uso de hongos entomopatógenos en condiciones de laboratorio, Ecuador

Biocontrole de *Cosmopolites sordidus* usando fungos entomopatogênicos em condições de laboratório, Ecuador

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Rev. Fac. Agron. (LUZ). 2025, 42(2): e254217
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v42.2.I

Crop production
Associate editor: Dr. Francisco Osorio
Postgraduate College, Veracruz Campus
United Mexican States

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Received: 01-12-2024
Accepted: 27-02-2025
Published: 30-03-2025

Keywords:
Fungus
Black weevil
Musaceae
Efficacy
Control

Abstract

Cosmopolites sordidus known as the black weevil, is an insect pest of economic importance for Musaceae crops such as bananas and plantain, resulting in production losses due to its damage. One of the alternatives for the management of the black weevil is biological control using entomopathogens that regulate the insect populations in Musaceae plantations. The objective of the research was to determine the action of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae,* and *Verticillium lecanii* on adults of the black banana weevil under controlled laboratory conditions. A completely randomized design was implemented with 8 treatments with the application of the entomopathogens individually and in combinations, as well as a control treatment. The results showed that the treatment with the combination of *B. bassiana* + *M. anisopliae* showed the best control of the insect pest with 100 % mortality 10 days after inoculation, followed by *B. bassiana* with 88 % and 24 % control with *M. anisopliae*, while the treatment with the fungus *V. lecanii* achieved 32 % control of the black weevil. Entomopathogens are an alternative for the management of the insect pest of Musaceae *C. sordidus*.

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Resumen

Cosmopolites sordidus conocido como picudo negro, es un insecto plaga de importancia económica para los cultivos de musáceas como banano y plátano, teniendo como resultado por su daño perdidas en producción. Entre unas de las alternativas para el manejo del picudo negro se encuentran el control biológico mediante el uso de entomopatógenos que regulen las poblaciones del insecto en las plantaciones de musáceas. El objetivo de la investigación fue determinar la acción de los hongos entomopatogenos *Beauveria bassiana, Metarhizium anisopliae* y *Verticillium lecanii* en el control del picudo negro del banano *C. sordidus* bajo condiciones controladas de laboratorio. Se implementó un diseño completamente al azar con 8 tratamientos con la aplicación de los entomopatógenos de forma individual y en combinaciones, al igual que un tratamiento testigo. Los resultados mostraron que el tratamiento con la combinación de *B. bassiana* + *M. anisopliae* mostró el mejor control sobre el insecto plaga con un 100 % en la mortalidad a los 10 días de la inoculación, seguido de *B. bassiana* con 88 % y un 24 % en el control con *M. anisopliae*, mientras que para el tratamiento con el hongo *V. lecanii* se alcanzó un 32 % en el control del picudo negro. Los entomopatógenos son una alternativa para el manejo del insecto plaga de musáceas *C. sordidus*.

Palabras claves: hongos, picudo negro, musáceas, eficacia, control.

Resumo

O *Cosmopolites sordidus*, conhecido como gorgulho da palmeira-preta, é um inseto praga de importância econômica para as culturas de musáceas, como a banana e o plátano, resultando em perdas de produção devido a seus danos. Uma das alternativas para o manejo do gorgulho da palmeira-preta é o controle biológico por meio do uso de entomopatógenos que regulam as populações do inseto nas plantações de musáceas. O objetivo da pesquisa foi determinar a ação dos fungos entomopatogênicos *Beauveria bassiana*, *Metarhizium anisopliae* e *Verticillium lecanii* sobre os adultos do gorgulho da palmeira preta em condições controladas de laboratório. Um projeto completamente aleatório com 8 tratamentos foi implementado com a aplicação dos entomopatógenos individualmente e em combinações, bem como um tratamento de controle. Os resultados mostraram que o tratamento com a combinação de *B. bassiana* + *M. anisopliae* apresentou o melhor controle do inseto-praga, com 100 % de mortalidade 10 dias após a inoculação, seguido por *B. bassiana* com 88 % e 24 % de controle com *M. anisopliae*, enquanto o tratamento com o fungo *V. lecanii* obteve 32 % de controle do gorgulho da palmeira preta. Os entomopatógenos são uma alternativa para o manejo do inseto-praga *C. sordidus*

Palavras-chave: fungos, black weevil, musaceae, eficácia, controle.

Introduction

The cultivation of bananas (*Musa* x *paradisiaca* L.) in Ecuador has a transcendence and importance at the socioeconomic level at the national level, ranking the first place within the exports of agricultural products in the country and worldwide occupies the first place in exports of Musacea; representing 2 % of the total gross domestic product (GDP) and 35 % of agricultural GDP in the last six years, and annual production has ranged between 6,023,390 and 6,583,477 tons with economic revenues ranging from 2,000 million to 3,600 million dollars (Zambrano and Gallardo, 2022). *Cosmopolites sordidus or* black weevil is considered the most important pest of banana and plantain cultivation (Tresson *et al.*, 2022). The insect pest causes damage to the plant and affects plant species of the Musaceae family (banana, plantain, abacá). It is native to Southeast Asia and has spread to all countries that export bananas. In the larval stage, it bores into the corm, damages the root and the vascular system, reducing the absorption of water and nutrients; and weakening the stability of the plant: in early attacks, it can cause crop loss (Gold and Tinzaara, 2008).

There are more than 750 species of fungi considered biopesticides used to control insects, among the most important and commercially used are *Metarhizium anisopliae* and *Beauveria bassiana*. The use of entomopathogens in agriculture focuses on identifying native strains with potential commercial use (Hernández *et al.*, 2019; Pacheco *et al.*, 2019). Studies carried out by Silva *et al.,* (2020), determined the affinity and efficacy of *B. bassiana* and *M. anisopliae* for the control of insect pests in the cultivation of cabbage and lettuce, in an aquaponics system, where it was evidenced that *B. bassiana* and *M. anisopliae* controlled whiteflies and aphids. Likewise, Castro and Martínez. (2019) provided that in an integrated management scheme for the control of *Trialeurodes vaporariorum, Chrysoperla externa* together with *B. bassiana* and *M. anisopliae* can be used in tomato cultivation. Membang *et al.* (2021) indicated that the success of bioinsecticides based on entomopathogenic fungi depends on favorable properties in three successive stages in the development of entomopathogenic fungi, conidia germination, mycelial growth and conidiogenesis, and their response to environmental variables such as the range of temperatures to which the fungus will be exposed.

Fungi are an important part of the biopesticide market for the biological control of arthropods. Entomopathogenic fungi produce spores that can attach to the cuticle of arthropods, germinate, penetrate, and cause infection. On the other hand, studies demonstrate another approach to the use of entomopathogenic fungi: the study of the biocontrol potential of the enzymes produced by these fungi (Motta and Murcia, 2011). Nowadays, the biological control of pests has increased since the impact of the application of synthetic pesticides is negative for both people and the environment, in addition, these entomopathogenic fungi can produce lytic enzymes, esterase, oxidoreductase and cytochrome P450, reacting with pesticides and decomposing into non-toxic substances (Kannan *et al.*, 2024). The present research aims to determine the action of the entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae,* and *Verticillium lecanii* in the control of the black weevil of the banana *C. sordidus* under controlled laboratory conditions

Materials and methods

Study area

This study was conducted in the entomology laboratory of the INIAP (National Institute of Agricultural Research - South Litoral Experimental Station) geographical coordinates 2°15’07”S and 79°38’36”W, km 26 via Durán-Tambo, Guayaquil, Ecuador (National Institute of Meteorology and Hydrology [INAMHI], 2018). The specimens of live adults of *Cosmopolites sordidus* were obtained from the “Angela María” banana farm located in the province of Guayas, Ecuador. For the bioassay with entomopathogens and their action on *C. sordidus*, a completely randomized statistical design was performed with 8 treatments and 5 replications, with a total of 40 experimental units (Table 1).

Table 1. Treatments with entomopathogens for the control of *Cosmopolites sordidus.*

Treatments	Combinations
T1	*Beauveria bassiana*
T2	*Metarhizium anisopliae*
T3	*Verticillium lecanii*
T4	*Beauveria bassiana* + *Metarhizium anisopliae*
T5	*Beauveria bassiana* + *Verticillium lecanii*
T6	*Verticillium lecanii* + *Metarhizium anisopliae*
T7	*Beauveria bassiana* + *Verticillium lecanii* + *Metarhizium anisopliae*
T8	Control

Collection of adults of *Cosmopolites sordidus*

The adult specimens of *C. sordidus* were obtained in the field, using plastic traps (20 L containers) with two openings in the upper part of 8 cm wide by 12 cm long. Pieces of pineapples, molasses (225 cc), and water (75 cc) were used as attractants. Ethological traps made from pseudostems of harvested banana plants were also used, the sandwich traps were randomly distributed, placing one every 9 plants at a distance of 9m x 9m (Figure 1), following the methodology of Farah *et al.* (2022).

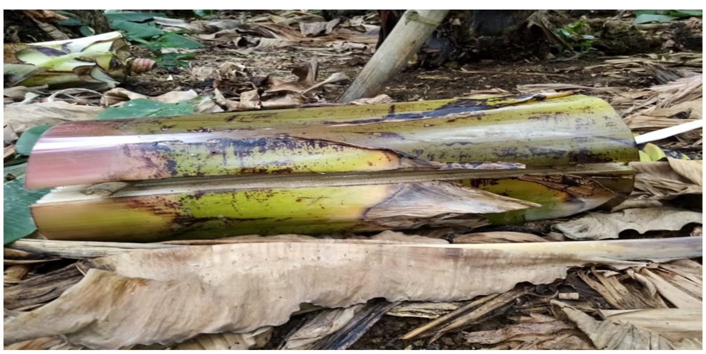

Figure 1. Sandwich-type trap for capturing *Cosmopolites sordidus.*

Fungi *Beauveria bassiana, Metarhizium anisopliae* and *Verticillium lecanii*

The entomopathogens for the assay were received from the Microbiolab laboratory. For the preparation of the solution to be applied in the treatments, 2 grams for each of the entomopathogens *B. bassiana*, *M. anisopliae,* and *V. lecani* were weighed on an electronic balance and mixed or diluted in sterilized water to obtain a solution of $1x10^6$ spores. mL^{-1}.

Bioassays of entomopathogenic fungi on *C. sordidus.*

To carry out the bioassays, live insects of *C. sordidus* in the adult stage were used, which were previously captured in the traps placed in the field, from where they were removed and taken to the entomology laboratory. The specimens used were those that showed greater biological activity, guaranteeing their viability during the execution period of the assay; they were disinfected with 0.5 % sodium hypochlorite solution applied with an atomizer and subsequently rinsed with disinfected deionized water. For the assays, 40 Petri dishes were used to confine the inoculated specimens (Figure 2). Food was provided with small pieces of banana pseudostems, changing them every day, and maintaining them at an average temperature of 27°C and 75 % relative humidity. In each Petri dish, 5 specimens of *C. sordidus* were placed, totaling 25 insects per treatment. These were inoculated with the entomopathogens using a sprayer or atomizer with the solution corresponding to each treatment.

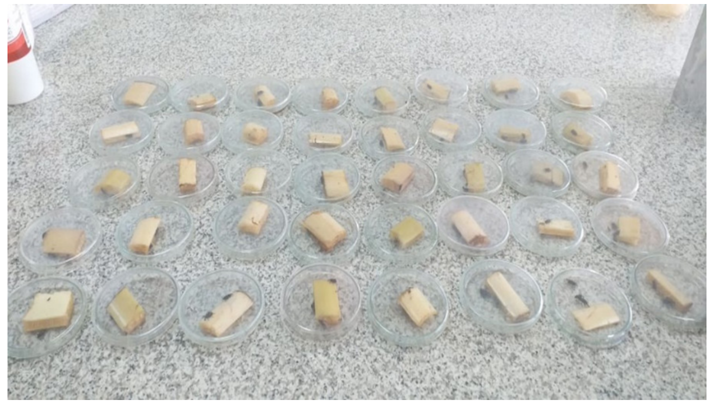

Figure 2. Experimental units in control bioassay for *Cosmopolites sordidus.*

To evaluate the mortality of the specimens and the efficiency of the treatments, monitoring began after the installation of the assay on day 6, giving time for the development of the inoculated microorganism in the host and its entomopathogenic action inside the insect. Subsequent evaluations were periodic (daily) until the tenth day with the culmination of the bioassay, completing a total of 5 evaluations. The dead Coleoptera in each evaluation were separated in Petri dishes individually and placed in wet chambers to observe the progress of the disease and consequently the sporulation of the fungus, with this the death of the insect by entomopathogenic microorganisms was confirmed.

Figure 3. Insect infected with the fungus, *Beauveria bassiana* + *Metarhizium anisopliae.*

Data processing and analysis

For the processing and analysis of the dependent variable number of dead insects and the independent variable based on entomopathogenic fungi, the non-parametric analysis of Kruskal Wallis at 5 % error was used, this was tabulated with the InfoStat program (2017). It is worth mentioning that the normality and homoscedasticity tests were significant (p>0.065).

Results and discussion

Mortality of *C. sordidus* in the application of entomopathogens

According to the results obtained in the assay of entomopathogens on the insect pest *C. sordidus*, the treatment of *B. bassiana* + *M. anisopliae* (T4) showed the highest mortality with a mean of 5.00 specimens on day ten of inoculation, followed by treatment with *B. bassiana* (T1) with a mean of 4.4 dead specimens, being the entomopathogenic activity and mycelial growth higher than in the other treatments under study, with T4 showing the beginning of *C. sordidus* mortality at 6 days after inoculation, and T1 at 8 days (Table 2). In studies carried out by Maharaj and Khan (2017), spray applications of mineral oil and water as spore carriers of *B. bassiana* were evaluated to improve virulence against *C. sordidus* at concentrations of 1 x 10^4, 1 x 10^5, 1 x 10^6, 1 x 10^7 and 1 x 10^8 pores.mL^{-1}, where the highest mortality was evidenced in the formulation of mineral oil, and *B. bassiana* 1 x 10^4 pores.mL^{-1} from 13 to 16 days after inoculation.

Table 2. Average mortality of *Cosmopolites sordidus*.

Treatments		Specimen mortality				
		Day 6	Day 7	Day 8	Day 9	Day 10
T4	*B. bassiana+ M. anisopliae*	0.4	0.4	2.0b	4.0c	5.0c
T1	*B. bassiana*	0.0	0.0	1.2b	2.2bc	4.4bc
T7	*B. bassiana + V. lecanii+ M.anisopliae*	0.0	0.0	0.0a	0.6a	1.6ab
T3	*V. lecanii*	0.0	0.0	0.4a	0.8ab	1.6ab
T5	*B. bassiana+ V. lecanii*	0.0	0.0	0.2ab	0.4ab	1.6a
T2	*M. anisopliae*	0.0	0.0	0.2a	0.6ab	1.2a
T6	*V. lecanii+ M. anisopliae*	0.0	0.0	0.0a	0.0a	0.8a
T8	Control	0.0	0.0	0.0a	0.0a	0.0a
	p-Value	N/A	N/A	0.0009	0.0004	0.0003

Mortality percentage

According to the treatments analyzed, it can be pointed out that T4 and T1, at the concentrations of 1x10^6 spores.mL^{-1} of the entomopathogens, exerted greater control in adults of *C. sordidus*, where T4 obtained 100 % mortality on day 10 after its application, followed by T1 with 88 % mortality, T3, T7, and T5 treatments obtained mortality of 32 % of specimens. For the T2 and T6 treatments, there was mortality of 24 % and 16 %, respectively, and for the control treatment (T8) there was no mortality of specimens 10 days after the installation of the assay in the control of the insect pest under laboratory conditions (Table 3). These results are consistent with Membang *et al*. (2021) who established that the combination of *B. bassiana* + *M. anisopilae* and *B. bassiana* individually act as promising biopesticides for commercial use to control *C. sordidus* in banana crops. However, in laboratory research conducted by Armendáriz *et al*., (2016) the *B. bassiana* strain was ineffective on adults of *C. sordidus*, even though Biryol *et al*. (2021) evaluated eight isolates of Indigenous fungi, four of *B. bassiana* and four of *Metarhizium brunneum*, with an oil base at maximum concentrations of 1 x 10^9 spore.mL^{-1} against nymphs and adults of the target pest *Orosanga japonica*, causing mortality against nymphs and adults of 97 % under field conditions, showing greater efficacy than commercial insecticides.

Table 3. Mortality percentage of *Cosmopolites sordidus.*

Treatments		Specimen mortality (%)				
		Day 6	Day 7	Day 8	Day 9	Day 10
T4	*B. bassiana+ M. anisopliae*	8.0	8.0	40.0b	80.0c	100.0c
T1	*B. bassiana*	0.0	0.0	24.0b	44.0bc	88.0bc
T5	*B. bassiana+ V. lecanii*	0.0	0.0	4.0a	8.0a	32.0ab
T7	*B. bassiana + V. lecanii+ M. anisopliae*	0.0	0.0	0.0a	12.0ab	32.0ab
T3	*V. lecanii*	0.0	0.0	8.0ab	16.0ab	32.0a
T2	*M. anisopliae*	0.0	0.0	4.0a	12.0ab	24.0a
T6	*V. lecanii+ M. anisopliae*	0.0	0.0	0.0a	0.0a	16.0a
T8	Control	0.0	0.0	0.0a	0.0a	0.0a
	p-Value	N/A	N/A	0.0009	0.0004	0.0003

Ahmed (2010) conducted an investigation with some strains of *Verticiliun lecanii* and *M. anisopliae* for the control of *Sitophilus zeamais* Motsch (Coleoptera) in stored corn kernel, where the insects treated with a higher concentration of 1 x 10^8 spores.mL^{-1} had greater control and mortality, compared to the other treatments. The estimates of dead Coleoptera were verified after application, that is, at 5, 10, and 15 days after infecting with entomopathogenic fungi in the various treatments, presenting the highest percentage of mortality in the application of 1x10^8 of conidia, reaching 1 to 5 dead individuals in the period of 5 to 10 days, while the other treatments in their different concentrations took longer. However, studies conducted by Kisaakye *et al.* (2021), used 8 isolates of *B. bassiana* ICIPE 273, ICIPE 648, and ICIPE 660, which were the most lethal, exhibited high levels of mortality and low median lethal time against the body weight of *C. sordidus* in the laboratory.

Regarding the statistical result, the control with the treatment of *B. bassiana* obtained 88 % mortality of the black weevil, while *M. anisopliae* obtained 24 %; considering that, by joining the two entomopathogens agents, 100 % control over *C. sordidus* was achieved, this considers that the action of entomopathogens when used separately tends to reduce their percentage of control of the insect pest. In this sense, Pelizza *et al*. (2015), provided that the comparative effects of the insecticides Rynaxypyr, Lufenuron, and Methoxyfenozide together with *B. bassiana* and *M. anisopliae*, were the ones that produced higher mortalities in the control of grasshoppers in field cage conditions. Likewise, González *et al.* (2022) determined that the mortality of *C. sordidus* reached 16 % and 7 %, using *M. anisopliae, B. bassiana*, separately and in a mixture of the two entomopathogenic fungi, reached 78 % in 24 days after the application of the biopesticides.

Conclusions

Regarding the results obtained in the entomopathogenic action of the microbial agents under analysis, it can be concluded that the combination of *B. bassiana* plus *M. anisopliae* and the treatment based on *B. bassiana* are more effective than when applied individually, having high mortality in adults of *C. sordidus*, being a viable option in the management of the pest under study.

The implementation of treatments on adults of *C. sordidus* did not cause damage to the insect's integrity during the initial evaluation phases, but later on, mycelial growth was subsequently observed on the insect's body, showing the colonization of the fungi in the inoculated specimens.

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