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Relation between *Helicoverpa armigera* (Hubner) (Lepidoptera/Noctuidae) mortality and entomopathogenic fungi persistence in soybean leaflets

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Abstract

Background: Entomopathogenic fungi have low persistence in the field because of UV light, high temperatures and low humidity. Thus, this study's objective was to correlate the persistence of *Beauveria bassiana* and *Metarhizium anisopliae* in soybean leaf by measuring the mortality and sub-lethal effects of *Helicoverpa armigera* (Hubner) (Lepidoptera/Noctuidae). In the 2017–2018 and 2019–2020 soybean seasons, the experiments were conducted on plots subdivided in a completely randomized design. After spraying each plot with the fungal, either *B. bassiana* or *M. anisopliae*, leaves were collected hourly for 8 h after application and transferred to the laboratory. The local air temperature was recorded each time. For persistence assessment, the fungal suspension was prepared from the leaf disks; thereafter, 150 µl of the suspension was removed and poured into a plate. After 72 h of inoculation, colony-forming units (CFUs) were counted. For the mortality test, the leaf disks were placed individually in pots and offered to second instar larvae of *H. armigera*. The surviving larvae were evaluated for sub-lethal effects and biological parameters.

Results: *Beauveria bassiana* was more persistent than *M. anisopliae*, which persisted for 3 h in the first year and 5 h in the second year when temperatures were milder; *B. bassiana* persisted throughout the evaluation period (8 h). The mortality of *H. armigera* decreased with an increase in the time since application fungal suspension, presenting a range of 86–30% for *B. bassiana* and 78–4.2% for *M. anisopliae*.

Conclusion: Assessments of sublethal effects showed an inverse correlation between CFUs and parameters such as fertility and weight.

Keywords: White Muscardine, Green Muscardine, *Helicoverpa armigera*, Virulence, Sublethal effects, Fungal control

Background

Glycine max. (L.) (soybean) is one of the world's largest agricultural crops. Brazil is the largest producer of soybean. Soybean exports in Brazil during the 2019/2020 harvest increased by 36.2% (124.8 million tons) compared to that during the previous harvest (Conab 2020).

Despite its extensive production, soybean is attacked by insect pests from germination to post-harvest, which increase the production cost due to the damage caused by these insects. Among the soybean pests, *Helicoverpa armigera* (Hubner) (Lepidoptera/Noctuidae) is the most prominent. The damage caused by this species occurs after plant emergence when the larvae cut the plants under the cotyledons and continue until the crop V3 stage when larvae behave as defoliators and attack the terminal buds (Suzana et al., 2018).

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In intensive farming systems, as is the case for most soybean crops in Brazil, this pest is often controlled using chemical insecticides (Bortolotto et al. 2015). The intensive use of broad-spectrum pesticides has accelerated the development of resistance and reduced the population of natural enemies, which is a major environmental concern (Bakker et al. 2020). The search for alternative measures that aim to reduce these impacts is hence crucial. Microbial control agents, especially entomopathogenic fungi (Sosa-Gómez 2012), are considered efficient and safe alternatives (Lacey et al. 2015).

The natural occurrence of entomopathogenic fungi has been an important factor in soybean crops for several years, where it causes a significant reduction in *Chrysodeixis includens* (Walker, 1857) populations. However, excessive fungicide uses against Asian rust caused a reduction in the occurrence of entomopathogenic fungi (Sosa-Gómez et al. 2010). The susceptibility of *H. armigera* to entomopathogenic fungi in the laboratory has been well-documented, showing efficacy of up to 97% mortality (Jarrahi and Safavi 2016).

The efficacy of these microorganisms against pests has aroused the interest of researchers. Currently, pathogens of the order Hypocreales have been shown to be effective in the form of an inundative control (Chandler 2017), and many companies around the world have invested in producing bioinsecticides that have entomopathogenic fungi as an active ingredient (Kumar et al. 2018), such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch) Sorokin.

Products formulated produced based on these fungi are used in agriculture worldwide, acting as a biological control agent for many insect pests. The increased usage of these microbial agents in Brazil (Mascarin et al. 2018) has necessitated studies into their efficiency and persistence in the field since a low persistence (Lacey et al. 2015) could be an important factor affecting their wider adoption by farmers.

Defoliating larvae come into contact with the fungal conidia when they move on the leaves. However, entomopathogenic fungi are very susceptible to UV radiation, high temperatures and low humidity (Rojas 2015) reducing their efficacy in the field. Little is known about the persistence of pathogens on the leaves of a specific species in the field. Regarding the percentage of viability or viable numbers, the useful life of outdoor fungal conidia is 3–4 h (Braga et al. 2001).

Thus, studies that assess these pathogens' potential in a specific ecosystem, measured by the effectiveness in insect mortality during their stay in the field, are essential. In this context, the objectives of the current study were to evaluate the persistence of *B. bassiana* and *M. anisopliae* in leaves' soybeans on the findings of *H. armigera* mortality and sublethal effects.

Methods

The experiments were carried out in the 2017–2018 and 2019–2020 soybean seasons in the experimental area of the Department of Plant Health and the Laboratory of Microbial Control of Arthropods Pests (LCMAP), Paulista State University (FCAV/UNESP), Jaboticabal, São Paulo.

Helicoverpa armigera rearing conditions

Approximately, 100 *H. armigera* larvae were obtained from the company Ouro Fino Agrociência, São Paulo, Brazil. The larvae were reared individually in a transparent plastic container (50 ml) containing approximately 50 g of an artificial diet (Greene et al. 1976) until they reached the pupal stage. The pupae were kept in plastic containers (11.5 cm in diameter and 3.5 cm in height) with filter paper until adult emergence (males and females). Subsequently, they were transferred to PVC cages (20 cm in diameter and 30 cm in height). The upper parts of the cages were sealed with voile fabric, and the lowest parts were supported on a plastic plate (28 cm in diameter) covered with filter paper. Internally, the cage was lined with paper towels, which were used as a substrate for oviposition. Adult insects were fed with a 10% honey solution soaked in cotton.

The eggs were collected from the cages every 3 days and transferred to a transparent plastic container (500 ml) containing approximately 50 g of the artificial diet. The larvae were separated and kept individually after 10 days when they reached the third instar larvae to avoid cannibalism. The larvae were kept in an air-conditioned room at a temperature of 25 ± 2 °C, 70 ± 10% RH, and 12-h.

Field trials photophase

An area of 1700 m^2 with plots subdivided in a completely randomized design was used for the field trials. Each plot (560 m^2) was sprayed with a suspension of *B. bassiana* IBCB-1363 and another one from *M. anisopliae* IBCB-425, and 0.003 µl of Tween 80®. The control was sprayed with distilled water. Fungal suspensions were prepared by washing rice grains that were used as a solid substrate for the production of both fungi with 15 days of cultivation. A 5 l of water were used to wash 1.5 kg of rice, after washing the suspension was filtered to determine the concentration.

The two fungal isolates were chosen to be taken to the field based on preliminary tests in the laboratory, where the isolates that showed the greatest virulence (100% mortality) against *H. armigera* larvae were selected. Parcels were sampled for eight collection periods after fungal

application. The applications were conducted at 7:00 AM using a hand sprayer, with a CO_2 pressure of 1 bar and a flow rate of 70 l ha^{-1}, for 15 s per line, shaking at the end of each line to maintain a uniform suspension of the conidia. At the time of spraying, the fungal suspension had a concentration of 108 conidia ml^{-1}, as determined by a Neubauer chamber (400× magnification) under a Carl Zeiss-Axio LabA1 microscope.

Leaf disk collection and evaluation

After spraying the fungi, soybean leaflets were collected at eight different times (0.5, 1, 2, 3, 4, 5, 6, and 7 h after application) and taken to the laboratory. The leaflets were cut into disks (3 cm ø). Each treatment evaluation consisted of 55 disks (50 disks for the mortality test and 5 disks for the persistence test), totaling 400 replicates for each evaluation of mortality and 40 for persistence. The local temperature was recorded during the collection of the leaflets using a thermometer (Tecpel®). At the farm's meteorological station where the experiment was carried out, the relative humidity was measured over the two seasons (harvests of 2018 and 2019). There was no rain during the experimental period. Each experiment was repeated twice each season.

Fungal persistence and mortality evaluation

For the persistence trials, fungal suspensions were prepared with leaflet disks shaken in Falcon tubes (15 ml) that contained 10 ml of distilled water and 0.001 µl of Tween 80® (Sosa-Gómez et al. 2010). 150 µl of the suspension was then removed and poured into a Rodac® plate (Replicate Organism Detection and Counting), with 5 ml of the potato dextrose agar (PDA) culture medium. The millimeter face of the plate was reversed, and the plates were sealed with Parafilm M®. They were incubated at 26 ± 1 °C over 12 h photoperiod. Each plate was considered a replicate, totaling five plates for each sampling time (0.5, 1, 2, 3, 4, 5, 6, and 7 h).

After 72 h of inoculation, the colony-forming units (CFUs) were directly counted using an optical microscope (ZEISS AX 10®) and a manual counter, which considered the standard plate count (the number of CFUs on the plate were counted and multiplied by the corresponding dilution).

For the mortality test, the disks were placed individually in bioassay pots (18 mm × 30 mm), and the surface of the pots was covered with a gelled mixture of 2.5% agar-water and filter paper in order to maintain the turgor of the leaves during the evaluation period. A second instar *H. armigera* larvae was transferred in each pot, totaling 400 larvae per fungal treatment. The trials were maintained under controlled conditions (RH: 75% ± 12%, T: 25 ± 2 °C, and 12-h photoperiod). Mortality was assessed 3 days after inoculation, for 7 days. The larvae were considered dead if they did not move when touched with a fine bristle brush.

Insect death due to the pathogen was confirmed as follows: the insects were washed in 70% alcohol for 10 s, rinsed in distilled water for 20 s for external decontamination, placed in plates, and left in a wet chamber for 15 days, making it possible to observe the extrusion of the pathogen, thereby confirming infection.

Sublethal effects on *Helicoverpa armigera*

The larvae that survived fungal exposure in the leaf disks were monitored daily in each treatment (collection time). The parameters of instar duration (days), larval weight (mg), pupal weight (mg), fecundity, egg viability (%), sex ratio, and adult longevity (male and female) were then evaluated.

Surviving larvae, pre-pupae, and pupae were weighed using an analytical balance (SHIMADZU-ATY224®). Pupae were sexed using a magnifying glass (ZEIZZ-STEMI 508®), with the males separated from the females. With these data, it was possible to establish breeding couples to assess the pre-oviposition period (APOP: Adult pre-oviposition period) and total pre-oviposition (TPOP: Total pre-oviposition period). The breeding couples were kept under the same conditions described for rearing. Oviposition and adult longevity were recorded daily. The larvae used in the experiments were not subjected to stresses of different natures that could interfere with their susceptibility to the isolates of the tested fungi.

For the analysis of the biological parameters of *H. armigera*, the raw data containing development time, survival, daily fertility, and male and female longevity were analyzed based on the constructed life table. In these assessments, all tested individuals were considered, including those that died during the immature stages (Chi and Liu 1985; Chi 1988). The parameters of intrinsic population growth rate (r), finite population growth rate (λ), net reproduction rate (R_0), average generation duration (T), and gross reproduction rate (GRR) were estimated using methodology of Chi and Liu (1985).

Statistical analysis

For all variables, the homogeneity of variance test was performed, using the Shapiro–Wilk test. All variables were normally distributed. To assess *H. armigera* mortality, an analysis of variance (ANOVA) was performed in the software Statistical Analysis System (SAS®) in a completely randomized design (CRD), with the treatments considered in a factorial arrangement. When a significant result was detected, the means were compared using Tukey's test at 5% probability. To assess the relationship between mortality and the number of

colony-forming units, Pearson's correlation coefficients were determined using the R statistical software program (R Core Team 2018).

The sublethal effects were analyzed with the bootstrap procedure, which was used to estimate the variations, means, and standard errors of the biological parameters of development time, fertility, and longevity using 100,000 bootstraps Reference. The comparison of population parameters of *H. armigera* that received fungal treatments with controls was performed using the paired bootstrap test ($p < 0.05$) based on the difference confidence interval (CI 95%) in the TWOSEX-MS-Chart software (2021) (Chi 2016).

Results

Fungal survival in relation to temperature

The number of CFUs evaluated during the experiment in 2018 was affected by time and temperature, with a significant inverse correlation for *B. bassiana* ($r = -0.8$) and *M. anisopliae* ($r = -0.75$) (Fig. 1). In the second season, the results were similar for *B. bassiana* ($r = -0.98$) and *M. anisopliae* ($r = -0.91$).

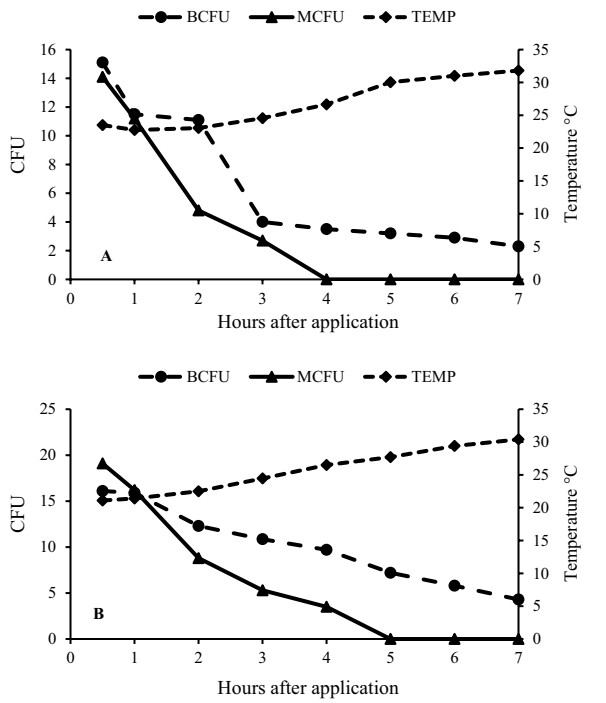

Fig. 1 Number of colony-forming units (CFUs) related to temperature at the hour of collection after fungal application. BCFU = CFU of *Beauveria bassiana*; MCFU = CFU of *Metarhizium anisopliae*. **A** First season of evaluation. **B** Second season of evaluation

Effect of time after application of *B. bassiana* and *M. anisopliae* on the mortality of *H. armigera*

There was a strong interaction between fungi and collection time ($p < 0.01$) for mortality in the first and second seasons of evaluation (Table 1).

The data presented a normal distribution and homogeneity of variance with an interaction at 1% probability and mortality in both seasons (Table 2).

Table 1 Synthesis of the analysis of variance for *Helicoverpa armigera* mortality in the first and second seasons of the field trials

Variation sources	Mortality (%) *F* values	
	First Season	Second Season
Fungi (*F*)	529.38**	239.98**
Time after application (TC)	25.49**	34.37**
*F**TC	12.32**	13.39**
CV%	80.74	102.65

CV% coefficient of variation

** Significant ($p < 0.01$)

Table 2 Persistence of the fungus in relation to time after application for the percentage of mortality in *Helicoverpa armigera* in the first (2018) and second (2019) seasons

Time after application (hrs)	Mortality (%)		
	Beauveria bassiana	*Metarhizium anisopliae*	Control
First Season			
0.5	86.02 aAB	78.05 aA	6.09 bA
1	94.05 aA	66.04 bA	4.32 cA
2	82.03 aABC	64.01 bA	2.15 cA
3	78.01 aABC	68.11 aA	0.09 bA
4	82.06 aABC	20.03 bB	2.02 cA
5	74.02 aABC	18.35 bB	6.08 bA
6	66.09 aBC	6.41 bB	2.03 bA
7	62.08 aC	4.26 bB	0.03 bA
Second Season			
0.5	66.06 aAB	62.09 aB	14.06 bA
1	74.09 aAB	90.03 aA	8.15 bA
2	82.07 aA	68.06 aAB	2.03 bA
3	76.03 aAB	56.03 bB	0.05 cA
4	70.01 aAB	24.11 bC	8.09 bA
5	58.06 aBC	4.13 bCD	10.08 bA
6	40.05 aCD	0.09 bD	6.03 bA
7	30.03 aD	0.09 bD	0.01 bA

A means the same lowercase letters in rows and uppercase letters in columns did not differ significantly by Tukey's test at 5% probability

Beauveria bassiana caused high mortality for 4–5 h after application, ranging from 94 to 62% in the first and 82 to 30% in the second seasons. In the second season, there was a more abrupt decrease in mortality for the last hours of collection, what happened also a drop in the first experiment but not so steep (Table 2). *Metarhizium anisopliae* showed different mortality pattern. The fungal persisted for only 3-h post-application in the first season, causing mortality ranging from 78 to 4%. In the second season and at milder temperatures, the persistence varies a little to 4-h post-application (Fig. 1), with a mortality rate ranging from 90 to less than 1% (Table 2).

The relationship between number of CFUs and mortality was observed as directly proportional, i.e., a decrease in mortality was observed with a reduction in the number of CFUs. The correlation between CFU and mortality was $r=0.76$ for *B. bassiana* and $r=0.82$ for *M. anisopliae* in the first season; it was $r=0.78$ for *B. bassiana* and $r=0.8$ for *M. anisopliae* in the second season (Fig. 2).

Sublethal effects of fungi on *Helicoverpa armigera*

There was no interaction between fungi and larval weight on the 10th day, and with pupal weight ($p>0.05$), a significant interaction was observed with pre-pupal weight, with the highest weight values noted after 4-h post-application. For *B. bassiana*, the larval weight difference was occurred at the first hours (1- and 3-h post-application). For *M. anisopliae,* a significant difference occurred only

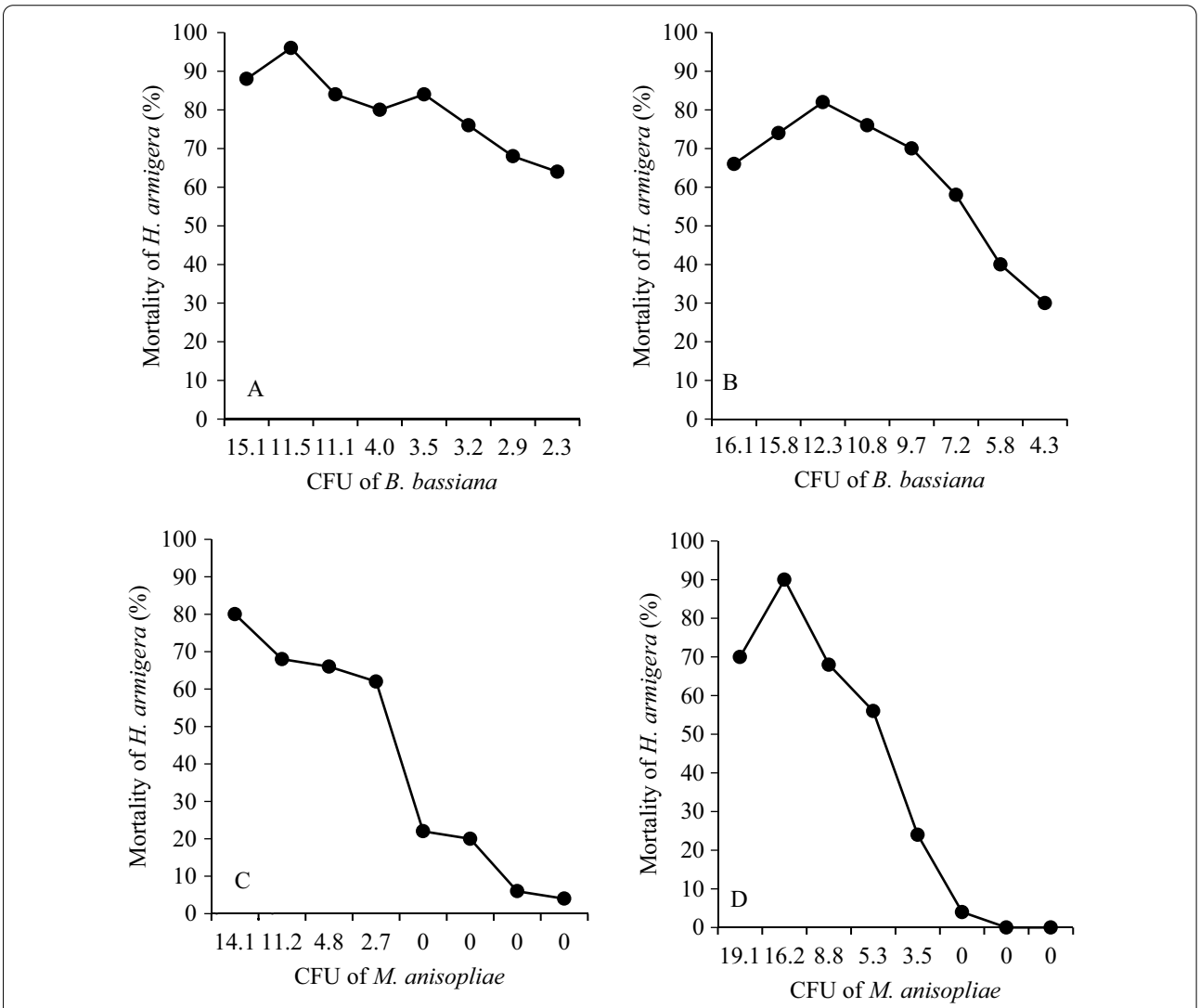

Fig. 2 Correlation between the number of colony-forming units (CFUs) of *Beauveria bassiana* in **A** 2018 and **B** 2019 and the mortality of *Helicoverpa armigera.* Correlation between the number of CFUs of *Metarhizium anisopliae* in **C** 2018 and **D** 2019 and the mortality of *H. armigera*

for pupal weight, with the highest values observed after 4 h (Table 3). Table 4 shows no significant difference ($p > 0.05$) in any treatment in the second season.

H. armigera life table

There was no significant difference in the average generation time (T) of *H. armigera* for treatment with *B. bassiana* at any time of collection of the leaves after the application of the fungal suspension. The net reproduction rate (R_0) was low in the first half an hour of collection (0.5 h), with an increase of 9.4% compared to that in the final hours. The intrinsic rate of increase (r) was significantly high in the first 4-h post-application, with differences only at 0.5 and 1 h. The finite rate of increase (λ) showed a difference in the first 2 h, with an increase of 1.14% post-application. The gross reproduction rate (GRR) of *H. armigera* at 6 h (296.14) was higher than that at the other evaluation times, however, but it did not differ from that at 1 and 2 h (Table 5).

The *M. anisopliae* isolate was higher than the other's average generation time (T) only during the first half an hour of collection (0.5 h). The net reproduction rate (R_0) showed differences at all evaluation times, with the highest average of 281.0 observed 6-h post-application. The intrinsic rate of increase (r) was the lowest in the first half an hour (0.5 h), followed by a significant increase of 88% until the end of collection (7 h). The finite rate of increase (λ) showed no significant difference in any of the leaf collection times after application.

Table 3 Weight of surviving *Helicoverpa armigera* larvae, pre-pupal, and pupal (g) following fungal application during the first season of collection

Collection time (hrs)	Weight of surviving *H. armigera*		
	10th day	Pre-pupae	Pupae
Control			
0.5	0.04 ± 0.01 Ns	0.20 ± 0.03 c	0.33 ± 0.02 Ns
1	0.06 ± 0.01 Ns	0.28 ± 0.03 abc	0.31 ± 0.02 Ns
2	0.05 ± 0.00 Ns	0.22 ± 0.02 bc	0.31 ± 0.01 Ns
3	0.07 ± 0.00 Ns	0.22 ± 0.02 bc	0.31 ± 0.01 Ns
4	0.05 ± 0.00 Ns	0.37 ± 0.02 a	0.34 ± 0.01 Ns
5	0.04 ± 0.00 Ns	0.37 ± 0.02 a	0.34 ± 0.01 Ns
6	0.05 ± 0.01 Ns	0.34 ± 0.02 ab	0.33 ± 0.01 Ns
7	0.05 ± 0.01 Ns	0.40 ± 0.04 a	0.33 ± 0.01 Ns
Beauveria bassiana			
0.5	0.03 ± 0.00 b	0.36 ± 0.06 Ns	0.30 ± 0.01 Ns
1	0.08 ± 0.03 a	0.22 ± 0.05 Ns	0.34 ± 0.03 Ns
2	0.03 ± 0.01 b	0.26 ± 0.03 Ns	0.31 ± 0.01 Ns
3	0.03 ± 0.00 a	0.28 ± 0.03 Ns	0.35 ± 0.02 Ns
4	0.04 ± 0.00 ab	0.29 ± 0.02 Ns	0.33 ± 0.02 Ns
5	0.05 ± 0.00 ab	0.30 ± 0.03 Ns	0.31 ± 0.01 Ns
6	0.03 ± 0.01 b	0.33 ± 0.02 Ns	0.30 ± 0.01 Ns
7	0.03 ± 0.00 b	0.26 ± 0.03 Ns	0.36 ± 0.01 Ns
Metarhizium anisopliae			
0.5	0.03 ± 0.00 Ns	0.30 ± 0.04 Ns	0.29 ± 0.01 ab
1	0.03 ± 0.00 Ns	0.29 ± 0.04 Ns	0.28 ± 0.00 bc
2	0.07 ± 0.02 Ns	0.31 ± 0.03 Ns	0.29 ± 0.00 ab
3	0.07 ± 0.01 Ns	0.33 ± 0.02 Ns	0.27 ± 0.00 c
4	0.05 ± 0.01 Ns	0.30 ± 0.01 Ns	0.34 ± 0.01 a
5	0.05 ± 0.00 Ns	0.34 ± 0.01 Ns	0.34 ± 0.01 a
6	0.05 ± 0.00 Ns	0.36 ± 0.02 Ns	0.31 ± 0.01 ab
7	0.05 ± 0.0 Ns	0.32 ± 0.02 Ns	0.33 ± 0.01 ab

Means followed by the same lowercase letters in rows did not differ significantly by Tukey's test at 5% probability

Ns Not significant

Table 4 Weight of surviving *Helicoverpa armigera* larvae, pre-pupal, and pupal following fungal application during the second season of collection

Collection time (hrs)	Weight of surviving *H. armigera*		
	10th day	Pre-pupae	Pupae
Control			
0.5	0.04 ± 0.00 Ns	0.27 ± 0.03 Ns	0.32 ± 0.01 Ns
1	0.04 ± 0.00 Ns	0.24 ± 0.03 Ns	0.31 ± 0.02 Ns
2	0.05 ± 0.02 Ns	0.38 ± 0.07 Ns	0.29 ± 0.02 Ns
3	0.03 ± 0.00 Ns	0.26 ± 0.04 Ns	0.34 ± 0.02 Ns
4	0.05 ± 0.00 Ns	0.27 ± 0.04 Ns	0.29 ± 0.01 Ns
5	0.04 ± 0.00 Ns	0.29 ± 0.04 Ns	0.35 ± 0.03 Ns
6	0.05 ± 0.01 Ns	0.32 ± 0.03 Ns	0.33 ± 0.03 Ns
7	0.05 ± 0.01 Ns	0.40 ± 0.03 Ns	0.33 ± 0.01 Ns
Beauveria bassiana			
0.5	0.04 ± 0.00 Ns	0.34 ± 0.04 Ns	0.34 ± 0.01 Ns
1	0.03 ± 0.00 Ns	0.35 ± 0.12 Ns	0.33 ± 0.03 Ns
2	0.01 ± 0.02 Ns	0.32 ± 0.08 Ns	0.35 ± 0.01 Ns
3	0.05 ± 0.00 Ns	0.34 ± 0.03 Ns	0.30 ± 0.00 Ns
4	0.06 ± 0.00 Ns	0.28 ± 0.11 Ns	0.32 ± 0.02 Ns
5	0.02 ± 0.00 Ns	0.31 ± 0.06 Ns	0.35 ± 0.02 Ns
6	0.02 ± 0.01 Ns	0.33 ± 0.03 Ns	0.31 ± 0.02 Ns
7	0.05 ± 0.01 Ns	0.25 ± 0.05 Ns	0.36 ± 0.01 Ns
Metarhizium anisopliae			
0.5	0.02 ± 0.00 ab	0.26 ± 0.03 Ns	0.35 ± 0.15 Ns
1	0.01 ± 0.00 b	0.37 ± 0.05 Ns	0.33 ± 0.00 Ns
2	0.02 ± 0.00 ab	0.23 ± 0.06 Ns	0.33 ± 0.02 Ns
3	0.03 ± 0.00 a	0.26 ± 0.04 Ns	0.26 ± 0.01 Ns
4	0.02 ± 0.00 ab	0.33 ± 0.07 Ns	0.37 ± 0.01 Ns
5	0.03 ± 0.00 a	0.40 ± 0.03 Ns	0.32 ± 0.01 Ns
6	0.02 ± 0.00 ab	0.36 ± 0.04 Ns	0.29 ± 0.01 Ns
7	0.02 ± 0.00 ab	0.33 ± 0.08 Ns	0.37 ± 0.01 Ns

Means followed by the same lowercase letters in rows did not differ significantly by Tukey's test at 5% probability

Ns not significant

Table 5 Mean life history parameters (± standard error) of the life table of *Helicoverpa armigera* at different collection times after the application of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* to the soybean crop in the first season (2019) over a single generation at 25 ± 1 °C and 70% ± 10% RH

Treatments (hrs)	Population parameters				
	T **(days)**	R_0	***r*** **(day^{-1})**	λ **(day^{-1})**	**GRR**
Beauveria bassiana					
0.5	38.60 ± 0.44 Ns	12.95 ± 6.19 e	0.066 ± 0.013 c	1.106 ± 0.014 b	187.8 ± 63.03 d
1	35.46 ± 0,11 Ns	38.79 ± 11.32 d	0.103 ± 0.009 b	1.108 ± 0,009 b	268.7 ± 50.85 ab
2	37.91 ± 0.35 Ns	81.29 ± 18.75 b	0.116 ± 0.006 a	1.123 ± 0.007 a	269.9 ± 55.02 ab
3	36.87 ± 0.53 Ns	50.62 ± 11.04 c	0.106 ± 0.006 ab	1.112 ± 0.007 b	210.4 ± 28.11 c
4	34.94 ± 0.13 Ns	63.7 ± 10.17 c	0.118 ± 0.004 a	1.126 ± 0.005 a	112.93 ± 15.16 d
5	34.72 ± 0.16 Ns	137.73 ± 20.17 a	0.142 ± 0.005 a	1.152 ± 0.005 a	245.88 ± 28.42 b
6	36.29 ± 0.18 Ns	119.74 ± 18.06 a	0.131 ± 0.004 a	1.140 ± 0.004 a	296.14 ± 56.61 a
7	36.29 ± 0.2 Ns	110.28 ± 16.60 a	0.129 ± 0.004 a	1.138 ± 0.004 a	203.79 ± 26.09 c
Metarhizium anisopliae					
0.5	41.26 ± 0.58 a	21.78 ± 7.80 f	0.074 ± 0.009 c	1.07 ± 0.001 Ns	211.19 ± 58.56 c
1	35.90 ± 0.12 b	163.61 ± 22.41 c	0.142 ± 0.004 a	1.15 ± 0.004 Ns	214.48 ± 26.86 c
2	36.45 ± 0.32 b	147.27 ± 18.77 c	0.137 ± 0.003 a	1.14 ± 0.004 Ns	287.31 ± 36.3 b
3	36.20 ± 0.32 b	109.75 ± 17.31 d	0.129 ± 0.004 ab	1.13 ± 0.005 Ns	190.36 ± 34.45 d
4	34.94 ± 0.13 b	63.7 ± 10.16 e	0.118 ± 0.004 b	1.12 ± 0.005 Ns	112.93 ± 15.13 d
5	34.71 ± 0.16 b	137.2 ± 20.15 c	0.141 ± 0.004 a	1.15 ± 0.005 Ns	244.86 ± 28.18 bc
6	35.72 ± 0.1 b	281.0 ± 24.74 a	0.157 ± 0.002 a	1.17 ± 0.002 Ns	323.32 ± 26.82 a
7	33.62 ± 0.14 b	237.1 ± 27.88 ab	0.162 ± 0.003 a	1.17 ± 0.004 Ns	275.54 ± 31.53 b
Control					
0.5	35.65 ± 0.17 Ns	283.32 ± 24.00 Ns	0.151 ± 0.003 Ns	1.16 ± 0.003 Ns	330.41 ± 41.47 Ns
1	35.91 ± 0.11 Ns	263.61 ± 22.45 Ns	0.141 ± 0.004 Ns	1.15 ± 0.004 Ns	316.56 ± 26.48 Ns
2	35.59 ± 0.12 Ns	287.93 ± 21.81 Ns	0.147 ± 0.003 Ns	1.58 ± 0.003 Ns	319.82 ± 23.60 Ns
3	35.77 ± 0.11 Ns	278.39 ± 22.54 Ns	0.144 ± 0.004 Ns	1.15 ± 0.004 Ns	319.18 ± 29.42 Ns
4	35.68 ± 0.12 Ns	282.38 ± 26.83 Ns	0.151 ± 0.004 Ns	1.16 ± 0.004 Ns	327.52 ± 30.44 Ns
5	35.72 ± 0.1 Ns	281.02 ± 24.74 Ns	0.150 ± 0.002 Ns	1.17 ± 0.003 Ns	323.32 ± 26.83 Ns
6	33.71 ± 0.1 Ns	281.02 ± 24.74 Ns	0.167 ± 0.002 Ns	1.18 ± 0.003 Ns	323.35 ± 26.80 Ns
7	33.62 ± 0.14 Ns	287.1 ± 27.87 Ns	0.162 ± 0.003 Ns	1.17 ± 0.004 Ns	325.54 ± 31.50 Ns

The data for each parameter were calculated using the 100.000 color initialization procedure. The means followed by the same letters did not differ between each other (paired initialization test: $p < 0.05$). *T* (day)—Average duration of a generation, R_0—net reproduction rate, *r* (day^{-1})—Intrinsic rate of increase, λ (day^{-1})—finite rate of increase, *GRR*—gross reproduction rate, *Ns*—not significant

The gross reproduction rate (GRR) of *H. armigera* was the highest (323.32) at 6-h post-application (Table 5).

The control did not significantly differ at any time of the assessment for any parameter (Table 5). When comparing parameters at different collection times between the fungal treatments and the control, it was observed that the average generation time (*T*) was not significantly different from the control for *B. bassiana* or *M. anisopliae*. The net reproduction rate (R_0) for treatment with *B. bassiana* in relation to the control was significantly low at all times. *Metarhizium anisopliae* did not significantly differ from the control from 4 h onwards. The intrinsic rate of increase (*r*) for the two fungi differed from the control only in the first half an hour of collection (0.5 h). The finite rate of increase (λ) showed no significant difference in any of the treatments compared to that of the control. The gross reproduction rate (GRR) differed significantly between the treatments of *B. bassiana* (296.14) and the control (330.41), whereas for *M. anisopliae* (323.32), there was no difference in the final hours of collection (Table 5).

In the second season of evaluation, there was a significant difference in the average generation time (*T*) for treatment with the *B. bassiana* isolate in the up to 2 h of collection after application. The net reproduction rate (R_0) was low in the first hour of collection (0.5 h), showing an increase of 162.19% after 3 h and a decline only in the collection of 6 h. The intrinsic rate of increase (*r*) and the finite rate of increase (λ) showed no difference. The gross reproduction rate (GRR) of *H.*

armigera for 2 and 3 h (319.85) was higher than that of the other hours of evaluation (Table 6).

There was a significant difference in *H. armigera* treated with isolate *M. anisopliae* the generation time (*T*) only for the first and third hrs of collection (0.5 and 2 h, respectively). The net reproduction rate (R_0) showed a difference at all evaluation times, with the highest average observed in samples collected 3-h post-application (253.66). The intrinsic rate of increase (*r*) the highest value was obtained for samples collected at 6 h (0.218). Averages at 0.5 and 2 h did not differ. The finite rate of increase (λ) showed no significant difference. The gross reproduction rate (GRR) of *H. armigera* was the highest (495.66) at 3 and 5 h after application (Table 6).

The control did differ significantly only R_0—net reproduction rate, *r*—the intrinsic rate of increase, and GRR—gross reproduction rate (Table 6). When collection times were compared after the application of fungal treatments and the control, it was observed that these results are aligned with the results of the first season of evaluation (Table 5).

Discussion

When applied under field conditions, entomopathogenic fungi are subject to the action of biotic and abiotic factors that can influence their survival and infection (Jaronski 2010). Among the abiotic factors, temperature and solar radiation can affect the long-term survival and virulence of fungi in a given environment (Lacey et al. 2015;

Table 6 Mean parameters (± SE) of the life table of *Helicoverpa armigera* submitted to different collection times after application of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* in the field in the soybean crop for the second year (2020) over a generation at 25 ± 1 °C and 70 ± 10% RH

Treatments (hrs)	Population parameters				
	T (day)	R_0	*r* (day^{-1})	λ (day^{-1})	GRR
Beauveria bassiana					
0.5	36.17 ± 0.64 b	42.96 ± 14.96 e	0.103 ± 0.010 Ns	1.109 ± 0.011 Ns	268.5 ± 36.33 b
1	38.41 ± 0.41 b	81.98 ± 19.60 b	0.123 ± 0.006 Ns	1.130 ± 0.007 Ns	258.76 ± 27.81 b
2	39.87 ± 0.17 b	112.64 ± 24.36 a	0.118 ± 0.005 Ns	1.125 ± 0.006 Ns	319.85 ± 28.11 a
3	47.22 ± 0.33 a	70.97 ± 18.05 c	0.090 ± 0.005 Ns	1.094 ± 0.006 Ns	267.54 ± 24.85 b
4	47.22 ± 0.32 a	70.97 ± 18.00 c	0.092 ± 0.005 Ns	1.097 ± 0.005 Ns	109.48 ± 24.83 c
5	47.18 ± 0.34 a	63.44 ± 16.07 d	0.087 ± 0.006 Ns	1.091 ± 0.006 Ns	144.77 ± 28.08 c
6	47.19 ± 0.35 a	58.61 ± 14.49 e	0.086 ± 0.005 Ns	1.090 ± 0.0061 Ns	220.92 ± 15.722 b
7	47.52 ± 0.38 a	72.14 ± 18.31 c	0.090 ± 0.006 Ns	1.094 ± 0.006 Ns	271.92 ± 24.37 b
Metarhizium anisopliae					
0.5	41.26 ± 0.58 a	21.78 ± 7.80 e	0.074 ± 0.009 d	1.07 ± 0.001 Ns	211.19 ± 58.56 c
1	36.24 ± 0.65 b	47.26 ± 17.15 d	0.106 ± 0.011 c	1.11 ± 0.012 Ns	294.25 ± 86.97 b
2	47.22 ± 0.32 a	47.22 ± 17.99 d	0.090 ± 0.005 cd	1.09 ± 0.006 Ns	109.48 ± 24.76 d
3	35.91 ± 0.26 b	253.66 ± 39.96 b	0.154 ± 0.004 b	1.16 ± 0.005 Ns	405.68 ± 70.24 a
4	35.82 ± 0.21 b	116.46 ± 32.49 c	0.132 ± 0.008 b	1.14 ± 0.006 Ns	159.64 ± 45.28 cd
5	36.06 ± 0.22 b	314.2 ± 45.49 a	0.159 ± 0.004 b	1.17 ± 0.004 Ns	495.66 ± 71.87 a
6	37.15 ± 0.31 b	118.61 ± 32.47 c	0.218 ± 0.005 a	1.21 ± 0.002 Ns	320.52 ± 85.25 b
7	37.22 ± 0.35 b	152.18 ± 38.32 c	0.190 ± 0.006 ab	1.29 ± 0.006 Ns	261.98 ± 25.38 c
Control					
0.5	35.65 ± 0.17 Ns	283.32 ± 24.00 b	0.151 ± 0.003 b	1.16 ± 0.003 Ns	330.41 ± 41.47 b
1	35.51 ± 0.14 Ns	388.31 ± 28.36 a	0.167 ± 0.002 b	1.18 ± 0.002 Ns	450.17 ± 49.53 a
2	35.92 ± 0.23 Ns	302.54 ± 38.27 ab	0.159 ± 0.003 b	1.17 ± 0.004 Ns	490.56 ± 63.73 a
3	35.92 ± 0.23 Ns	303.26 ± 38.27 ab	0.159 ± 0.003 b	1.12 ± 0.003 Ns	485.33 ± 37.41 a
4	35.92 ± 0.26 Ns	254 ± 39.85 b	0.154 ± 0.004 b	1.16 ± 0.005 Ns	399.62 ± 69.09 ab
5	37.18 ± 0.34 Ns	63.45 ± 16.03 d	0.087 ± 0.005 c	1.09 ± 0.006 Ns	339.77 ± 21.75 b
6	37.15 ± 0.31 Ns	288.61 ± 28.52 b	0.316 ± 0.005 a	1.00 ± 0.002 Ns	326.02 ± 65.02 b
7	36.72 ± 0.41 Ns	148.12 ± 25.31 c	0.291 ± 0.006 a	1.05 ± 0.006 Ns	391.95 ± 27.32 ab

The data for each parameter were calculated using the 100.000-color initialization procedure. The means followed by the same letters did not significantly differ from each other (paired initialization test: $p > 0.05$). *T* (day)—average duration of a generation, R_0—net reproduction rate, *r* (day^{-1})—intrinsic rate of increase, λ—finite rate of increase, *GRR*—gross reproduction rate, and *Ns*—not significant

Chandler 2017), as the leaf surface. The entomopathogenic fungi are not protected against the harmful effects of temperature, including *B. bassiana* and *M. anisopliae* (Braga et al. 2001).

The current results clearly indicated that there was variability in the susceptibility of the two tested fungal isolates when exposed to field conditions, with an abrupt loss of viable conidia as temperatures increased. This fact, acting directly or indirectly, limited the action of fungi and justified the decrease in mortality of *H. armigera* after hours of fungal application. Ambient variation temperatures can affect the effectiveness of fungi in a field, decreasing their control (Jaronski 2010). Decrease in the effectiveness of *B. bassiana* against *Dendrolimus punctatus* Walker, 1855, in small field tests, conidia growth in general decreased rapidly above 30 °C, and ceased for the most isolates between 34 and 37 °C (Dannon et al. 2020).

A caveat of some studies published on this subject is that photodegradation of conidia is faster on the leaf surface (Jaronski 2010). However, the most studies of entomopathogenic fungi persistence have focused on the persistence of these pathogens in soil, given that they are facultative saprophytic and their presence is ubiquitous, soil persistence is very high, reaching years (Bidochka et al. 2010), satisfactory effects on different pests, especially lepidopterans, have been reported (Kalvnadi et al. 2018). Studies conducted by Fernandes et al. (2007) reported that there was high variability in the thermotolerance of *Beauveria* spp. 2-h post-exposure to 45 °C, where different germination rates (80–20%) were obtained. Souza et al. (2014) evaluated the thermotolerance of several species of entomopathogenic fungi, and the results showed that *M. anisopliae* was more sensitive to high temperatures, as well as in the present study.

The rapid inactivation of entomopathogens under field conditions was due to high temperatures, solar irradiation, and the surface on the leaf surface, including pH and the unknown in activators released by plants (Brancini et al. 2022). Castrillo et al. (2010) evaluated the persistence of *B. bassiana* fungal in leaves and tree bark and observed a significant decline in the recovery of colony-forming units within 1 week of application. The decline was more pronounced in the leaves than in the peels, and the loss of virulence was also evident with increasing time after application.

Beauveria bassiana and *M. anisopliae* conidia are highly susceptible to solar radiation. Sunlight in the 290 to 400 nm range can affect the persistence of fungi deposited on the foliage (Acheampong et al., 2019). Environments with temperatures above 35 °C reduced persistence, but with low temperatures have lesser effects (Mcoy et al. 2006).

High temperature tolerance is also related to the hydrophobicity of entomopathogenic fungal conidia (Kim et al. 2010). *Beauveria bassiana* isolates have high amounts of hydrophobin proteins and consequently have high thermotolerance (Ying and Feng 2004). For this reason, even with temperature variations, *B. bassiana* remained viable but falling throughout the evaluation period in our study. In the 2 years of the study, the persistence of fungi was greater at milder temperatures, such as at 25 °C, consistent with the results of Coombes et al. 2016.

In general, a reduction in the number of CFUs was observed throughout the evaluations, reinforcing the hypothesis that abiotic factors affect conidia's persistence under field conditions. These variations, characterized by increases and decreases in CFU number during the 8-h evaluation, were also reflected in the susceptibility of *H. armigera* since fungal virulence is linked to conidia concentration and its ability to germinate when it comes in contact with the pest. In this way, the initial and final number of conidia was reflected in the percentage of insect mortality, showing the limited efficacy of these control agents.

The efficacy of entomopathogenic fungi in the protection of a crop depends on the persistence of the inoculum in the field. Although there are exceptions, the persistence of entomopathogenic fungi on different plant parts is a few days or hours. *Beauveria bassiana* remains viable for 5–21 days on the surface of plants (Islam et al. 2021). Leaf surface survival during a *B. bassiana* persistence study in Southern California revealed that conidia viability on the lowest and upper surfaces of melon (*Cucumis melo* L.) leaves decreased by approximately 9–11% day^{-1}. On the upper surfaces of the leaves, viability fell by 47% on day^{-1} (Jaronski 2010).

As shown in the present results, viable conidia were still found on *B. bassiana* even 8-h post-application; thus, the fungal was infectious throughout the evaluation period. As *H. armigera* larvae have the habit of walking on the leaves, the not permanence and viability of these fungi on the leaves can delay infection, since the effect on the host plant in the expression of epizootic diseases is not well-known (Sosa-Gómez 2012). Thus, in addition to temperature variations, the low persistence of fungi in the present study may be related to the chemical composition and morphological structure (distribution of trichomes, "hairiness") of the leaf surface, which can influence the adhesion of the conidia, which in turn it affects germination or inhibits the virulence of these fungi (Łaźniewska et al. 2012). Research on the interaction of entomopathogenic fungi, morphology, and chemical composition of leaves is necessary to confirm the interference in the persistence of fungi in the field.

Another important factor that must be taken into account is the time of application of fungal control in the field; in the current study, it was applied at 7 am, which resulted in high exposure of the infectious structures of the fungi (conidia), greater temperature variations and radiation, making it more evident that escaping the intensity of these factors on fungi is the best way to obtain more promising results from fungi in the field.

Like our results, Leite et al. (2011) reported that *B. bassiana* was more efficient against *H. armigera* than *M. anisopliae*. By considering the decrease in the number of CFUs in the treatments, the susceptibility of *H. armigera* larvae in the developmental period of surviving individuals was observed.

The mortality allowed us to infer that the isolates, besides being infectious, can change larvae fitness, such as by decreasing development time even within hours after fungal application, an assertion based on the sublethal effects evaluated and demonstrated in the results. In fact, even with a low amount of viable conidia over the hours, the tested fungal isolates affect the survival, development, and fertility of the *H. armigera* generations. For the weights of larvae and pupae, the expected results would not find differences between 0.5 and 3 h; however, there were variations in weights occurring even in the first hours; these variations may be due to the non-standardization of leaves, or not homogeneity of sprays, considering that the leaves were randomly collected within the treated plots (Jarrahi and Safavi 2016; Dannon et al. 2021).

Thus, the importance of selecting appropriate fungal isolates for good performance in the field under certain conditions is important since the literature is full of examples of fungi that performed well in laboratory tests and showed "great potential" but failed when tested in the field, thus prompting the search for new isolates. It is necessary to study the underlying factors contributing to success or failure, such as the methods used for applying these pathogens in the field, which are currently scarce and limited to the same spraying techniques developed for chemical products (Garcia et al. 2005).

Conclusions

Beauveria bassiana fungus was infectious during the 8-h evaluation period and was more persistent than *M. anisopliae,* which only persisted for 3 h in the first season and 5 h in the second one when temperatures were milder. The percentage of *H. armigera* mortality decreased with an increase in time after fungal application, and in the first 3 h, both fungi caused high mortality of *H. armigera*. In addition to being infectious, the tested isolates can alter the physiological factor of the larvae, reducing development time and weight even with few viable conidia after application.

Abbreviations

IBCB: Biological Institute of Campinas; PDA: Potato dextrose agar; LC: Lethal concentration; APOP: Period from the appearance of adults to the first oviposition; TPOP: Total pre-oviposition; *r*: Population growth rate; λ: Finite population growth rate; R_0: Net reproduction rate; *T*: Average generation duration; GRR : Reproduction rate; Sxj: Age-specific survival rate; Fxj: Age-specific fertility of the stage; mx: Age-specific fertility; MTD: Mortality; LOGOV: Egg stage duration; LOGLA: Caterpillar longevity; PRPU: Pre-pupa longevity; PUPA: Pupa stage duration; LOGTO: Total longevity; FECU: Fertility; OVI: Oviposition; PC: Principal components; CFUs: Colony-forming units.

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Authors' contributions

TDS is Master's student responsible for the project, participated in the planning and execution of bioassays, data analysis, and writing of the manuscript. FOF assisted in the setup and evaluation of experiments in the laboratory and in the analysis of the data. ACS assisted in the setup of the experiments in the laboratory. JN assisted in the setup of the experiments in the laboratory. AAP assisted in the setup and evaluation of experiments in the laboratory and in the analysis of the data. RAP is the project supervisor; guided the data collection; and reviewed the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

All data of the study have been presented in the manuscript, and high quality and grade materials were used in this study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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