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RESEARCH ARTICLE

Determination of LD50 and LT50 at Different Doses of *Beauveria bassiana* on *Cosmopolites sordidus*

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ABSTRACT

Banana is a crop of global importance, both for its economic value and its role in food security. It is threatened by pests such as the banana weevil, which causes damage and can lead to considerable economic losses. Given the need to control this pest in a sustainable manner, the use of biopesticides, such as *Beauveria bassiana*, has been explored as an alternative to chemical pesticides. In this study, the pathogenicity of five isolates of this fungus was compared. Through the calculation of mean lethal doses (LD50) and mean lethal times (LT50), the efficacy of each isolate in pest insect mortality was determined. Statistical analysis was initiated from the mortality records obtained at 96 hours. The data were organized in an Excel table and subjected to normality and homogeneity of variance tests. Subsequently, an analysis of variance (ANOVA) was performed with InfoStat to compare treatments, and the differences between means were established by Tukey's Test ($p \leq 0.05$) the isolate that presented the highest virulence was BBT35 at 144 hours, followed by BBT31. The isolates evaluated showed different levels of effectiveness in controlling Cosmopolites sordidus. Some isolates were more effective at lower concentrations, while others required higher concentrations to achieve similar control. These results contribute to a better understanding of the interaction between the fungus and the fungus.

Keywords: Banana, bioassays, biological control, entomopathogens.

1. Introduction

The banana is a product of great economic importance due to the income generated by its exports around the world. It is produced all over the world and is a staple food for countless people on the planet [1], [2]. In terms of productivity, it represents in terms of productivity, it is one of the leading export fruits and, from an economic point of view, second only to citrus fruits, according to the Food and Agriculture Organization of the United Nations [3], [4].

Cosmopolites sordidus, a coleopteran that attacks bananas, causes damage to the corm that weakens the plant and can lead to its fall [5], [6]. Traditional chemical methods to control this pest are expensive and have generated problems of resistance and environmental contamination. In response to these limitations, the potential of entomopathogenic fungi, especially *Beauveria bassiana* and *Metarhizium anisopliae*, as biological control agents have been explored. These fungi have been shown to be effective against a wide range of agricultural pests and offer a safer and more economical alternative for integrated pest management in banana crops [7]–[9].

The purpose of this research was to evaluate the pathogenicity of five native isolates of *Beauveria bassiana* on the banana weevil. For this purpose, the mean lethal concentrations (LC50) and mean lethal times (LT50) of the five isolates were calculated and contrasted.

2. Materials and Methods

The research was carried out at the biopesticide laboratory of the Universidad Nacional Agraria, located at km 12½ of the North Highway, Managua, Nicaragua at coordinates 12° 08′ 52″ North Latitude and 86° 09′ 41″ West Longitude Fig. 1.

Five isolates of entomopathogenic fungi were selected from a previous study of isolation and characterization of Beauveria Bassiana in the department of Rivas, Nicaragua,

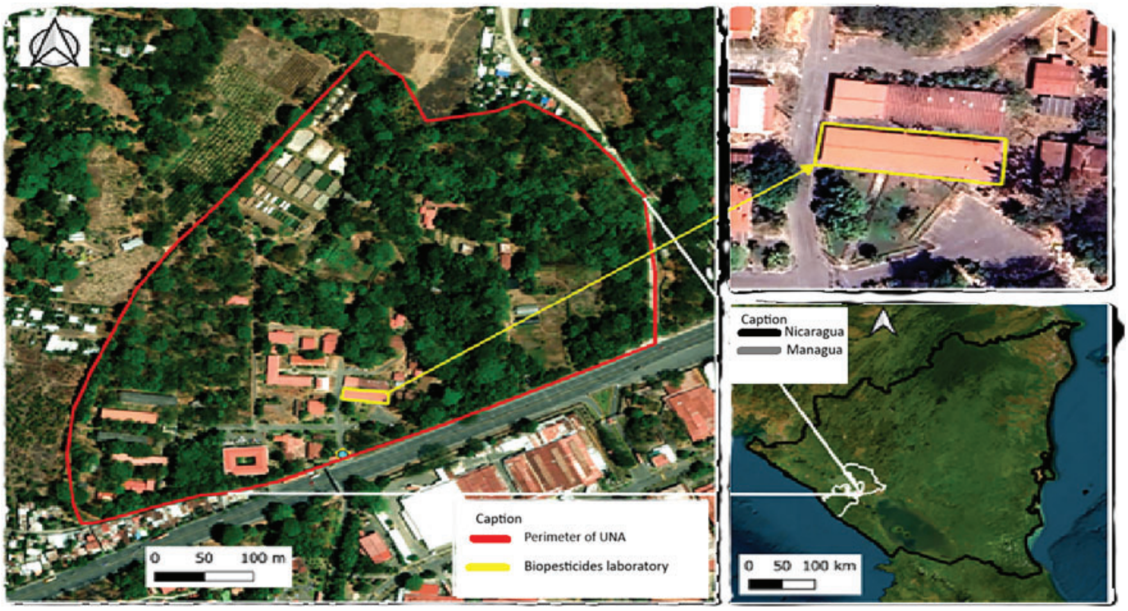

Fig 1. Location of the study.

TABLE I: Experiment Treatments

Isolate	Origin	Concentration	Organism	Conidia/300 g rice
BBT34	UNA	1.00E+06	Biological	1×10^6
		1.00E+08		1×10^8
		1.00E+12		1×10^{12}
BBT41	UNA	1.00E+06	Biological	1×10^6
		1.00E+08		1×10^8
		1.00E+12		1×10^{12}
BBT11	UNA	1.00E+06	Biological	1×10^6
		1.00E+08		1×10^8
		1.00E+12		1×10^{12}
BBT23	UNA	1.00E+06	Biological	1×10^6
		1.00E+08		1×10^8
		1.00E+12		1×10^{12}
BBT23	UNA	1.00E+06	Biological	1×10^6
		1.00E+08		1×10^8
		1.00E+12		1×10^{12}
Absolute control		0.00E+00		0

and evaluated in a dose-response bioassay against adults of Cosmopolites sordidus. The isolates were evaluated at three concentrations 1×10^6, 1×10^8 and 1×10^{12} conidia (Table I).

Five hundred adults of banana weevils were collected from infested plants in the department of Rivas. These were kept in the Biopesticides laboratory of the Universidad Nacional Agraria in plastic containers at room temperature for one week before being used in the experiments, the covers of the containers were perforated for ventilation, banana corms were cut and placed in the containers.

A completely randomized experimental design (CRD) was used, where mean lethal dose (LD50) and mean lethal time (LT50) were evaluated.

2.1. *Inoculation of Weevils*

Five experimental units were prepared, each in a 90 mm × 14 mm plastic Petri dish. In each dish, five weevils were placed on absorbent paper and sprayed with an atomizer, using 1500 μL of a conidia solution at one of the following concentrations: 1×10^6, 1×10^8 or 1×10^{12}. This methodology made it possible to evaluate the response of weevils to different initial loads of conidia [10].

2.2. *Evaluation of Mortality*

To evaluate the pathogenicity of the isolates, five weevils per Petri dish were exposed to the strains and concentrations described above, recording the percentage of mortality (LD50) and determining the time at which 50% of the population died (LT50) for each isolate evaluated [6].

To calculate mortality, the methodology described by Álvarez *et al*. [11] was used, dividing the number of deaths in a period by the average population in that period and multiplying the result by 1000.

Bioassay readings were taken every 12 hours until reaching 144 hours, recording the percentage of mortality and determining the time at which 50% of the population died (TL50) for each isolate and concentration under study, which allowed comparison of their virulence.

2.3. *Abbott Efficacy Index*

To correct the mortality of the control in the treatment, the Abbott formula modified by Castillo-Arévalo [6] was used.

$$\text{Efficacy} = \frac{\%\text{ITr} - \%IT}{\%\text{ITr}} \times 100 \quad (1)$$

where
% ITr—the number of parasitized insects at the end of the trial
ITr—the number of infested insects of the control at the end of the trial.

2.4. *Statistical Analysis*

For the statistical analysis, data was considered from 96 hours, which is when mortality records were presented. After the data were collected, they were arranged by variables in a data table in Excel, then each variable was compared between treatments, the Kolmogorov-Smirnov and Levyn normality assumptions test was performed to measure constant variances, carrying out an analysis of variance, using the InfoStat program (2020). The level of significance in the analysis was ($p = 0.05$).

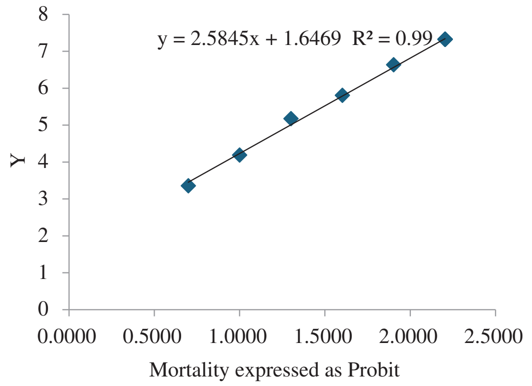

Fig. 2. Mortality expressed in probit.

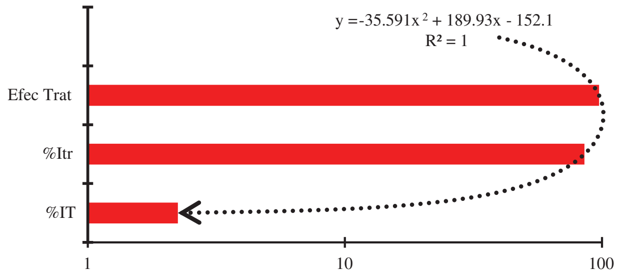

Fig. 3. Effectiveness of treatments with different isolates of *B. bassiana* against black boll weevil (*Cosmopolites sordidus*).

3. Results

In the analysis of variance, a significant difference was found ($p = \leq 0.05$). In general, it was observed that the mean lethal time (TL50) was significantly lower for fungal isolate BBT11 indicating lower virulence, followed by isolate BBT34. The other isolates also presented TL50 values lower than four and five days, obtaining better results than in studies, like those of (3), (6), presenting values close to seven days and those of Álvarez *et al*. [11] that presented results higher than these, the isolate that presented higher virulence was BBT41 at 144 hours.

Fig. 2 shows the relationship between mortality, expressed in probit, and the Y variable. There is a clear positive linear relationship between the two variables, indicating that as mortality increases the Y variable also increases proportionally. 2.5845 is the slope of the line, indicating the change in Y for each unit change in mortality, 1.6469 is the point of intersection with the Y axis (the value of Y when mortality is zero). The coefficient of determination (R^2) is 0.99, indicating that the linear regression model explains 99% of the variability in the Y variable. This high value of R^2 suggests an excellent fit of the model to the data, which reinforces the conclusion of a strong linear relationship between mortality (probit) and the Y variable. Finally, the figure evidences a direct and highly significant linear relationship between mortality expressed in probit and the Y variable, with a regression model that explains almost all the observed variation.

The graph shows that the efficacy of Beauveria bassiana treatments varies according to the isolation used.

The isolates evaluated showed different levels of efficacy in controlling Cosmopolites sordidus.

Some isolates were more effective at lower concentrations, while others required higher concentrations to achieve similar control.

The equation in Fig. 3 shows that the relationship between fungus dose and treatment efficacy follows a curve with an optimum concentration point at which efficacy is maximal.

The fungus Beauveria bassiana shows potential as a biological control agent for Cosmopolites sordidus. Although studies such as those in De la Rosa [12] and Quesada-Moraga and Vey [13], and Castillo-Arévalo [14] have demonstrated the potential of *Beauveria bassiana* for crop pest control, the efficacy of this entomopathogenic fungus may be limited by several factors. Spore viability, environmental conditions and timing of application are some of the elements that may influence the success of biological control with B. bassiana.

The weevils were found dead, rigid and with the presence of mycelia colonizing the entire integument, indicating that the fungus fulfilled its function, infecting and killing the insects, this proves the results of Rosales and Suárez [15], [16] and Castillo-Arévalo [6] (Fig. 4).

Since the first application of the different isolates of Beauveria bassiana showed a high effectiveness in the elimination of weevils after 96 hours, [17], Fig. 4 shows a rapid colonization of their bodies by the fungi. The results obtained indicate that the fungus is a promising tool for weevil management, reaching mortality rates higher than 95% under laboratory conditions [18].

4. Discussion

The present in vitro study aimed to evaluate the virulence of different isolates of the entomopathogenic fungus Beauveria bassiana on the banana weevil, Cosmopolites sordidus, a key pest in the cultivation of musaceae. The

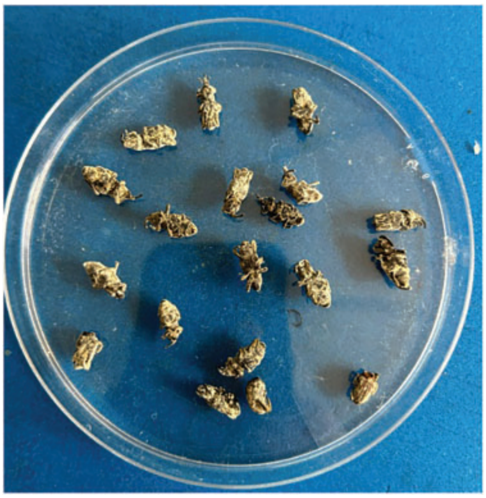

Fig. 4. Cosmopolites sordidus parasitized with different isolates of *B. bassiana*.

results obtained (Table II) demonstrate significant variability in the pathogenic capacity of the isolates tested, which underlines the potential of B. bassiana as a biological control agent for C. sordidus, but also the importance of selecting highly virulent strains for development into biopesticides.

Isolates BBT35 and BBT31 showed the highest efficacy, inducing mortalities of 98.67% and 97.60%, respectively. These figures are remarkably high and suggest that these isolates possess superior virulence characteristics under the test conditions. The absence of significant differences between BBT35 and BBT31 (grouped with the letter 'A' according to Tukey's test) indicates comparable efficacy, positioning them as promising candidates for future research and development. Importantly, despite the high mortalities, the EE (Standard Error) and TL50 (Time to Lethal Mean) values were consistent for all isolates and the control, which could indicate similar dynamics once established, or alternatively, the need to adjust the methodology for calculating these metrics to detect finer differences. The constancy of the TL50 at 144 hours for the control could be an artifact or indicate a basal mortality of the insects in the assay at that time, regardless of treatment. In the case of isolates, a TL50 of 144 hours with such high mortalities suggests relatively rapid action of the fungus once infection is established. On the other hand, isolate BBT11 exhibited a 91.47% mortality, significantly different (letter 'B') from BBT35 and BBT31, but still a considerably high percentage mortality. This suggests that BBT11 also has good insecticidal potential, although slightly lower than the two best isolates. The lowest efficacy was observed with isolate BBT34, which caused only 62.93% mortality (letter 'C'), statistically differentiating it from the other active isolates. This variability in virulence among B. bassiana isolates is a phenomenon commonly reported in the literature and may be attributed to genetic differences among strains, physiological factors, or the ability to produce diverse toxins and extracellular enzymes that facilitate infection and host death.The absolute control group, with a mortality of 5.60% (letter 'D'), establishes a crucial baseline for the interpretation of the results, confirming that the mortalities observed in the treated groups are attributable to the action of the B. bassiana isolates and not to external factors or natural mortality of the insects under the trial conditions. The statistical significance ($p > 0.05$) between means with a common letter, as noted in the table, reaffirms the robustness of the observed differences between virulent groups.

TABLE II: Percentage of Mortality and Lethal Half-Time (LT50) Caused by Isolates of *B. bassiana* on *Cosmopolites sordidus*

Isolated	Mortality	EE	TL50	Tukey
BBT35	98.67	2.25	144	A
BBT31	97.60	2.25	144	A
BBT11	91.47	2.25	144	B
BBT34	62.93	2.25	144	C
Absolute control	5.60	2.25	144	D

Note: Means with a common letter are not significantly different ($p > 0.05$).

Consistency in EE and TL50 values for all treatments and control could be a critical point of analysis. While mortality varies significantly, the uniformity in EE and TL50 (144 hours) suggests that, once infection is successful, the time course of disease may be similar, or, alternatively, that these parameters are not sensitive enough to discriminate between subtle differences in infection kinetics that may be occurring at the individual insect level. It would be valuable to discuss whether the methodology for calculating EE and TL50 is optimal for capturing these variations or whether other kinetic parameters are needed for further characterization.

5. Conclusion

The results of this vitro assay demonstrate the differential virulence of Beauveria bassiana isolates on Cosmopolites sordidus. Significant mortality was observed in most treatments with fungal isolations compared to absolute control. Specifically, isolates BBT35 and BBT31 showed the highest efficacy, inducing mortalities of 98.67% and 97.60%, respectively, and did not differ significantly from each other (Tukey, $p > 0.05$). These two isolates stood out for their high pathogenicity compared to the other treatments.

Isolate BBT11 also exhibited considerable mortality of 91.47%, although it was significantly lower than that observed with BBT35 and BBT31, but higher than BBT34. In contrast, isolate BBT34 showed significantly lower mortality (62.93%) compared to the other isolates evaluated, although still superior to the control. The absolute control group recorded a baseline mortality of 5.60%, confirming the specificity of the effect of fungal treatments

Importantly, while mortality varied among isolates, the "EE" (2.25) and "TL50" (144) parameters were consistent for all treatments, including the control, suggesting that these parameters may not be the most sensitive for differentiating virulence in this context, or that concentrations and exposure times reached a threshold where these values stabilized. The major differentiation in virulence between isolates was clearly reflected in the final percentage mortality.

In summary, isolates BBT35 and BBT31 are promising candidates for the development of biopesticides against C. sordidus due to their high efficacy under the conditions of this in vitro assay. Future research should include in vivo tests to confirm these findings and evaluate their performance under field conditions.

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Conflict of Interest

The author declares that he has no conflict of interest.

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