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Presentation: April, 2024 Acceptance: Juny, 2024 Publication: August, 2024

Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the mortality of *Cosmopolites sordidus* and *Metamasius hemipterus*

Efecto de Beauveria bassiana y Metarhizium anisopliae en la mortalidad de Cosmopolites sordidus y Metamasius hemipterus

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Cita sugerida (APA, séptima edición)

Arias Murillo, J. y Barrezueta Unda, S. (2024). Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the mortality of *Cosmopolites sordidus* and *Metamasius hemipterus*. *Revista Científica Agroecosistemas*, 12(2), 12-19. https://aes.ucf.edu.cu/index.php/aes

Abstract

The objective of the study was to evaluate the effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the survival of *Cosmopolites sordidus* and *Metamasius hemipterus* in a laboratory trial. First, several specimens of *C. sordidus* and *M. hemipterus* were collected in a banana plantation using traps with natural attractants. Subsequently, in the laboratory, different liquid doses of *B. bassiana* and *M. anisopliae* were applied to measure their mortality at various times. The results demonstrated the remarkable efficacy of both fungi as biological control agents, although they exhibited disparities in the speed of action. *M. anisopliae* demonstrated superior effectiveness at higher doses for both species, while *B. bassiana* exhibited a more gradual effect. It is recommended that further investigation be conducted to ascertain the effectiveness of the treatment under field conditions. Additionally, the optimal doses should be determined under various circumstances and the possible long-term impacts on the banana crop environment evaluated.

Keywords:

Banana, Biological control; *Curculionidae*, Black weevil, Striped weevil.

Resumen

El objetivo del estudio fue evaluar el efecto de *Beauveria bassiana* y *Metarhizium anisopliae* en la supervivencia de *Cosmopolites sordidus* y *Metamasius hemipterus* en un ensayo de laboratorio. Primero se recolectaron varios especímenes de *C sordidos* y *M hemipterus* en una plantación de banano utilizando trampas con atrayenbtes naturales. Luego en laboratorio se aplicaron diferentes dosis en liquido de *B bassiana* y *M anisopliae*, para medir su mortalidad a varios tiempos. Los resultados revelaron una destacada eficacia de ambos hongos como agentes de control biológico, si bien mostraron disparidades en la rapidez de acción. *Metarhizium anisopliae* se destacó por su efectividad en dosis más altas para ambas especies, mientras que *Beauveria bassiana* exhibió un efecto más gradual. Se sugiere continuar investigando su efectividad en condiciones de campo, así como determinar las dosis óptimas bajo diversas circunstancias y evaluar los posibles impactos a largo plazo en el entorno del cultivo de banano.

Palabras clave:

Banano, Control biológico; *Curculionidae*, Picudo negro, Picudo rayado.

Introduction

The banana (***Musa acuminata)*** is Ecuador's most significant export crop. However, phytosanitary management represents a significant challenge for banana growers (Duque, 2023). The insects ***Cosmopolites sordidus*** (black weevil) and ***Metamasius hemipterus*** (striped weevil), both of the family Curculionidae, have emerged as critical threats to banana and plantain crops in Ecuador (Castillo-Arévalo, 2022; Delaplace et al., 2024). The black weevil, Cosmopolites sordidus, is native to Southeast Asia, a region where the Musaceae are also native. The striped weevil, Metamasius hemipterus, has its habitat in palm plantations in the Americas. Both species have adapted their behavior to the ecological conditions of commercial banana crops (Maymon et al., 2020; Saquicela Cruz et al., 2023).

Behavior of M. hemipterus and C. sordidus

M. hemipterus and C. sordidus are primarily nocturnal in their habits. They prefer dark and humid areas, such as plant debris of banana crops in process or in a state of decay (Selvaraj et al., 2019), because they are predominantly nocturnal and survive in humid environments, making them vulnerable to desiccation.

Infestation by ***C. sordidus*** and ***M. hemipterus*** occurs in the chord and roots, resulting in reduced nutrient uptake, delayed flowering, and increased susceptibility to other pathogens such as black sigatoka (Bakaze et al., 2022; Farah Asang et al., 2022). Furthermore, banana wilt, usually associated with nematode infestation, has been observed to occur under severe C. sordidus infection (Buena Diaz et al., 2021; Guzman, 2019; Ristaino & Records, 2020).

The use of traps impregnated with chemical insecticides is the main control method for banana weevils. However, not only does this method of suppression have a negative impact on the environment, but it also causes resistance to these insecticides in these insects (Londoño-Caicedo et al., 2023). For these reasons, biological control is being considered as an alternative for the control of C. sordidus and ***M. hemipterus*** (Selvaraj et al., 2019).

Although the use of entomopathogenic fungi such as Beauveria spp. and Metarhizium spp. can be effective in eliminating some insect pests, they can also have long-term negative effects on populations of beneficial insects such as ***Hololepta quadridentata*** (Coleoptera: ***Histeriidae***), ***Camponotus spp.*** (Hymenoptera: ***Formicidae***), among others, in soils where bananas are grown (Londoño-Caicedo et al., 2023; Viswakethu et al., 2021; Kisaakye et al., 2021).

Effects of *Beauveria Bassiana and Metarhizium anisopliae*

Entomopathogenic fungi ***Beauveria Bassiana*** (Clavicipitaceae) and ***Metarhizium anisopliae*** (Deuteromycetes), upon contact with the host organism inoculate it with the spores adhering to the cuticle of the exoskeleton of the host insect. The conidia then perforate the insect's cuticle to eventually colonize the insect and cause its death, after which the fungus germinates until the cycle begins again (Castillo-Arévalo, 2022).

Therefore, ***Beauveria bassiana*** and ***Metarhizium anisopliae*** are promising ecological alternatives for the control of ***C. sordidus*** and ***M. hemipterus*** (Brunner-Mendoza et al., 2019). Their mortality time on the insect varies from 2 to 14 days, depending on the dose applied, the age and biological state of the insect, as well as the environmental conditions and evaluation methodology.

On the other hand, the lack of information on ***Beauveria bassiana*** and ***Metarhizium anisopliae*** to control both target and non-target populations is a critical issue (Castillo-Arévalo, 2022). In addition, the long-term consequences of continued use of high doses of these biocontrol agents are unknown, including the potential development of resistance in populations of M. hemipterus, an insect that adapts to different habitat types.

Therefore, it is necessary to evaluate different doses of B. bassiana and M. anisopliae under laboratory conditions to develop dosage strategies that maximize lethality for M. hemipterus and C. sordidus without altering the ecological balance of the agricultural environment. The aims of the study were to: Evaluate the effect of Beauveria bassiana and Metarhizium anisopliae on Cosmopolites sordidus and Metamasius hemipterus mortality using a laboratory bioassay.

Materials and methods

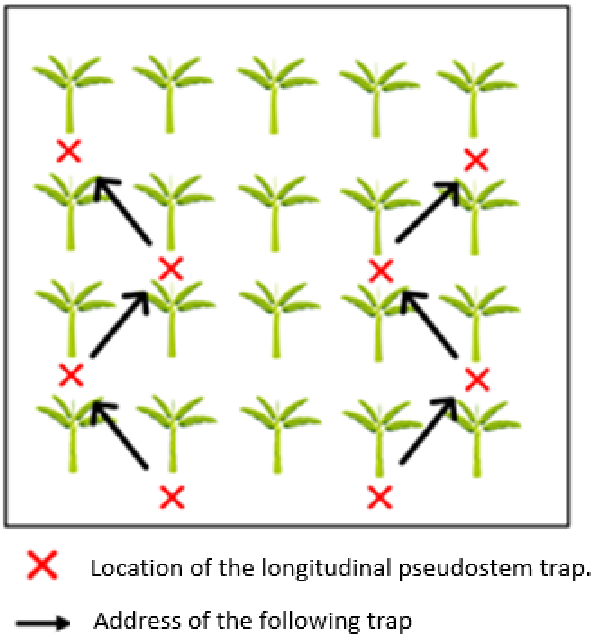
Study Site

The study was divided into two phases. First, the collection of ***C. sordidus*** and ***M. hemipterus*** was carried out in the banana plantation of the Technical University of Machala (UTMACH), located between the coordinates: 3°17'28.2"S 79°54'44.3"W, at an altitude of 6 meters above sea level. The average temperature of the area is 28°C and the annual rainfall is 1012 mm (Villaseñor-Ortiz & Pereira Da Silva, 2022). The second phase, corresponding to the bioassay, was carried out at the Phytosanitary Laboratory of the UTMACH.

Collection of specimens

To capture ***C. sordidus*** and ***M. hemipterus***, it was necessary to set 200 traps in a W-shaped pattern throughout the banana plantation (Figure 1).

Fig. 1. Placement and distribution of traps within the banana plantation.



Source: self made.

The traps were made with pieces of freshly harvested pseudostem, about 40 cm long, cut in two with a machete (Figure 2A). Then, wilted banana leaves were placed on the ground about 30 cm away from the pseudostem plants, avoiding proximity to the sprout to avoid disturbing pollination (Figure 2B). This procedure is carried out in attempt to improve the capture of the weevils, especially the striped weevils, due to the odor emitted by the banana leaves, which initiates the process of decomposition.

The attractant used was 300 g of ripe pineapple. The attractant was placed on the leaf and to facilitate the entry of the weevils, two fragments of the central rib of the banana leaf with a length of 20 cm were placed on the leaf. The trap was closed by placing two 20-30 cm long banana leaf midrib fragments to create a separation between the attractant leaf and the pseudostem (Figure 2C). Finally, the trap was covered with dry banana leaves as shown in Figure 2D. Seven days after installation, the traps were reprocessed because the pseudostem, along with the attractant and other parts of the plant that made up the trap, were undergoing a process of decomposition that would render them useless.

C. sordidus and M. hemipterus species were collected and counted every 3 days for 28 days. The collected specimens were placed in plastic containers with the remains of the harvested banana pod and covered with plastic gauze.

Fig. 2. A: Longitudinal section of the pseudostems, **B:** placement of the base leaf and central support vein, **C:** placement of the attractant, **D:** placement of the pseudostem covering the attractant.

Source: self made.

Bioassay Preparation

A selection of 25 live adult specimens of ***M. hemipterus*** and ***C. sordidus*** was made from the collected specimens. These specimens were immersed in distilled water for 30 seconds in plastic containers in groups of 5 specimens.

To prolong the life of the captured specimens, freshly harvested banana corms were cut into uniform slices of 300 grams each. To prevent contamination, the corms were disinfected in a 1% sodium hypochlorite solution and then dried, thus preserving the integrity of the plant material, which is essential for the validity of the assay.

In the laboratory, five treatments were performed by inoculating the samples in a 3L solution of distilled water and the control by immersion for 30 seconds. The treatments were: one dose of 1.5 g of ***Beauveria bassiana*** (D1Bb), two doses of 3 g of ***Beauveria bassiana*** (D2Bb) and the control treatment without ***Beauveria bassiana***. The ***Metarhizium anisopliae*** treatments were: dose four of 1.5 g ***Metarhizium, anisopliae*** (D3Ma), dose five of 3 g ***Metarhizium anisopliae*** (D4Ma) and the control without ***Metarhizium anisopliae***. ***Beauveria bassiana*** strains at a concentration of 2x109 CFU (colony forming units) and ***Metarhizium anisopliae*** at a concentration of 5x1010 CFU.

Mortality assessment

Five inoculated samples of each treatment and the control were placed in plastic boxes. The effect of ***B. bassiana*** and ***M. anisopliae*** as biological control agents on ***M. hemipterus*** and ***C. sordidus*** was evaluated by counting the live specimens after 4 observations at intervals of: 24 hours (F1), 72 hours (F2), 168 hours (F3), and 240 hours (F4). The second step to evaluate the mortality was to calculate the percentage of mortality, which was calculated using Abbott's equation (1) of 1925, proposed by Solarte Quintero et al. (2010).

$$Percentage\ of\ Corrected\ Deaths = \frac{\%\ of\ deaths\ on\ treatment - \%\ dead\ in\ control}{100 - \%\ dead\ in\ control}$$

Statistical Design

SPSS software was used for descriptive and inferential statistical analysis. Normality of data was tested using the Shapiro-Wilk test and Levene's test to examine homogeneity of variance between groups. Data from live specimens that did not follow a normal distribution were transformed using the natural logarithm of the base 10. A one-way analysis of variance was then performed on F2, F3, and F4 with the corrected percentages that followed a distribution.

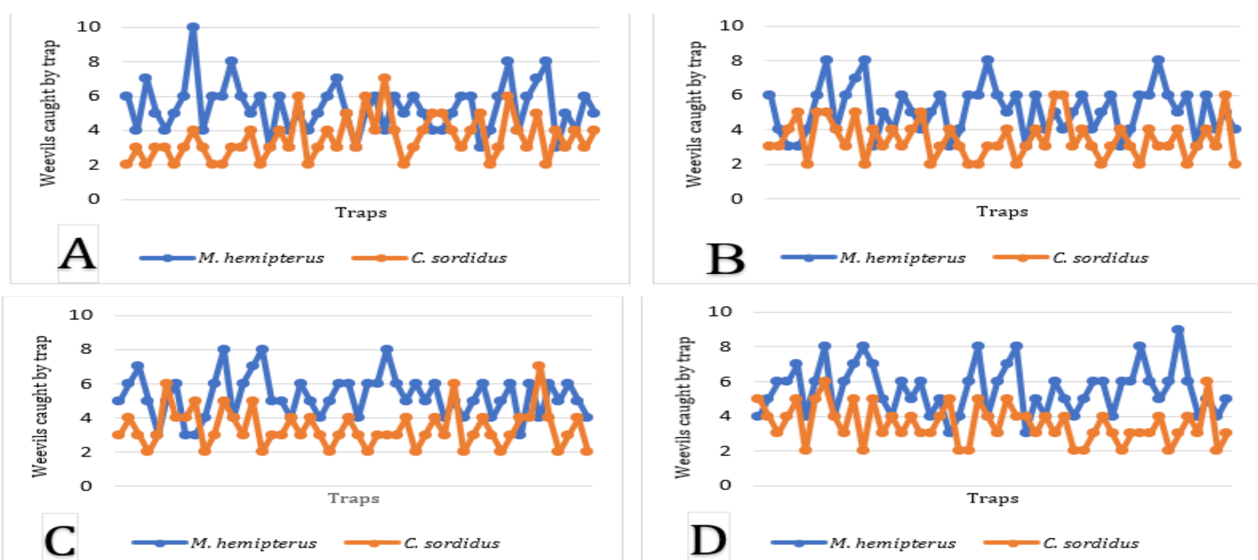
Results-discussion

Records of captures of Metamasius hemipterus and Cosmopolites sordidus

In general, Figure 3 shows that ***M. hemipterus*** has a greater variability in the number of individuals captured per trap, reaching peaks of up to 10 individuals, while ***C. sordidus*** has a more stable trend with generally lower numbers of captures, reaching a maximum of 6 individuals. Previous research has shown that the variability in the capture of billbills can be very different depending on the species and the environmental conditions. For example, studies in different regions of Latin America have documented that ***M. hemipterus*** tends to show highly variable capture patterns like those observed in this sampling, with high peaks at certain times of the year due to factors such as food availability and shelter.

C. sordidus, on the other hand, could adapt to a wider range of environmental conditions and has a more homogeneous dispersal strategy, which has been consistently reported in several studies. Research in banana plantations in Africa and Southeast Asia has shown similar results, with ***C. sordidus*** showing stable and less variable catches regardless of seasonal or management variations (Tinzaara et al 2005).

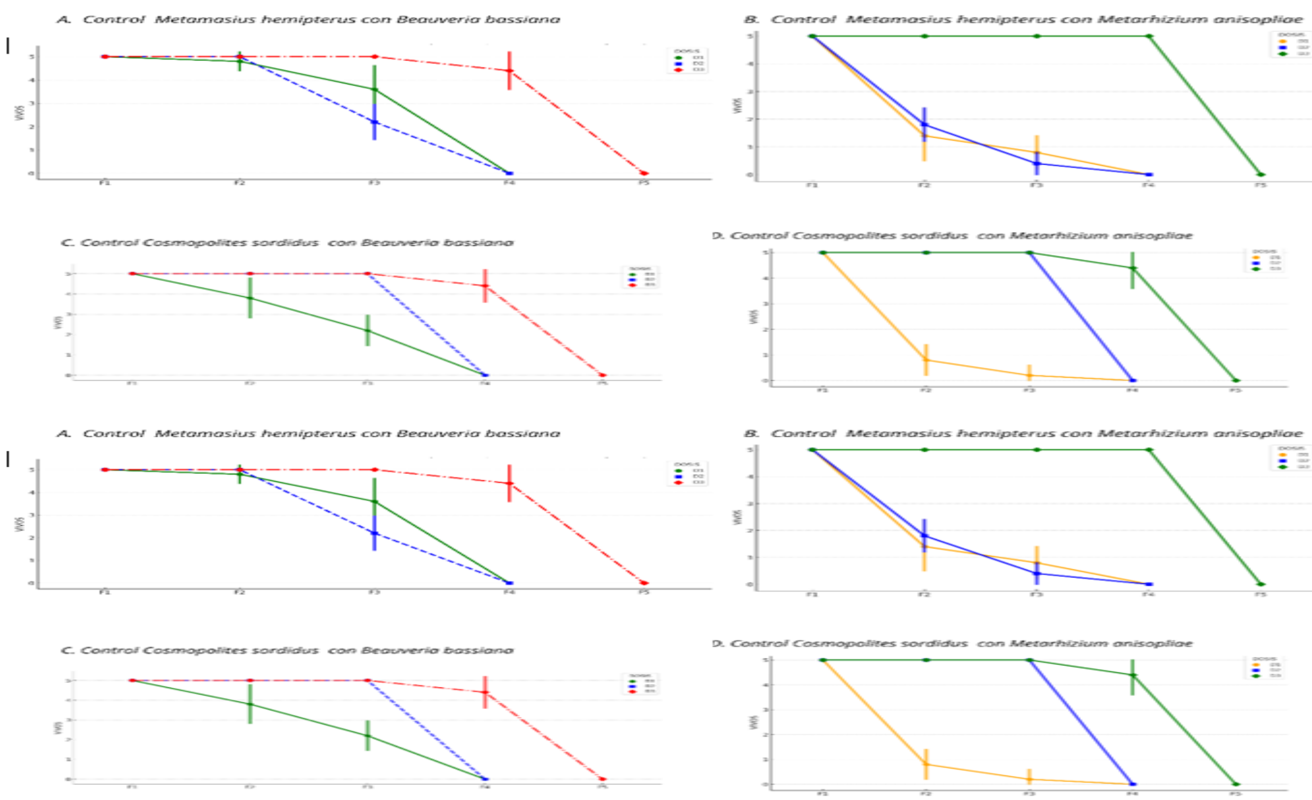
Fig. 3. Catch record of ***M. hemipterus*** and ***C. sordidus*** in the different sampling dates (FM) where **A:** Sampling date one (FM1); **B:** Sampling date two (FM2); **C:** Sampling date three (FM3); **D:** Sampling date four (FM4).



Source: self made.

Figure 4 shows the survival curves of ***M. hemipterus*** and ***C. sordidus*** at different doses inoculated with ***Metarhizium anisopliae*** and ***Beauveria bassiana***. The highest number of live specimens was recorded in the experiment without control agents. On the other hand, the lowest dose of ***B. bassiana*** (D1Bb) in the control of ***M. hemipterus*** showed a gradual decrease in the number of live insects from F2 to F4, where zero live specimens were reached. At the highest doses of B. bassiana (D2Bb), the lethality effect started at 168 h (F3) and reached 0 live insects at F4. In the treatments with the lowest dose of ***M. anisopliae*** (D3Ma) for both ***M. hemipterus*** and ***C. sordidus***, mortality records started at 72 h and reached 0 live insects at 240 h (F4) for both species.

Fig. 4. Survival curves of *M. hemipterus* and *C. sordidus* at different doses inoculated with *Metarhizium anisopliae* and *Beauveria bassiana.*



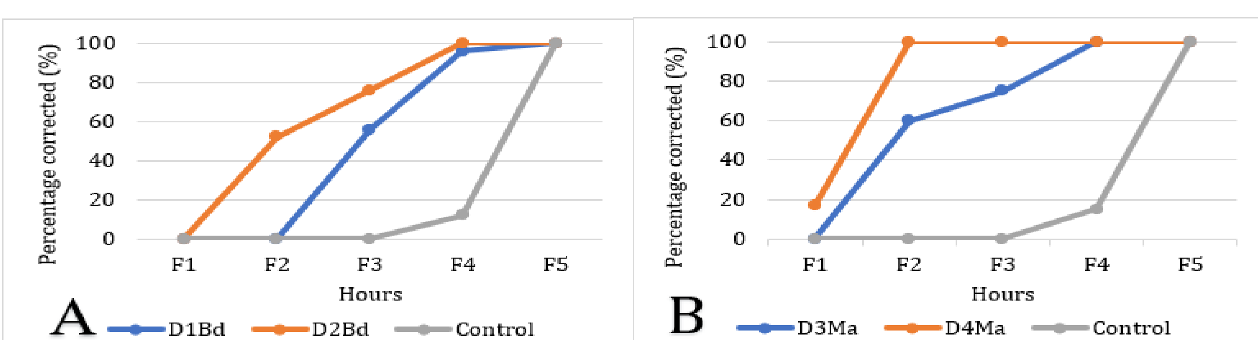
Source: self made.

Mortality curve for Metamasius hemipterus.

Figure 5 shows the mortality curves adjusted with the Abbott equation (1925) for *M. hemipterus* when different insects were inoculated with *B. bassiana* and *M. anisopliae*. The D2Bb dose proved to be the most effective treatment, with onset of action at 24 hours (F1) and a steady increase in mortality, reaching 100% at F4 (Figure 5A). While the D1Bb treatment started its control action at F2 and reached its maximum at F4 (100%).

Figure 6B shows the control of *M. hemipterus* with *M. anisopliae*. The D4Ma treatment proved to be the most effective treatment, with a rapid onset of action in F1, reaching 100% total control in F2, while the D3Ma treatment with a lower dose of *M. anisopliae* showed a gradual increase in mortality, starting in F1 and reaching its maximum in F4. In the control bioassay, where no controls were applied, mortality started in F3 and reached 100% mortality in F5.

Fig. 5. Corrected mortality curve of *Metamasius hemipterus* control. **A:** Effect of *Beauveria bassiana.* **B:** Effect of *Metarhizium anisopliae.*



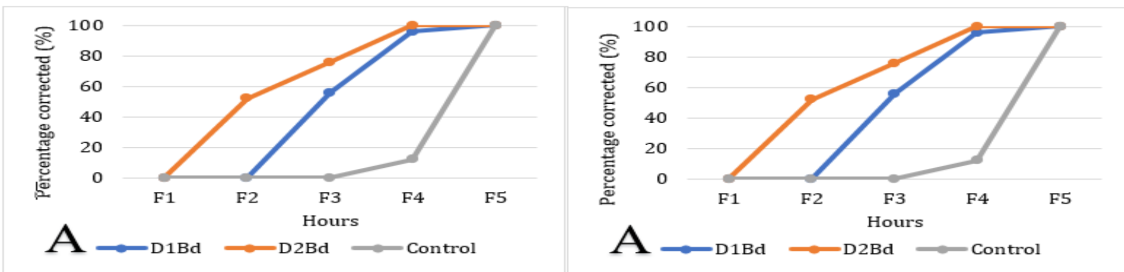
Source: self made.

Mortality Curve for Cosmopolites sordidus

Figure 6 shows the efficacy of ***Beauveria bassiana*** as a biological control agent against ***Cosmopolites sordidus.*** The D2Bb treatment showed a rapid mortality effect from F1 to reach 100% in F4, different from the D1Bb treatment where there was a constant increase in mortality, starting in F1 with 20%, increasing in F2 to 60% and reaching 100% in F5.

Figure 5B shows the corrected percentage of mortality for the two treatments with *Metarhizium anisopliae* to control *Cosmopolite sordidus.* D4Ma showed a steady increase in mortality, starting at about 60% in F1 and reaching its maximum of 100% in F3. D3Ma showed a more gradual onset, starting with 20% mortality in F1, reaching a maximum of 90% in F4 and 100% in F5. While the control reached 100% control in F5. The data suggest that all treatments evaluated with ***M. anisopliae*** are effective for the control of ***C. sordidus***, reaching similar levels of efficacy (approximately 70-90%) in F3. In previous studies, both ***Beauveria bassiana*** and ***Metarhizium anisopliae*** have been shown to be effective for the control of various curculioid species, supporting the results observed in this study (Shapiro-Ilan et al., 2012; Negrete et al., 2018).

Fig. 6. Corrected mortality curve of *Cosmopolites sordidus* control. **A:** Effect of *Beauveria bassiana.* **B:** Effect of *Metarhizium anisopliae.*



Source: self made.

Comparison of efficacy between treatments

The results showed that the control *M. anisopliae* had a significant effect at the D2Ma dose on both days, with means of 68.0% and 56.0%, respectively, placing it in group AB according to Tukey's test at 5% significance. On the other hand, the D1Ma and D3Ma doses showed no relevant effects, remaining in group A with means of 0.0 in D3 and 0.0 to 8.0% in D1Ma (Table 1). On the other hand, the B. bassiana agent showed a significant effect mainly at the D1Bb dose on day F3, with a mean of 56.0 in group AB, while the D2Bb and D3Bb doses showed no noticeable effects, both with means of 0.0. These results suggest that the efficacy of the control agents varies considerably with dose and time of application, with ***M. anisopliae*** being more effective at higher doses over a short period of time, and ***B. bassiana*** showing efficacy at a specific dose but with variability over time. The lack of effect at D3 doses for both agents suggests the need for dose adjustment to optimize control of ***M. hemipterus.*** This finding is consistent with recent studies suggesting that the efficacy of B. bassiana can vary considerably with time and dose (Lara et al., 2020).

Table 1. Tukey 5% test for ***M. hemipterus*** at three doses of ***Beauveria bassiana*** and ***Metarhizium anisopliae.***

Specimens/control agent	Date	Dosage	Mean (%)	Tukey
M. hemipterus/ M. anisopliae	F2	D3Ma	0,0	A
	F2	D4Ma	68,0	AB
	F2	Control	0,0	A
	F3	D3Ma	8,0	A
	F3	D4Ma	56,0	AB
	F3	Control	0,0	A
M. hemipterus/ B. bassiana	F2	D1Bd	24,0	AB
	F2	D2Bd	0,0	A
	F2	Control	0,0	A
	F3	D1Bd	56,0	AB
	F3	D2Bd	0,0	A
	F3	Control	0,0	A

Source: self made.

The results in Table 2 show significant differences in the efficacy of the control agents ***M. anisopliae*** and ***B. bassiana*** on C. ***sordidus*** according to the dose applied and the date of evaluation.

For ***M. anisopliae***, on day F2, the D3Ma dose showed a mean of 24.0% and was placed in group AB, indicating a significant effect. The D4Ma doses and the control showed no effect, with a mean of 0.0% in group A. On day F3, the D3Ma dose increased its efficacy, showing a mean of 56.0% in the AB group, while D4Ma and the control again showed no effect, with a mean of 0.0%.

In the case of ***B. bassiana***, on day F2, the D2Bd dose showed a mean of 68.0% and was placed in the AB group, while D1Bd and the control had no effect, both with means of 0.0% in the A group. On day F3, the D2Bd dose maintained its efficacy with a mean of 56.0% in the AB group, and the D1Bd dose showed a mild effect with a mean of 8.0% in the A group, while the control remained without effect with a mean of 0.0%.

These results are consistent with recent studies suggesting that the efficacy of biological agents such as ***M. anisopliae*** and ***B. bassiana*** depends on the dose applied and the environmental conditions (Pérez et al., 2016). The variability in efficacy observed at D3Ma and D2Bd doses may be due to factors such as inoculum density and persistence of the biological agent in the environment (Lara et al., 2020).

Table 2. Tukey 5% test for C sordidus at three doses of *Beauveria bassiana* and *Metarhizium anisopliae.*

Specimens/control agent	Date	Dosage	Mean (%)	Tukey
	F2	D3Ma	24,0	AB
	F2	D4Ma	0,0	A
	F2	Control	0,0	A
C. sordidus/ M. anisopliae	F3	D3Ma	56,0	AB
	F3	D4Ma	0,0	A
	F3	Control	0,0	A
	F2	D1Bd	0,0	A
	F2	D2Bd	68,0	AB
	F2	Control	0,0	A
C. sordidus/ B. bassiana	F3	D1Bd	8,0	A
	F3	D2Bd	56,0	AB
	F3	Control	0,0	A

Source: self made.

Conclusion

This research has demonstrated the high efficacy of both Beauveria bassiana and Metarhizium anisopliae as biological control agents for both C sordidus and M hemipterus under laboratory conditions. Although C sordidus showed faster mortality than M hemipterus with both fungi and all doses, M anisopliae is more effective at high doses for both species, while B bassiana shows moderate and gradual efficacy, which is crucial to optimize field biological control.

Future research should focus on validating field efficacy, determining optimal doses under different environmental conditions, studying interactions with other control agents, and evaluating long-term effects on banana ecosystems.

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