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# Endophytic establishment of *Beauveria bassiana* and *Metarhizium anisopliae* in maize plants and its effect against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae

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## Abstract

The aim of the present study was to evaluate the artificial establishment of *Beauveria bassiana* and *Metarhizium anisopliae* as endophytes in maize plants, and its effect in controlling the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. The commercial strains *B. bassiana* Bb-18 and *M. anisopliae* Ma-30 were concentrated at $1 \times 10^8$ conidia $ml^{-1}$, and the soil drench method was used to establish them as endophytes in maize plant parts. The biological control assays were conducted under laboratory conditions on second and fourth larval instars of *S. frugiperda*. *B. bassiana* colonized roots, stems, and leaves of maize tissues. However, a high occurrence of *B. bassiana* was obtained in roots than leaves and stems with 25, 10, and 5 isolations, respectively, whereas *M. anisopliae* was only acquired on roots. Both entomopathogenic fungi caused (100%) mortality on the second instar larvae. In addition, *B. bassiana* and *M. anisopliae* killed (87 and 75%) of the fourth larval instars, respectively. The fungus *M. anisopliae* caused the highest sporulation rates during the study. These results suggest that the entomopathogenic fungi might contribute to a sustainable *S. frugiperda* management in maize production in Cuba.

**Keywords:** Biological control, Endophytes, Entomopathogenic fungi, *Spodoptera frugiperda*, Maize

## Background

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is distributed in tropical and subtropical regions (Pogue 2002). This insect pest causes damage to several host plants (maize, peanuts, cotton, soybean, and forage grasses); however, the maize (*Zea mays* L.) constitutes the main agricultural crop preferred by *S. frugiperda* (Virla et al. 2008). The most serious damage produced by this pest is continuous consumption of the young shoots reducing the photosynthetic area of the plant.

Currently, the most employed method to reduce populations of *S. frugiperda* is the spraying of chemical insecticides, but despite its fast mode of action, the larvae have developed resistance as effects of this method of control, and it causes environmental pollution (Berón and Salerno 2006). Furthermore, *S. frugiperda* larvae remain feeding inside the plant shoots reducing the contact with insecticides applied for their control (Braga Maia et al. 2013). For these reasons, the use of biological control as an eco-friendly alternative could be effective to control *S. frugiperda*. Among the most biological control measures used stand out the entomopathogenic fungi (EPF); *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin, and *Metarhizium anisopliae* (Metschnikoff) Sorokin, because they can cause

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infection at all life stages (Hajek and St Leger 1994). These EPF can also act as saprophytes on the organic matter and live as endophytes of several plants (Vega 2008). However, the endophytic colonization by EPF might be more widespread than currently realized and may provide a source of indirect interaction between fungi and insects. Furthermore, studies related to the endophytic colonization of *B. bassiana* and *M. anisopliae* in maize in Cuba and its use as biological control on *S. frugiperda* is already scarce.

The aims of this study were therefore to evaluate the artificial establishment of *B. bassiana* and *M. anisopliae* as endophytes in maize plants, and their effect in controlling *S. frugiperda* larvae.

## Material and methods

### Experimental setup and soil treatment

The experiment was carried out at the laboratory of Microbiology belonged to Universidad Central "Marta Abreu" de Las Villas, Santa Clara (22° 24′ 49″ N, 79° 57′ 58″ W), Cuba. Twenty kilograms of an Inceptisol soil (USDA Soil Taxonomy) were collected from an agroecological maize field located in "Encrucijada" municipality (22° 37′ 01″ N, 79° 51′ 58″ W). The soil was autoclaved 3 times at 121 °C for 1 h. The efficacy of the sterilization was evaluated by diluting 1 g of soil in 20 ml sterile distilled water and then by inoculating 100 μl (soil + water solution) on Sabouraud Dextrose Agar (SDA) culture medium without antibiotics. Petri dishes (9 cm diameter) with the soil inoculations were incubated at 25 °C and 75% relative humidity (RH) in the dark. Effectiveness of the soil sterilization was reached when no growth of bacterial or fungal microorganisms were detected.

### Endophytic colonization of *B. bassiana* and *M. anisopliae* on maize plants

The EPF used in the study were the commercial strains *B. bassiana* Bb-18 and *M. anisopliae* Ma-30. These fungi were previously isolated from *Hypothenemus hampei* Ferrari and stored at the EPF collection of the Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas. Sterile soil was deposited into 40 polyethylene bags (500 g capacity), and then 20 bags were inoculated with 10 ml of the commercial strain of *B. bassiana* Bb-18, and the other 20 bags were inoculated with 10 ml of *M. anisopliae* Ma-30 at a concentration of $1 \times 10^8$ conidia $ml^{-1}$. The soil contained into the bags was incubated during 7 days in a climatic chamber (Memmert, Germany) at 25 °C and 75% RH in the dark to propitiate the establishment of the entomopathogenic fungi. Afterwards, maize grains cv 'P 78-45' were sown into the soil contained in the bags at a ratio of 1 grain per bag, and then the bags were placed into a germination chamber (TP, China) at 25 °C, 75% RH, and 16 h of light and 8 h of dark (L16:D8). Once the maize plants reached the growth stage BBCH 12 (2 leaves unfolded) (Meier 2001), roots, stems, and leaves were collected and surface sterilized by dipping for 3 min in 1.5% sodium hypochlorite, 2 min in 70% ethanol, and then rinsed 3 times in sterile distilled water. The efficacy of sterilization was evaluated by inoculating 100 μl of the last rinse water on SDA culture medium.

Damaged plants resulting from the sterilization procedure were discarded to avoid the death of the endophyte tissue (Douglas et al. 2012). Afterwards, plants were dried by sterile paper towels and aseptically cut into small pieces (1 $cm^2$) in a laminar flow hood. The plant pieces were then inoculated on SDA culture medium contained in Petri dishes (9 cm diameter) with addition of chloramphenicol (250 mg/l w/v). The Petri dishes with the plant pieces were incubated before. A single spore of each EPF was harvested and re-inoculated on SDA culture medium to obtain colonies without contamination. For each EPF studied, 4 replications were done.

### Insects assay

Both EPF strains, *B. bassiana* Bb-18 and *M. anisopliae* Ma-30, were supplied by the Plant Health Center from Villa Clara province. Conidia of each fungal strain were adjusted to a final concentration of $1 \times 10^8$ conidia $ml^{-1}$ through a Neubauer hemocytometer chamber (Brand, Germany). The EPF were diluted in distilled water. Additionally, distilled water was used as the control treatment. To evaluate the effect of each fungal formulation against *S. frugiperda*, a pedigree of this insect was obtained under laboratory conditions (Chacón-Castro et al. 2009). Twenty larvae from the second instar of *S. frugiperda* and 20 from the fourth instar were dipped for 1 min into 15 ml of a conidial suspension of *B. bassiana* Bb-18 and *M. anisopliae* Ma-30, as well as 15 ml of distilled water (control treatment). After 10 min of treatments exposure, each larva was placed individually in a sterile Petri dish (15 cm diameter) with a portion of leaves and stem of maize plants (growth stage BBCH 12) for feeding. Petri dishes with *S. frugiperda* were incubated at 25 °C, 90% RH, and L16:D8 photoperiod. Larvae were checked every day for mortality and production of mycosis. The experiment was repeated 4 times, and for each treatment 4 replications were used.

### Data analysis

Data on frequencies of occurrence of endophytic *B. bassiana* and *M. anisopliae* in maize plant parts were analyzed by chi-square test. Chi-square statistics were calculated through a frequency table, and $p$ values were corrected using Cramer's V. Analysis of variance (ANOVA) was applied to compare the mortality and sporulation induced by *B. bassiana* and *M. anisopliae* on *S. frugiperda* larvae. Means were separated using the

**Table 1** Endophytic colonization of *Beauveria bassiana* and *Metarhizium anisopliae* in maize plant tissues

| Plant tissue | *Beauveria bassiana* | | *Metarhizium anisopliae* | |
|---|---|---|---|---|
| | Mean ± SE | Fungal colonization (%) | Mean ± SE | Fungal colonization (%) |
| Roots | 25 ± 0.25a | 62.5 | 32 ± 0.29 | 73.5 |
| Stems | 5 ± 0.65c | 12.5 | 0 ± 0.00 | 0 |
| Leaves | 10 ± 0.29b | 25.0 | 0 ± 0.00 | 0 |

Means followed by different lowercase letters in the same column differ statically by the chi-square test ($p < 0.05$)
*SE* standard error

Tukey DHS test. Chi-square, ANOVA, and LSD tests were run using STATGRAPHICS Plus 5.1 (Manugistics Inc.) with significance level of 0.05.

## Results and discussion

### Endophytic colonization of *B. bassiana* and *M. anisopliae* on maize plant tissues

The EPF, *B. bassiana* and *M. anisopliae*, resulted positive for endophytic colonization of maize plant tissues. *B. bassiana* colonized roots, stems, and leaves of maize plants. However, a high occurrence ($\chi^2 = 5.06$, df = 2, $p = 0.0014$) of this fungus was obtained in roots than leaves and stems with 25 ± 0.25, 10 ± 0.29, and 5 ± 0.65 isolations, respectively (Table 1). These results revealed that the *B. bassiana* colonization (62.5%) occurred on the maize roots showing differences ($\chi^2 = 2.25$, df = 1, $p = 0.0001$) with the 12.5% of stems colonized by this fungus (Table 1). *M. anisopliae* was greater ($\chi^2 = 42.67$, df = 2, $p = 0.0001$) acquired from roots, 32 ± 0.29 isolations, than from leaves and stems, where no fungal colonization was detected (Table 1).

The obtained results showed that *B. bassiana* was endophyticaly established on roots, stems, and leaves of maize plants. These findings are in accordance with the results obtained by Mahmood et al. (2019), who revealed that all inoculated maize plants in their experiment contained endophytic *B. bassiana.* In addition, the highest colonization levels were (61%) in the oldest inoculated leaves and (19%) in the youngest non-inoculated leaves indicating the movement of the endophyte inside plants. It has been shown that after a foliar spray of *B. bassiana* on maize plants the hyphae can penetrate inside the xylem and act as entophyte in leaves tissues (Wagner and Lewis 2000). However, the present study showed that *M. anisopliae* was only detected on roots. Besides, Pilz et al. (2011) demonstrated that *M. anisopliae* spores applied on maize leaves were able to survive for no longer than 3 days after application, whereas on the soil surface a high increase of fungus densities were found after treatments. Other studies have been demonstrated that *M. anisopliae* was commonly associated with plant roots (Keyser et al. 2015). Other experiments revealed that *B. bassiana* reached a higher colonization on leaves than roots of *Sorghum bicolor* (L.) Moench when the grains were treated by a formulation of this fungus (Tefera and Vidal 2009). This result suggested that the endophytic colonization of *B. bassiana* can vary with the

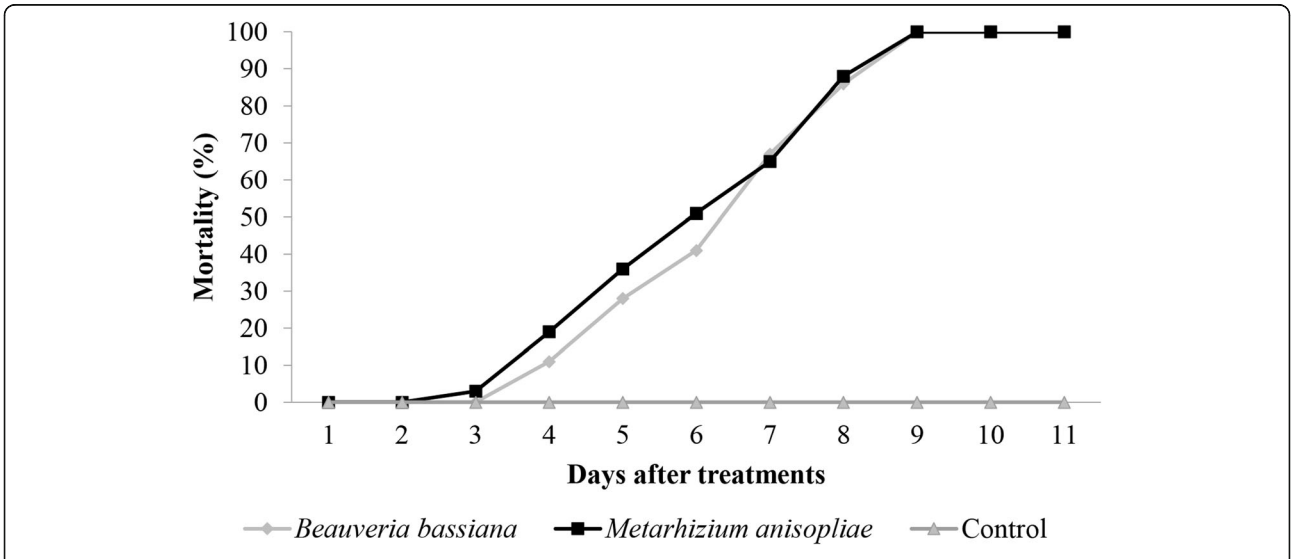


**Fig. 1** Effectiveness of the commercial strains *Beauveria bassiana* Bb-18 and *Metarhizium anisopliae* Ma-30 in the control of the second instar of *Spodoptera frugiperda* larvae

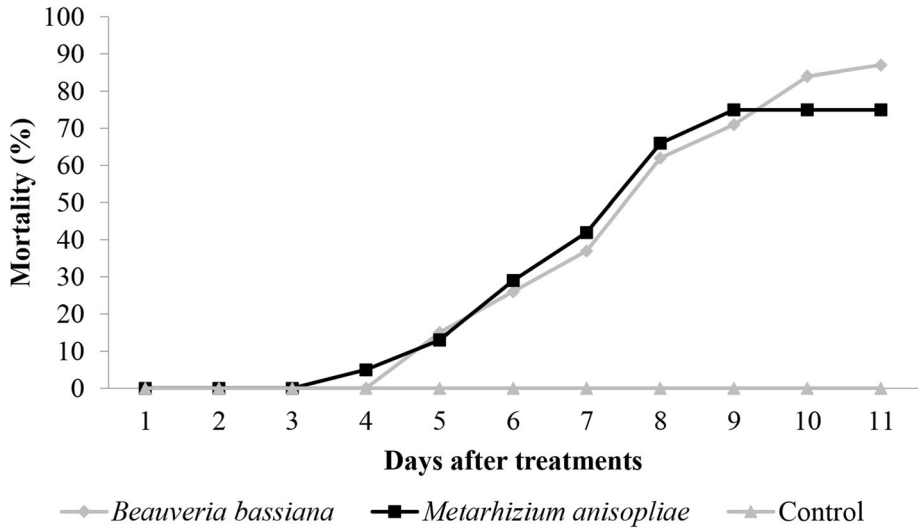


**Fig. 2** Effectiveness of the commercial strains *Beauveria bassiana* Bb-18 and *Metarhizium anisopliae* Ma-30 in the control of the fourth instar of *Spodoptera frugiperda* larvae

host plant part and the method used. Renuka et al. (2016) observed that stems of *Z. mays* were also colonized by *B. bassiana*; however, the availability and persistence on the plant tissues varied according to their age (high persistence in young tissues).

Despite several authors have demonstrated that *B. bassiana* as endophyte of maize, others have been revealed that after the inoculation of this EPF on maize seeds, no colonization of plant tissues was obtained (Tall and Meyling 2017).

### Effect of *B. bassiana* and *M. anisopliae* on *S. frugiperda*

The survivorship experiments showed that *B. bassiana* started to kill larvae from the second instar of *S. frugiperda* on the fourth day after treatment, reaching 11% mortality rate. After that, mortality increased by this fungus reaching 100% mortality rate on ninth day after treatment. Meanwhile, *M. anisopliae* started its mortality on the third day after treatment with 19% larval mortality (Fig. 1).

At the fourth instar, the EPF *M. anisopliae* and *B. bassiana* started their mortality at third and fourth days after treatments, respectively. The mortality caused by *B. bassiana* was increased, reaching up to 87% on 11th day after the application. However, the fungus could not kill 100% of the larvae. On the other hand, *M. anisopliae* caused a maximum of 75% mortality rate (Fig. 2). The control treatment did not have any effect on second and fourth instars of *S. frugiperda* larvae.

No differences were detected ($p > 0.05$) on the mortality rates of *S. frugiperda* larvae treated with *B. bassiana* (19.45 ± 0.23) and *M. anisopliae* (19.36 ± 0.28). However, the sporulation level obtained with *M. anisopliae* (9.1 ± 0.20) was significantly higher than that obtained of *B. bassiana* (5.9 ± 0.19) (Table 2).

The results are in contrast with the findings obtained by Akutse et al. (2019), who demonstrated that *B. bassiana* ICIPE 676 caused moderate mortality of 30% to the second instar larvae of *S. frugiperda*, whereas *M. anisopliae* ICIPE 78, ICIPE 40, and ICIPE 20 caused egg mortality of 87, 83, and 79.5%, respectively. This denotes that the virulence of these EPF may vary according to the strain origin.

Studies conducted to compare the sporulation level of *B. bassiana* and *M. anisopliae* demonstrated that the effectiveness of 3 *B. bassiana* strains was greater than the effect caused by *M. anisopliae,* when these EPF were applied on *Spodoptera litura* F. Furthermore, *B. bassiana* caused the best candidate related to virulence and germination rates, whereas the highest enzymatic activity

**Table 2** Mortality and sporulation caused by *Beauveria bassiana* and *Metarhizium anisopliae* on *Spodoptera frugiperda*

| Entomopathogenic fungi | Mortality | | Sporulation | |
|---|---|---|---|---|
| | Mean ± SE | Percentage (%) | Mean ± SE | Percentage (%) |
| *Beauveria bassiana* | 19.45 ± 0.23a | 95.20 | 5.9 ± 0.19b | 31.00 |
| *Metarhizium anisopliae* | 19.36 ± 0.28a | 95.00 | 9.1 ± 0.20a | 45.00 |

Means followed by different lowercase letters in the same column differ statically by the Tukey DHS test ($p < 0.05$)
*SE* standard error

was resulted by the use of *M. anisopliae* (Petlamul and Prasertsan 2015).

Generally, several studies have been conducted to evaluate the effect of *B. bassiana* against lepidopteran species, but the effect of *M. anisopliae* on *S. frugiperda* larvae has been less reported and the present research constitute an advance in this regard.

Although there were no differences between the mortality produced by *B. bassiana* and *M. anisopliae* on *S. frugiperda* larvae, the sporulation rate was higher in the case of *M. anisopliae* than in *B. bassiana*. The EPF *M. anisopliae* had the ability to reach a 52.8% of sporulation on cadavers of *S. frugiperda* at 3 days after the mortality (Ibarra-Aparicio et al. 2005).

This result is important because the sporulation of the EPF from its host means the formation of a new source of inoculum for the infection of new populations of insect pests. Therefore, the fungus that shows the highest percentage of sporulation is more likely to be disseminated by biotic and abiotic agents.

## Conclusion

The present study indicated that *B. bassiana* and *M. anisopliae* occurred as endophytes in maize plant parts, mainly in root tissues. Further, it highlights the importance of these EPF as potential biological control agents of *S. frugiperda* in maize fields in Cuba. These results suggest that the EPF studies could contribute to sustainable pest management of maize production.

### Abbreviations

BBCH: Phonological development stages of plants from Biologische Bundesanstalt Bundessortenamt und CHemische Industrie; df: Degrees of freedom; DHS: Honestly significant difference; EPF: Entomopathogenic fungi; L16:D8: 16 h of light and 8 h of dark; LSD: Least significant difference; *p*: *p* value; RH: Relative humidity; SDA: Sabouraud Dextrose Agar; SE: Standard error; $\chi^2$: Chi square

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### Authors' contributions

YR and OP conceived and designed the experiment. YR, AT, and JJ executed the experiment. YR and OP analyzed the data. YR, OP, AT, and JJ wrote and reviewed the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

This manuscript is in accordance with the guide for authors available on the journal's website. Also, this work has not been published previously and is approved by all authors and host authorities

### Consent for publication

All authors approve to publication.

### Competing interests

The authors declare that they have no competing interests.

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## Publisher's Note