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In Vitro and Field Evaluation of Three Bio Controllers of the Black Banana Weevil (Cosmopolites Sordidus)

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Abstract **Original Research Article**

The black banana weevil is the most prevalent and destructive pest of Musaceae in all pantropical regions. At present, there is no other method more effective than pesticide applications for its total control, which has caused contamination of water sources and human health, so biological control techniques have been implemented, This research evaluated the performance of *Heterorhabditis bacteriophora*, *Beauveria bassiana* and *Metarhizium anisopliae*, a mixture of *Beauveria bassiana* and *Metarhizium anisopliae* for the management of this pest in the biopesticide laboratory and at the El Plantel farm of the National Agrarian University (UNA). In relation to the control of the pest, the bioformulation that presented the best results was the base mixture of *Beauveria bassiana* and *Metarhizium anisopliae* with 91. 25% effectiveness followed by the formulation of *Beauveria bassiana* with 87. 52% and *Heterorhabditis bacteriophora* with 2. 45% effectiveness in the field, no significant differences were found in the three laboratory controls, a significant difference was found between the fungi and the nematode in the field.

Keywords: Cosmopolites sordidus, biological control, *Beauveria bassiana*, *Metarhizium anisopliae*, *Heterorhabditis bacteriophora*.

INTRODUCTION

The banana is an item of great economic importance because of the income generated by its exports worldwide. It is produced all over the world and is a staple food for countless people on the planet (Frison and Sharrock, 1999). In terms of productivity, it represents one of the first export fruits and in economic terms it is second only to citrus, according to the Food and Agriculture Organization of the United Nations (FAO, 2017).

Black boll weevil is the pest with the highest aggressiveness index in Musaceae plantations on the planet. It causes great damage to the pseudo stem of the plant, reducing production when control strategies are not implemented (Castillo Arévalo and Jiménez-Martínez, 2018). In the department of Rivas, reductions of up to 65% in bunch weight have been reported in plantations where it is not controlled (Castillo-Arévalo, and Martínez Machado, 2022).

The most effective curative method to control the pest is with systemic pesticides, which causes environmental contamination in established plots (Astorga, 1999; Vindas *et al.*, 2004).

At present, the best technique to control the disease is by means of the control method, with up to 40 sanitation sprays in the first cycle, which means three quarters of the production price, causing resistance genes of the pathogen to chemical products (Sagder, 1995). These pesticides are manufactured to be applied every seven, fourteen and twenty-one days to break the insect cycle, which means up to 50 applications in the mother generation (Chica *et al.*, 2004).

Environmental contamination is the result of the indiscriminate use of agricultural pesticides; their traces in the water table, topsoil and condensation cause the death of many animals, as well as the cause of chronic diseases in humans, and despite all this damage, the pest cannot be controlled satisfactorily (Michel, 2001).

On the other hand, the misuse of insecticidal inputs has provoked resistance in the pest, causing an increase in the disease over time, developing the insect's

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growth, decreasing production rates, and increasing harvest costs (Marín *et al.*, 2003).

The U.S. Food and Drug Administration (FDA, 2013) reported two rejections of fresh plantain from Rivas for pesticide residues, destined for Costa Rica. Similarly, in 2019, it again reported the rejection of bananas with residues that were destined for Costa Rica.

All this, plus the demand for environmentally safe control measures, make it evident the need to investigate and implement sustainable management strategies such as biological control. Currently, microorganisms have been reported that exert growth and pest control at laboratory and greenhouse scale (Nadal-Medina *et al.*, 2009, (Gold and Messiaen, 2000). The black banana weevil Cosmopolites sordidus. Information sheet, 4, 1-4.) For this reason, this field research was carried out.

MATERIAL AND METHODS

Study number 1:

It was carried out at the biopesticide laboratory of the National Agrarian University, located at km 11.5 Pan-American Highway, Managua, Nicaragua.

Five treatments and four replicates were evaluated, *Heterorhabditis bacteriophora*, *Beauveria bassiana* and *Metarhizium anisopliae*, a mixture of *Beauveria bassiana* and *Metarhizium anisopliae*, and the control without any type of application. Each experimental unit consisted of a 90 x 14 mm plastic petri dish with 10 adult individuals of C. sordidus. In each one was placed filter paper that was inoculated with 1 ml of a suspension with 200 JI.ml^{-1} (40 JI. larva^{-1}) of *Heterorhabditis bacteriophora*. And in the case of Hypocreales fungal treatments the experimental unit was arranged by a petri dish with antagonistic fungi inoculated with 200 µl of a spore solution (5 000 to 7 500 spores/µl), which was distributed throughout the plate with a Drigalski spatula.

The fungi under study were strains from the National Agrarian University (UNA); in the case of *Heterorhabditis bacteriophora*, it was isolated in the municipality of Cardenas, Rivas. It was reproduced according to the insect trap method (De Doucet, 1998); Cepeda-Siller *et al.*, 2018).

For the laboratory study, a completely randomized experimental design was used, where only one single factor was studied, the mortality of banana weevils.

Table 1: Laboratory treatment

Active Principle	Microorganism	Dosage	Treatment number
Heterorhabditis bacteriophora	Biological	5X10^{7}	T1
Beauveria bassiana	Biological	1X10^{12}	T2
Metarhizium anisopliae	Biological	1X10^{12}	T3
Beauveria bassiana y Metarhizium anisopliae	Biological	1X10^{12}	T4
No Application			T0

Statistical Analysis of the Data

After the data were collected, they were arranged by variables in a data table in Excel, then each variable was compared between treatments, Shapiro-Wilk and Levyn normality assumptions test was performed to measure constant variances, performing an analysis of variance, using the InfoStat program (2020). The significance level used in the analysis was (p = 0.05).

Study number 2:

It was carried out at the farm El Plantel (Universidad Nacional Agrarians), located at km 30 Masaya -Tipitapa highway, Nicaragua.

Five treatments were evaluated in the study: Heterorhabditis bacteriophora, Beauveria bassiana and Metarhizium anisopliae, a mixture of Beauveria bassiana and Metarhizium anisopliae, and the control without any application.

Sample selection

The sample was selected using the finite population sample calculation formula used by (Castillo-Arévalo, 2022), which is used when the universe or total number of units under study is known.

$$n = \frac{n\,N \ast z2\;p \ast q}{(N-1)\,e2 + z2 \ast p \ast q}$$

Meaning of the letters:

n = Sample size.
N = Universe population.
Z = 95% confidence level
P = Proportional estimate of the population
Q = (1-P)
E = Standard error of the sample, 5% (0.05)

Experimental Design

An experimental design with a completely randomized block distribution, was used, with 4 replications of each treatment, each block had 5 experimental units, for an area of 783.2 m² per block.

The experimental unit was 71.2 m long and 65.33 m wide.

The distance between each experimental unit was 10.3 m, the distance between blocks was 16.16 m and the experimental area was 6.4 m wide and 9.13 m long at the perimeter, established in a commercial area.

Methodology of Application of the Treatments in the Field

Five sandwich type disc traps with pieces of rhizome were installed 20 cm from the base of the plant stem, where 200 ml of the bioformulated mixture and the *nematode H. bacteriophora* were applied per trap, every seven days for four weeks, each experimental unit was composed of three traps. The variables evaluated were Mortality of weevils and treatment efficacy index.

Table 2: Field treatments

Commercial Product	Active Principle	Microorganism	Dosage/ Ha	Treatment
Strains UNA	*Heterorhabditis bacteriophora*	Biological	300 ml ($5X10^7$)	T1
Strains UNA	*Beauveria bassiana*	Biological	250 g ($1X10^{12}$)	T2
Strains UNA	*Metarhizium anisopliae*	Biological	250 g ($1X10^{12}$)	T3
Strains UNA	*B. bassiana + M. anisopliae*	Biological	250 g ($1X10^{12}$)	T4
Absolute control of the experiment	No Application			T0

Abbott Efficacy Index

The number of pest insects per trap was calculated, with this data the efficacy index of the treatments in reducing the densities of the final pest insect populations was calculated using the Abbott formula modified by (Fernandez-Santillan et al., 2016).

$$eficacia = \left[1 - \left(\frac{Ni}{NMax}\right)\right] x100$$

Meaning of the letters:

Ni = is the number of pest insects per plant at the end of the trial.

N max = is the maximum number of pest insects per plant obtained among all treatments and replicates at the end of the experiment.

Statistical Analysis of the Data

After collecting the data, they were arranged by variables in a data table in Excel, then each variable was compared between treatments, Shapiro-Wilks and Levyn's normality assumptions test was performed to measure constant variances, performing an analysis of variance, using the InfoStat program (2020). The level of significance in the analysis was (p = 0.05).

RESULTS

Table 3: Evaluation of the effect of bio controllers on the black palm weevil in vitro

	% Mortality
Treatment	Average ± ES
Heterorhabditis bacteriophora	96.25±1.87 a
Beauveria bassiana	97.31±1.87 a
Metarhizium anisopliae	94.95±1.87 a
Beauveria bassiana + Metarhizium anisopliae	98.25±1.87 a
Absolute control of the experiment	1.37±1.87 b
C.V.	13.59
p-valor	0.0001
F; df; n	19.42; 19; 20

ES=Standard error; SD=Significant Difference; C.V.=Coefficient of Variation; p=Probability; F=Fisher calculated; df=Degrees of freedom of the error; n=Number of data used in the analysis. *Means with different letters: significant differences exist.

Comparison of the Average Mortality of the Black Weevil of Musaceae in Vitro

In the analysis of variance, a significant difference was found (p=≤0.05). In general, it was observed that the *Beauveria bassiana* + *Metarhizium anisopliae* treatment had better control over the insect with (98.25%), followed by the *Beauveria bassiana* treatment (97. 31 %); followed by *Heterorhabditis bacteriophora* (96. 25%).

Parasitized black weevil adults decreased their movement; while, once dead, they showed no change in coloration or flaccidity and remained rigid and with their legs folded.

When the adults killed by *H. bacteriophora* were analyzed, it was found that eggs and other stages of the nematode (J1, J2, J3, JI J4, females and males) were found in the abdomen and head. The nematodes were alive and with good mobility. This is evidence of several generations within the insect. Weevil mortality values differed statistically between the control (no nematode application) and the treated weevils, with a mortality ratio higher than 90% (R^2= 0.1662) in the in vitro study Figures 1 and 2. In the case of the weevils parasitized by *Beauveria bassiana*, they were dead without mycelia at day five of the inoculation without showing mycelia to the naked eye, which were shown from day 9 onwards as well as the mixture of *Beauveria bassiana* + *Metarhizium*, in the case of *Metarhizium* *anisopliae* the death of the insect began to be observed from day seven, and the presence of conidia from day 12 onwards. In all cases, the weevils remained rigid and with their legs folded.

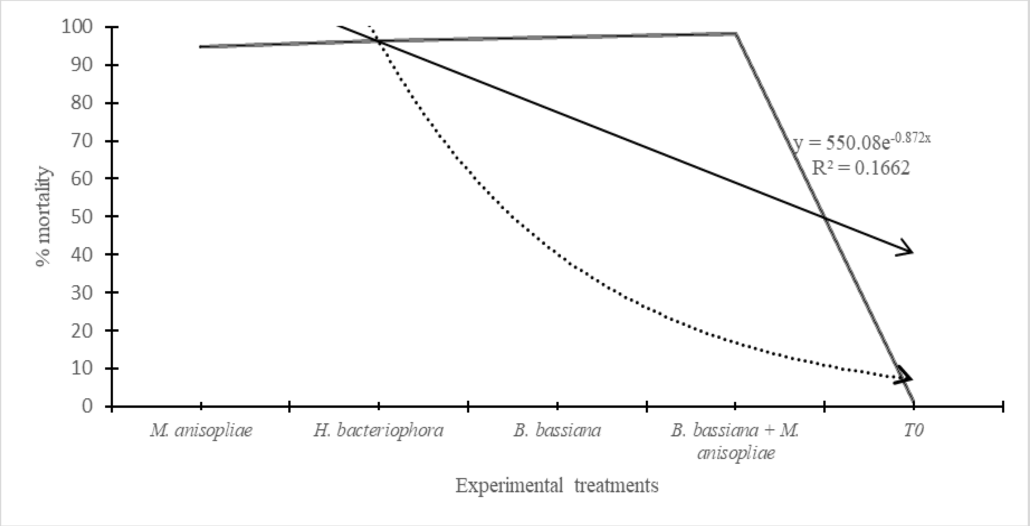

Figure 1: % mortality of three bio-controllers of the black Musaceae weevil (*Cosmopolites sordidus*).

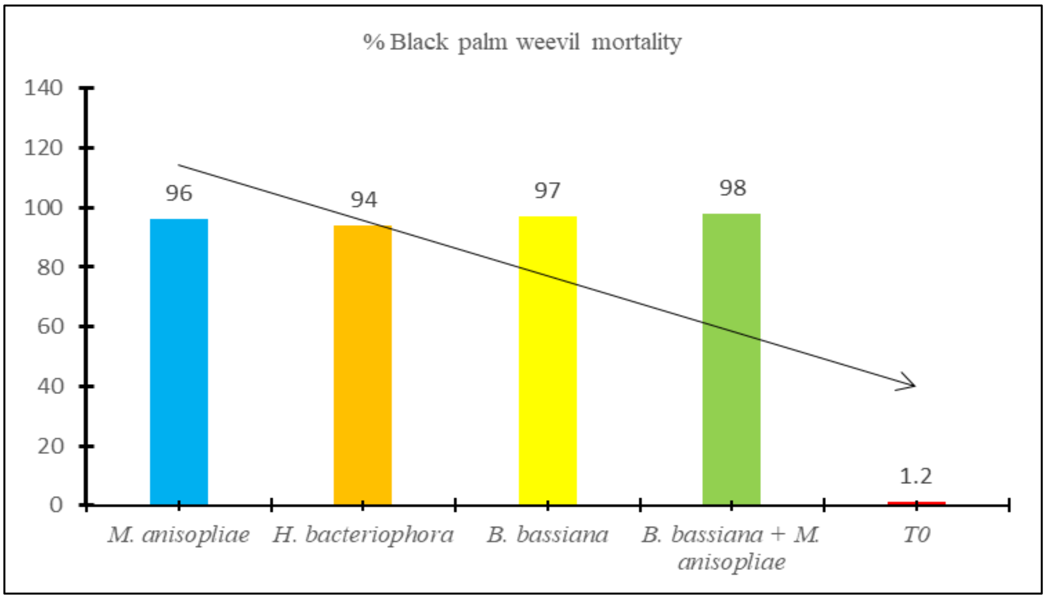

Figure 2: Percentages of adult mortality of *C. sordidus* caused by different doses of bio controller strains UNA per ml applied in vitro

Table 4: Percentages of adult mortality of *C. sordidus* caused by different doses of bio controller strains UNA per ml applied in vitro

	% mortality	**% effectiveness (Abbott)**
Experimental treatments	Average ± ES	Average ± ES
Heterorhabditis bacteriophora	2..45±2.56 b	1.45±2.57 b
Beauveria bassiana	87.52±2.56 a	87.93±2.57 a b
Metarhizium anisopliae	74.95±1.87 a	74.09±.2.57 ab
Beauveria bassiana + Metarhizium anisopliae	91.25±1.87 a	91.26±2.2.57 a
Testigo	1.37±1.87 c	1.30±2.57 c
C.V.	13.59	15.82
p-valor	0.0001	0.0001
F; df; n	19.42; 19; 20	16.60; 19; 20

ES=Standard error; SD=Significant Difference; C.V.=Coefficient of Variation; p=Probability; F=Fisher calculated; df=Degrees of freedom of the error; n=Number of data used in the analysis. *Means with different letters: significant differences exist.

Comparison of the Average Mortality of the Black Weevil of Musaceae in the Field

In the analysis of variance, a significant difference was found ($p=\leq 0.05$). In general, it is observed that the *Beauveria bassiana* + *Metarhizium anisopliae* treatment had better control over the insect with (91.25 %), followed by the *Beauveria bassiana* treatment (87. 52 %); followed by *Metarhizium anisopliae* (74. 95 %), *Heterorhabditis bacteriophora* (2. 45%). As shown in Figure 3.

The effectiveness of the treatments with the entomopathogenic fungus *Beauveria bassiana* was observed from the first application, the weevils were found dead, rigid and with the presence of mycelia colonizing the entire integument of the weevil, in the same way as the treatments *Metarhizium anisopliae*, This behavior was observed during the entire time the research was carried out, the treatment of the bioformulated *Beauveria bassiana* + *Metarhizium anisopliae*, the growth of conidiophores was observed to be more aggressive than the previous treatments. In the case of the *Heterorhabditis bacteriophora* treatments, it was not as effective in the field as in the laboratory study. The few weevils found dead in this treatment were found dead nematodes at an angle of 90 degrees. This is evidence that this bio controller is not viable in this area of the country. Weevil mortality values differed statistically between the control (no nematode application) and the treated weevils, with a mortality ratio higher than 70 % (R^2= 0.1754) Figures 3 and 4. In the case of the weevils parasitized by *Beauveria bassiana*, they were dead without mycelia at day seven of the application, in the same way as the mixture of *Beauveria bassiana* + *Metarhizium*, and in the same way as *Metarhizium anisopliae*. In all cases death weevils remained stiff and with shrunken legs.

The results of the analysis of variance applied to the mortality and effectiveness of the treatments indicate that the bioformulated *Beauveria bassiana* + *Metarhizium anisopliae* and only *Beauveria bassiana* are superior to the other experiments with 87% efficacy in open field Figure 5.

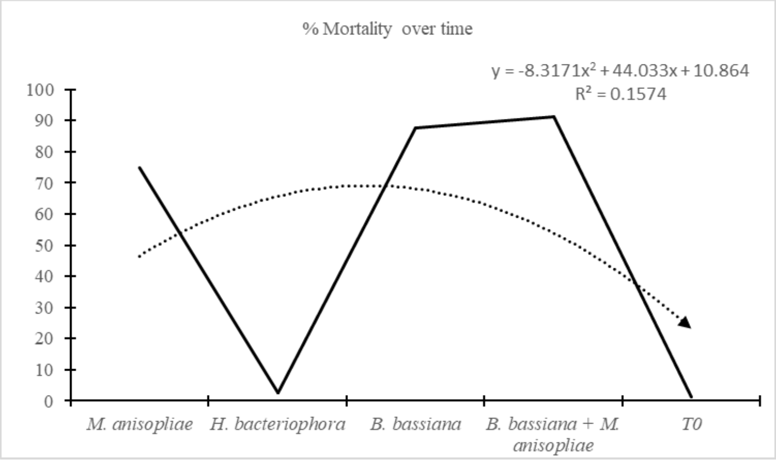

Figure 3: Mortality percentages of *C. sordidus* adults originating from different UNA bio controller strains applied in sandwich disk traps in the field

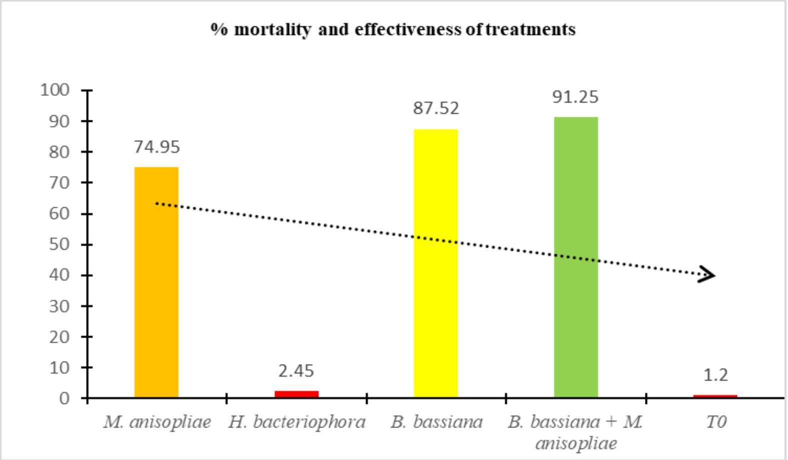

Figure 4: Mortality percentages of *C. sordidus* adults in days of exposure to treatments caused by bio controller strains UNA applied in sandwich disk traps

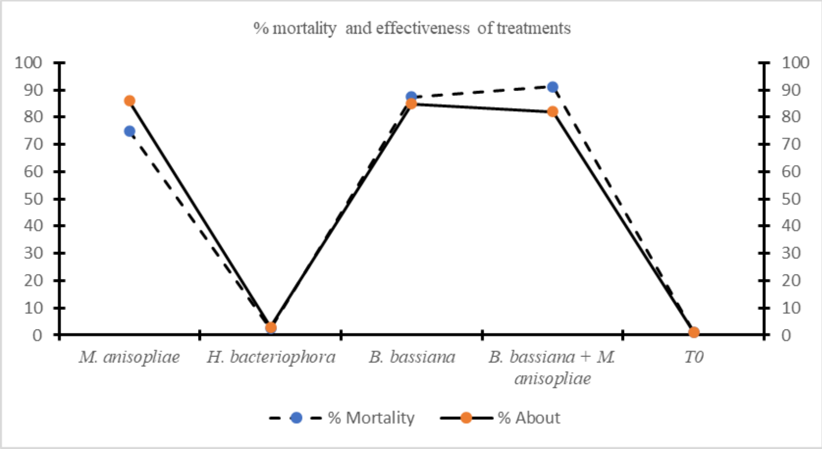

Figure 5: Mortality and effectiveness of open field treatments using UNA bio controller strains applied in sandwich disk traps

DISCUSSION

Studies by Amador *et al.*, 2015 found that *Heterorhabditis amazonenesis* caused high percentages of mortality in weevil adults under laboratory conditions in the same way as in this research, in the same way (Rosales and Suárez, 1998). The mortality caused is like those found in this research. In the same way (Treverrow & Bedding, 1993).

They obtained a maximum mortality of 70% of weevil adults, after 14 days of being exposed to the entomopathogenic nematodes, then the entry of the nematodes does not occur until several days after contact between the two organisms and only 1 to 3 nematodes penetrate. Occurring in the same way in this study from day 15, besides being effective entomopathogenic nematodes in parasitizing banana weevil adults in laboratory conditions, several strains were also effective in field conditions, which was confirmed by (Waturu *et al.*, 1998), being different from the result of this research (Kaaya and Hassan, 2000) and (Caballero, 2014). They indicate that *B. bassiana* infects more than 200 species of insects of different orders, confirming in this research that C. sordidus is one of these species, while *M. anisopliae*, with a broader toxicity spectrum, has been observed in 300 to 400 taxa, disagreeing in this research because the highest percentages of mortality were caused by *B. bassiana*. Furthermore, in studies with *B. bassiana* and *M. anisopliae* (Gandarilla *et al.*, 2013).

The strains analyzed were considered as promising for the development of technology for biological control, agreeing with the results of this study.

CONCLUSION

It is demonstrated that the nematode Heterorhabditis bacteriophora is capable of biological control under controlled conditions, but it is not effective in field conditions, further studies should be conducted to find methods of inoculation in open field and exploit its potential as a biological controller.

It was found that the use of biological organisms to control this pest is as effective as pesticides and safer for the environment and public health.

It is demonstrated that biological control of the pest shows an effectiveness of 0.16 % (day^{-1}), in the department of Masaya, Nicaragua.

Of the biological treatments, it is shown that the bioformulated Beauveria bassiana + Metarhizium anisopliae is the best management option to control the insect pest, followed by the entomopathogenic fungus Beauveria bassiana.

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