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Sublethal effects of different fungal isolates on *Helicoverpa armigera* (Lepidoptera: Noctuidae)



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Abstract

Background: Broad-spectrum pesticides and *Bt* crops have been used against the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), but their effect on the environment and the development of resistance are the main limitations for these control measures. The use of mycobiopesticides is an ecofriendly and efficient alternative. Fungicides have a wide distribution and a high genetic variability, which allows their screening for more virulent isolates. Therefore, the objective of this work was to evaluate the interactions of *H. armigera* larvae with *Metarhizium anisopliae* and *Beauveria bassiana*, on mortality, virulence, sublethal effects, and life table aspects.

Main body: The isolates that caused mortality rates of *H. armigera* above 80% were selected for further assays. Virulence was measured by LC₅₀ estimates, using Probit analysis to sublethal effects, and the bootstrap procedure to estimate the biological parameters: time for development, fertility, longevity, pre-ovipositional period, egg viability, and sex ratio. The *H. armigera* mortality rates caused by *B. bassiana* and *M. anisopliae* ranged from 45 to 100% and from 40 to 90%, respectively. Longevity, fertility, and survival rates are affected in all treatments. The pupal weight of individuals treated with fungi was lower than in the control. Deformations were observed in *H. armigera* pupae developed from larvae treated with *B. bassiana*.

Conclusion: The use of sublethal concentrations of isolates of *M. anisopliae* and *B. bassiana* resulted in an adverse effect on the biological parameters of *H. armigera*.

Keywords: *Helicoverpa armigera*, *Metarhizium anisopliae*, *Beauveria bassiana*, Pathogenicity, Sublethal effects, Life table

Main text

Background

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is one of the most important insect pests of cotton in the world. It attacks more than 200 host plants (Challa et al. 2013). Its high polyphagia, reproductive capacity, and wide geographic distribution facilitated its adaptation to different agroecosystems (Tay et al. 2013). The severity of the damages caused

and the frequency of applications of chemical insecticide to control this pest have accelerated its resistance development and increased environmental problems (Yang et al. 2013). Thus, efficient alternatives for its control with less aggressiveness to the environment are necessary. Entomopathogenic fungi (EPF) are important to control insect pests from several orders, including lepidopterans (Lacey 2017). These microorganisms present high efficiency of control and have been used in bioinsecticide formulations due to their harmlessness to humans, the environment, and natural enemies (Jaronski et al. 2007).

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The fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales), are generalists, with a wide geographic distribution and a high frequency in soils and plants (Zimmermann 2007; Rezende et al. 2015 and Botelho et al. 2019). Selecting promising isolates to control specific insect pests is possible due to their wide distribution, high intraspecific genetic variability, and spectrum of action (Goettel et al. 2010). *B. bassiana* and *M. anisopliae* have been studied for controlling *H. armigera* with lethal and sublethal rates, evaluating the parameters survival rate, adult longevity, sex ratio, and fertility (Ahmad and Ansari 2013 and Jarrahi 2016).

Pest species fertility, mortality rates at different instars, and sex ratio of the population are required data to determine the pathogenicity of certain concentrations of EPF. The life table with inclusion of males increases knowledge about the population dynamics of the pests (Peroni and Hernández 2011). Therefore, interaction studies of isolates are essential to predict the effect of EPF on pest's population.

The objective of the study was to evaluate the changes in biological parameters of *H. armigera* larvae treated with different isolates of *B. bassiana* and *M. anisopliae* and compare the efficiency of these pathogens.

Materials and methods

Fungal isolates

Twenty-three isolates of the fungi *B. bassiana* and *M. anisopliae* were obtained and used in the bioassays. They were isolated from the collection of EPF cultures (ARSE F-USDA) of the School of Agriculture Luiz de Queiroz (ESALQ, Piracicaba, SP, Brazil) and Biological Institute (IBCB, Campinas, SP, Brazil) (Table 1). Suspensions of each fungus used in the bioassays were prepared by scraping the conidia produced in Potato Dextrose Agar (PDA) culture medium with 0.002 µl of Tween 80°. Each fungal suspension was subjected to serial dilutions of conidia counting in a Neubauer chamber (Alves 1998), following a concentration pattern of 10⁸ conidia ml⁻¹ for each treatment.

Conidia viability test

The conidia viability test carried out as follows: a thin layer of PDA (approximately 2.0 ml) was placed in a plate after the solidification of the medium. Each plate received 0.1 ml of the fungal suspension, which was prepared by each fungal isolate in 10 ml of sterile distilled water and 0.02% Tween 80°. The suspensions spread, using a Drigalski loop, after flamed and properly cooled.

Table 1 Origin of entomopathogenic fungi used in the bioassays

Isolates	Pathogens	Origin	Host
ARSEF 3293	<i>Metarhizium anisopliae</i>	México, MEX	<i>Spodoptera frugiperda</i>
ARSEF 1080	" "	Gainesville, USA	<i>Helicoverpa zea</i>
ESALQ 114	" "	Goiânia, GO	<i>Mahanarva posticata</i>
ESALQ 1051	" "	Porto Alegre, RS	<i>Solenopsis</i>
ESALQ 860	" "	Piracicaba, SP	<i>Macropsis</i> sp.
ESALQ 935	" "	Itabúna, BA	<i>Diatraea saccharalis</i>
IBCB 418	" "	Iporanga, SP	Soil - Atlantic Forest
IBCB 383	" "	Araras, SP	<i>Mahanarva fimbriolata</i>
IBCB 425	" "	Iporanga, SP	Caterpillar
IBCB 364	" "	Córrego Rico, SP	Soil
ARSEF 1149	<i>Beauveria bassiana</i>	Córdoba, ARG	<i>Helicoverpa armigera</i>
ARSEF 1788	" "	Spain, ES	<i>Helicoverpa virescens</i>
ESALQ 986	" "	Piracicaba, SP	<i>Blatella germanica</i>
ESALQ 1289	" "	Caçú, GO	<i>Leptopharsa heveae</i>
ESALQ 1075	" "	Arapongas, PR	Soil
ESALQ 480	" "	Cuiabá, MT	<i>Solenopsis invicta</i>
ESALQ 1288	" "	Pontes e Lacerda, MT	<i>Rhynchophorus palmarum</i>
IBCB 36	" "	S. J. do Rio Pardo, SP	<i>Hypothenemus hampei</i>
IBCB 170	" "	Goiânia, GO	<i>Cosmopolites</i> sp.
IBCB 38	" "	Araras, SP	<i>Cosmopolites sordidus</i>
IBCB 35	" "	Cruz das Almas, BA	<i>Cosmopolites sordidus</i>
IBCB 241	" "	Pindamonhangaba, SP	<i>Oryzophagus oryzae</i>
IBCB 1363	" "	Pariquera-Açu, SP	Soil - rubber tree plantation

The plates were then kept in a greenhouse at $25 \pm 2^\circ\text{C}$ and 12-h photophase for 24 h. The plates were examined under an optical microscope with $\times 400$ magnification for counting of 100 conidia, germinated and non-germinated, establishing their proportion.

***Helicoverpa armigera* mortality test**

Second-instar larvae of *H. armigera* were used for mortality bioassays. The population of the pest was at the 5th generation. The trials consisted of 23 treatments, 10 of which were isolates from *M. anisopliae*, 13 from *B. bassiana*, and a control. Two hundred caterpillars were used in each treatment. They are placed in polyethylene containers (18×30 mm), under an artificial diet (Greene et al. 1976), and then inoculated with $0.2 \mu\text{l}$, using a $10\text{-}\mu\text{l}$ pipette. The same procedure was used at the control, but replacing the fungal suspension with distilled water plus 0.02 ($v v^{-1}$) of Tween 80°. The plastic containers with the insects were closed and kept in an air-conditioned room (25°C , RH of $70 \pm 10\%$, and photophase of 12 h). Mortality evaluations started 3 days' post inoculation, considering the total number of dead insects at the end of 7 days. The caterpillars were identified as dead when presented no movement to the touch of a soft bristle brush. The death of the insects due to the pathogen was confirmed as follows: the insects were washed by 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. This made it possible to observe the extrusion of the pathogen, confirming the infection. The isolates that caused deaths of *H. armigera* above 80% up to the 7th day of evaluation were selected for the subsequent stages of the study.

Lethal concentration (LC_{50}) of *M. anisopliae* and *B. bassiana* for *H. armigera* caterpillars

Mean lethal concentration (LC_{50}) was estimated using 2nd-instar *H. armigera* caterpillars that were exposed to 7 concentrations (10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 conidia ml^{-1}) of isolates previously selected in the mortality tests. One hundred caterpillars/treatment were used. The methodology for these bioassays, storage, and evaluation were the same as those used in the mortality tests.

Evaluation of sublethal effects

Fifty caterpillars that survived the exposure to LC_{50} concentrations of the most promising isolates (*B. bassiana* ARSEF 1149, ARSEF 1788, ESALQ 986, ESALQ 1288, IBCB 36, IBCB 170) (*M. anisopliae* ESALQ 860, ESALQ 1051) were monitored daily to assess the biological parameters: instar duration (days), caterpillar weight (mg), pupal weight (mg), sex ratio, fecundity, egg viability (%), and male and female adult longevity.

The effects of different treatments on caterpillar, prepupa, and pupa weights were evaluated by weighing them on an analytical balance (SHIMADZU-ATY224*) at the 7th day. Sexing the pupae was carried out according to the observations of the anal and genital openings, according to Zenker et al. (2007), with the aid of a magnifying glass (ZEIZZ-STEMI 508°). The males were separated from females, and couples were formed to estimate the adult pre-ovipositional period (APOP) and total ovipositional period (TPOP). The formed couples were kept at the same conditions as they reared. Ovipositional period and longevity were recorded daily. Raw data of time for development, survival, daily fertility, and male and female longevity were used for the analysis of biological variables of *H. armigera* and development of the life table. All tested individuals were considered for these evaluations, including those who died during the development of immature stages (Chi and Liu 1985 and Chi 1988). 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 by the Chi and Liu's methodology (1985), using the equations:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \quad (1)$$

$$l_x = \sum_{j=1}^{\beta} S_{xj} \quad (2)$$

$$m_x = \frac{\sum_{j=1}^{\beta} S_{xj} f_{xj}}{\sum_{j=1}^{\beta} S_{xj}} \quad (3)$$

where β is the number of life stages; s_{xj} is the age-specific survival rate, considering the difference and standard deviation; l_x is the age-specific survival rate; f_{xj} is the age-specific fertility of the stage; and m_x is the age-specific fertility.

Statistical analysis

A completely randomized design with 24 treatments and 200 replications was used to select the isolates; each caterpillar was a replication. Statistical analysis was performed using the SAS program (University 2002); when the means were significant, they were compared by the Tukey test at 5% probability. The mortality data found for the different concentrations were subjected to Probit analysis, using the POLO PC Software (Leora-software 1987) to estimate the LC_{50} .

Regarding the sublethal effects, the bootstrap procedure was used to estimate the variations, means, and standard error of the biological parameters: time for

development, fertility, and longevity, using 100,000 bootstraps. The parameters of the *H. armigera* populations under fungal treatments and the control were compared by the paired bootstrap test ($p < 0.05$), based on the differences in confidence interval (CI = 95%) using the TWOSEX-MS-Chart program (Chi 2016). Hierarchical cluster analysis was used, with the Euclidean dissimilarity measured by the coefficient similarities. Ward's clustering method was used to identify the similarity between groups. Non-hierarchical cluster analysis was used by the K-means method, with k corresponding to the number of groups indicated in the hierarchical cluster analysis. The data of the variables were processed in the R program (R Core Team, 2018).

Results and discussion

Mortality rates

Mortality rates of 2nd-instar *H. armigera* caterpillars caused by the isolates of *M. anisopliae* and *B. bassiana* were different. The highest means were found at the treatments of IBCB 425 and ESALQ 860 for *M. anisopliae*, and IBCB 1363 and IBCB 36 of *B. bassiana* (Fig. 1). The number of highly virulent isolates against *H. armigera* was high at the fungus *B. bassiana*, corroborating the results of Hassani (2000), who tested the virulence of the fungus *B. bassiana* against *H. armigera* and *Spodoptera littoralis* caterpillars and found that several isolates of *B. bassiana* were highly virulent against noctuid species.

Conidial viability

Conidial viability of the isolates ranged 42.0–98.0% at the concentration between 1×10^2 and 1×10^8 , respectively. The isolates ARSEF 1149, ESALQ 1288, IBCB 36, IBCB 170, and IBCB 1363 of the *B. bassiana* fungus presented a high virulence and the highest numbers of viable conidia. The isolates ESALQ 860, ESALQ 1051, and IBCB 425 of *M. anisopliae* fungus had the highest number of viable conidia, differing from the other isolates, presenting the same virulence in the mortality test (Fig. 1). The 8 isolates that caused deaths of *H. armigera* above 80% were used in the tests to estimate LC_{50} .

LC_{50} estimates (Table 2)

H. armigera longevity and fecundity

Ovipositional period was affected in 2 *M. anisopliae* isolates—ESALQ 860 (3.59 days) and ESALQ 1051 (4.2 days). The control presented 5.2 days, but with non-significant difference (Tables 3 and 4), as shown by the paired bootstrap method. Total time for development in the control (46.66 days) was shorter than that at the treatments ESALQ 860 (48.88 days) and ESALQ 1051 (58.8 days), but did not differ from that of the isolate IBCB 425

(47.7 days). A high susceptibility of *H. armigera* larvae to *B. bassiana* isolates was found and also observed by Nguyen et al. (2007) and Kalvnadi et al. (2018). However, the results of larval developmental period differed from those presented by Nguyen et al. (2007).

The total pre-ovipositional period (TPOP) in the treatments of ESALQ 860 and ESALQ 1051 was significantly longer than that in the control (38.52 days), but did not differ from that of the IBCB 425 treatment. The adult pre-ovipositional period (APOP) in the treatments was similar to that in the control. The average fertility of females in all treatments was lower than in the control. The lowest egg viability was found at the isolate IBCB 425 (60.14%) (Table 3).

The ovipositional duration of caterpillars exposed to *B. bassiana* isolates was different from that in the control (5.2 days) (Table 4), but similar to those of the other isolates. The developmental periods of the larvae and pupae of the isolates were different and were affected in all treatments than in the control. Strategies to avoid or overcome the actions of the pathogens are different. Each host population evolves and presents the different immune responses (Lu et al. 2016). The isolates may have intrinsic characteristics, which related to virulence. One of these characteristics is the production of extracellular enzymes; the production of enzymes can be different depending on the isolate and present temporal variations (Wang et al. 2009).

The maintenance of this virulence above the levels of the enzyme production may also be related to the genetic diversity of these fungi, which may be influenced by the habitat in which each isolate was obtained (Mora et al. 2016). A research comparing the location and isolation method showed that fungi isolated from soil resulted to a high mortality rate of *H. armigera* caterpillars (Kalvnadi et al. 2018). In the present study, the highest virulence was found for caterpillars exposed to isolates from soil; the isolate IBCB 1363, for example, was isolated from the soil of a rubber tree plantation. However, *M. anisopliae* isolates showed non-effect of the habitat.

Adult longevity is affected by the treatments of both sexes. It was shorter at some isolates and longer in others than the control (45.00 for females and 48.07 for males). The female longevity decreased at *B. bassiana* isolates, consistent with the results of Wu et al. (2014), and increased at *M. anisopliae* isolates, differing from the results found by Jarrahi and Safavi (2016). Longevity was low in 3 treatments IBCB 36 (35.16 days), IBCB 170 (29.46 days), and IBCB 1363 (42.78 days), differing significantly from the control (46.66 days). The isolates ARSEF 1149 (47.72 days) and ESALQ 1288 (47.08 days) did not differ from the control (Table 4). TPOP was affected by the most treatments; it was short at the isolate IBCB 36 (30.00), long at the isolates ESALQ 1288

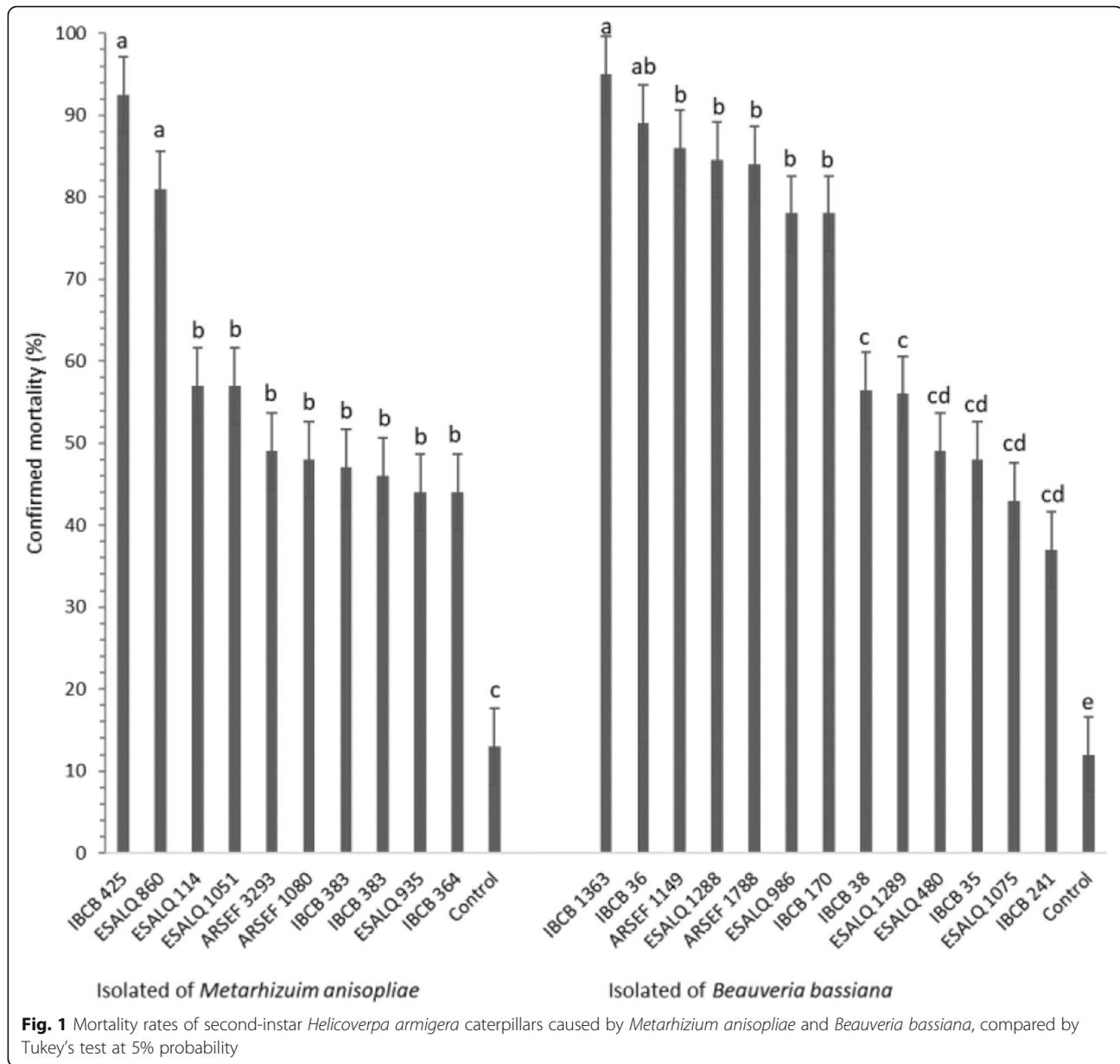


Fig. 1 Mortality rates of second-instar *Helicoverpa armigera* caterpillars caused by *Metarhizium anisopliae* and *Beauveria bassiana*, compared by Tukey's test at 5% probability

(48.88), and similar at the isolates IBCB 170 (40.36), IBCB 1363 (37.53), and ARSEF 1149 (37.45), when compared to the control (38.52).

The APOP in the control was 2.70 days, similar to those of the isolates ARSEF 1149 (2.45), IBCB 170 (2.36), and IBCB 1363 (2.53). The APOP of the ESALQ 1288 (3.44) and IBCB 36 (3.00) was significantly different from those of the other isolates. The average fertility of females in all treatments was lower than that in the control (470.83), but did not differ from each other at the isolates IBCB 1363 (328.27), ARSEF 1149 (221.23), ESALQ 1288 (338.12), and IBCB 1363 (328.27). The egg viability was low, mainly at the isolates IBCB 1363 (75.24%) (Table 4).

Jarrahi and Safavi (2016) found different results than Arthurs and Thomas (2000), who reported that the adult pre-ovipositional period and total ovipositional period extended in *H. armigera* at *M. anisopliae*. The egg viability of females treated with the isolates was low, as found by Kalvnadi et al. (2018); however, it differed from the results of Arthurs and Thomas (2000), who found no reduction in fertility of adults treated with *M. anisopliae*.

Grouping of parameters

Complementing the results of the bootstrap analysis, hierarchical and non-hierarchical classifications were performed for each fungal isolate. The hierarchical

Table 2 Estimates of LC₅₀ of *Beauveria bassiana* and *Metarhizium anisopliae* isolates for second-instar *Helicoverpa armigera* larvae, using the Polo-PC program

Treatments	LC ₅₀ (conidia mL ⁻¹)	Slope ± SE	N	χ ²
<i>Beauveria bassiana</i>				
ARSEF 1149	1.4 × 10 ⁶ (5.8 × 10 ⁵ –8.1 × 10 ⁶)	0.36 ± 0.05	100	8.39
ESALQ 1288	1.5 × 10 ⁵ (3.3 × 10 ⁴ –5.6 × 10 ⁶)	0.46 ± 0.05	100	3.88
IBCB 36	2.9 × 10 ⁵ (1.8 × 10 ⁴ –7.1 × 10 ⁶)	0.55 ± 0.05	100	2.99
IBCB 170	5.4 × 10 ⁴ (2.8 × 10 ³ –5.1 × 10 ⁶)	0.29 ± 0.05	100	6.26
IBCB 1363	4.5 × 10 ⁴ (2.3 × 10 ³ –8.3 × 10 ⁶)	0.25 ± 0.05	100	1.75
<i>Metarhizium anisopliae</i>				
IBCB 425	6.8 × 10 ⁵ (1.4 × 10 ⁴ –2.7 × 10 ⁷)	0.30 ± 0.05	100	7.2
ESALQ 860	7.8 × 10 ⁶ (1.8 × 10 ⁶ –3.7 × 10 ⁷)	0.22 ± 0.05	100	9.25
ESALQ 1051	5.6 × 10 ⁶ (2.1 × 10 ⁷ –1.4 × 10 ⁸)	0.23 ± 0.05	100	4.68

N number of caterpillars tested, χ² chi-squared value, LC₅₀ lethal concentration, total conidia ml⁻¹

cluster analysis, represented by a dendrogram, formed 4 groups: group 1 was formed by the ESALQ 1051 isolate, which was characterized by a lower mortality rate, and greater caterpillar longevity and pupa and total pre-ovipositional periods. Group 2 was formed by IBCB 36, characterized by a low egg, pupa, caterpillar, fecundity, and oviposition. Group 3 was formed by ESALQ 1288 and IBCB 425, and group 4 was formed by ARSEF 1149, IBCB 1363, ESALQ 860, and IBCB 170, characterized by average values for all parameters (Figs. 2 and 3). The results described were confirmed by the clustering analysis (Figs. 2 and 3); groups were formed despite the isolates had different characteristics. The differences among isolates tend to be lower when considering all parameters

of the sublethal effect, promoting an effective control for *H. armigera*.

Principal component analysis

The groups obtained in the previous analysis were confirmed by subjecting the results to principal component analysis (Fig. 4). The eigenvalues of the principal components (PC) PC1 and PC2 explained 70% of the response variation of *H. armigera* caterpillars to the exposure to fungal isolates. Therefore, the total amount of information contained in the evaluated parameters formed 4 distinct groups, which were similar to those observed in the hierarchical cluster analysis. PC1 explained 45.45% of the variance (eigenvalue of 4.54); the parameters with greater discriminatory power in PC1 were BAT (0.76), LOGOV (-0.65), LOGLA -0.78), PUPA (-0.86), LOGTO (-0.95), and TPOP (-0.88). PC2 explained 24.51% of the variance (eigenvalue of 2.45); the parameters with the greatest discriminatory power were PRPU (0.68), FECU (0.90), and OVI (0.69). The biplot graph (Fig. 4) shows the distribution of lethal and sublethal parameters of *H. armigera* caterpillars exposed to fungal isolates. The figure shows the parameters that best discriminate each isolate.

The positive correlations discriminated the isolates at the right of PC1 (IBCB 425, ESALQ 1288, IBCB 1363, and IBCB 36), and the negative correlation discriminated the isolates at the left of PC1 (ESALQ 1051, ESALQ 860, ARSEF 1149, and IBCB 170) (Fig. 4). The variables with positive correlation discriminated the isolates in the upper part of PC2 (IBCB 425 and ESALQ 1288), and the

Table 3 Developmental periods (days) of different stages, longevity, fecundity, pre-ovipositional, and total ovipositional period of *Helicoverpa armigera* caterpillars infected with different isolates of *Metarhizium anisopliae*, and a control (untreated)

Parameters	Treatment with <i>Metarhizium anisopliae</i>			
	IBCB 425	ESALQ 860	ESALQ 1051	Control
Egg (days)	3.00 ± 0.00 a	3.00 ± 0.00 a	3.00 ± 0.00 a	2.89 ± 0.00 a
Caterpillars (days)	9.00 ± 0.00 d	14.7 ± 0.36 c	24.36 ± 0.16 a	17.00 ± 0.00 b
Pre-Pupa (days)	10.39 ± 0.12 a	5.92 ± 0.16 b	5.26 ± 0.41b	2.00 ± 0.00 c
Pupa (days)	16.00 ± 0.00 b	25.26 ± 0.22 a	25.04 ± 0.22 a	15.00 ± 0.25 b
Adult female longevity (days)	47.52 ± 0.73 c	53.05 ± 0.69 b	63.93 ± 0.61 a	45.00 ± 0.72 c
Adult male longevity (days)	51.64 ± 0.36 c	60.71 ± 0.74 b	65.42 ± 0.46 a	48.07 ± 0.39 c
Total Longevity (days)	47.7 ± 1.09 bc	48.88 ± 0.70 b	57.78 ± 0.19 a	46.66 ± 0.44 c
APOP (days)	2.71 ± 0.12 a	2.41 ± 0.10 a	2.53 ± 0.13 a	2.70 ± 0.26 a
TPOP (days)	40.71 ± 0.12 c	48.32 ± 0.25 b	58.8 ± 0.34 a	38.52 ± 0.58 c
Fertility (egg/F ₁ female)	411.52 ± 46.65 b	289.18 ± 21.42 d	308.27 ± 23.67 c	470.83 ± 24.5 a
Oviposition in days	5.2 ± 0.51 a	3.59 ± 0.23 b	4.2 ± 0.42 b	5.2 ± 0.34 a

APOP adult pre-oviposition period, TPOP total pre-oviposition period—both calculated using females that produced fertile eggs. Average oviposition days were calculated for days when the number of eggs laid was greater than zero. Standard errors were estimated using 100,000 bootstraps and compared using the paired bootstrap test based on the differences in confidence intervals. Means followed by different letters in the rows are significantly different (p < 0.05) by the paired bootstrap test

Table 4 Developmental periods (days) of different stages, longevity, fecundity, pre-ovipositional, and total ovipositional period of *Helicoverpa armigera* caterpillars infected with different isolates of *Beauveria bassiana*, and a control (untreated)

Parameters	Treatment with <i>Beauveria bassiana</i>					
	ARSEF 1149	ESALQ 1288	IBCB 36	IBCB 170	IBCB 1363	Control
Egg (days)	3.00 ± 0.00 a	3.00 ± 0.00 a	2.90 ± 0.00 a	2.95 ± 0.00 a	3.00 ± 0.00 a	2.89 ± 0.00 a
Caterpillars (days)	13.88 ± 0.16 b	8.2 ± 0.21 c	5.76 ± 0.14 d	9.00 ± 0.00 c	15.00 ± 0.00 ab	17.00 ± 0.00 a
Pre-Pupa (days)	2.6 ± 0.22 b	8.2 ± 0.21 a	8.71 ± 0.14 a	2.64 ± 0.14 b	3.32 ± 0.07 c	2.00 ± 0.00 b
Pupa (days)	16.24 ± 0.06 b	16.56 ± 0.41b	11.00 ± 0.00 d	23.45 ± 0.19 a	15.35 ± 0.28 c	15.00 ± 0.25 c
Adult female longevity (days)	46.00 ± 0.32 a	41.29 ± 0.92 b	34.43 ± 0.20 c	45.27 ± 0.27 a	42.6 ± 0.4 b	45.00 ± 0.72 a
Adult male longevity (days)	49.07 ± 0.37 ab	50.06 ± 0.88 a	37.81 ± 0.49 c	47.18 ± 0.42 b	48.56 ± 0.34 b	48.07 ± 0.39 b
Total Longevity (days)	47.72 ± 0.32 a	47.08 ± 0.92 a	35.16 ± 0.86 c	29.46 ± 2.13 d	42.78 ± 0.67 b	46.66 ± 0.44 a
APOP (days)	2.45 ± 0.19 b	3.44 ± 0.16 a	3 ± 0.0 a	2.36 ± 0.15 b	2.53 ± 0.13 b	2.70 ± 0.26 b
TPOP (days)	37.45 ± 0.19 b	48.88 ± 0.35 a	30 ± 0.0 d	40.36 ± 0.15 b	37.53 ± 0.12 b	38.52 ± 0.58 bc
Fertility (egg/F ₁ female)	221.23 ± 7.35 d	338.12 ± 31.86 b	250.05 ± 25.1 d	289.00 ± 45.07c	328.27 ± 44.39 b	470.83 ± 24.5 a
Oviposition in days	4.18 ± 0.17 b	3.72 ± 0.23 c	3.14 ± 0.30 c	3.90 ± 0.31 bc	4.06 ± 0.31 b	5.2 ± 0.34 a

APOP adult pre-oviposition period, TPOP total pre-oviposition period—both calculated using females that produced fertile eggs. Average oviposition days were calculated for days when the number of eggs laid was greater than zero. Standard errors were estimated using 100,000 bootstraps and compared using the paired bootstrap test based on the differences in confidence intervals. Means followed by different letters in the rows are significantly different ($p < 0.05$) by the paired bootstrap test

variables with negative correlation discriminated the isolates in the lowest part of PC2 (ESALQ 1051, ESALQ 860, ARSEF 1149, IBCB 170, IBCB 1363, and IBCB 36). According to the results for the variables of the isolates, the isolates IBCB 425 and ESALQ 1288 potentially had a great pre-pupa weight, adult pre-ovipositional period, and mortality, and the isolates ESALQ 1051, ESALQ 860, ARSEF 1149, and IBCB 170 had a high pupal weight and caterpillar longevity.

Sex ratio and caterpillar and pupa weights
Effect of *B. bassiana* on biological parameters of *H. armigera*

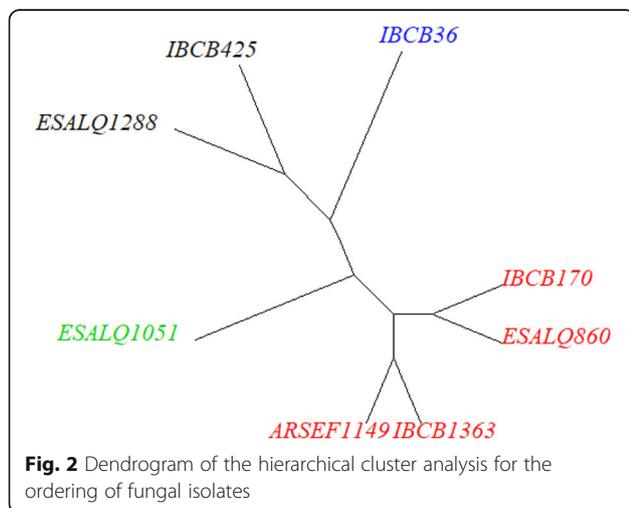
The weight of caterpillars after 10 days of exposure to the treatments was lower than that in the control, with differences among treatments. The pre-pupal

and pupal weights of the isolate IBCB 1363 were affected, with consequent abnormal pupae and defective adults (Fig. 5).

Fungi act via tegument, which is an advantage over other groups of pathogens that only enter the insects orally. This contact action is due to the secreting of a series of compounds, including bassianin, bassiacridin, oosporeins, cyclosporine, and destruxins (Molnar et al. 2010; Wang et al. 2010 and Gibson et al. 2014), which were dependent on the specificity of the host and can suppress their immune response (Amiri-Besheli et al. 2000).

The exoskeleton and digestive tract of insects are barriers that can be broken and trigger several cellular and hormonal mechanisms, resulting in an immune response (Levy et al. 2004). During these responses, insects begin to lose lipids and proteins that are essential to complete their cycle (Qu and Wang 2018), explaining their weight loss at the pupal stage and the non-emergence of adults found in the present study.

Kaur et al. (2011) reported morphological abnormalities in *Spodoptera litura*, using EPF. Most caterpillars treated with *B. bassiana* isolates were unable to complete their larval phase, and extending this stage and early forming pupae. This is explained as the actions of enzymes, and the insects' immune system, which accelerate the molting process, reducing the production of youth hormone and maintaining the production of ecdysone, which induce the ecdysis process (Qu and Wang 2018). The concentrations of these hormones in the hemolymph determine the completion of metamorphosis and the reproductive process of insects, which may also explain the decrease in fertility of adults treated



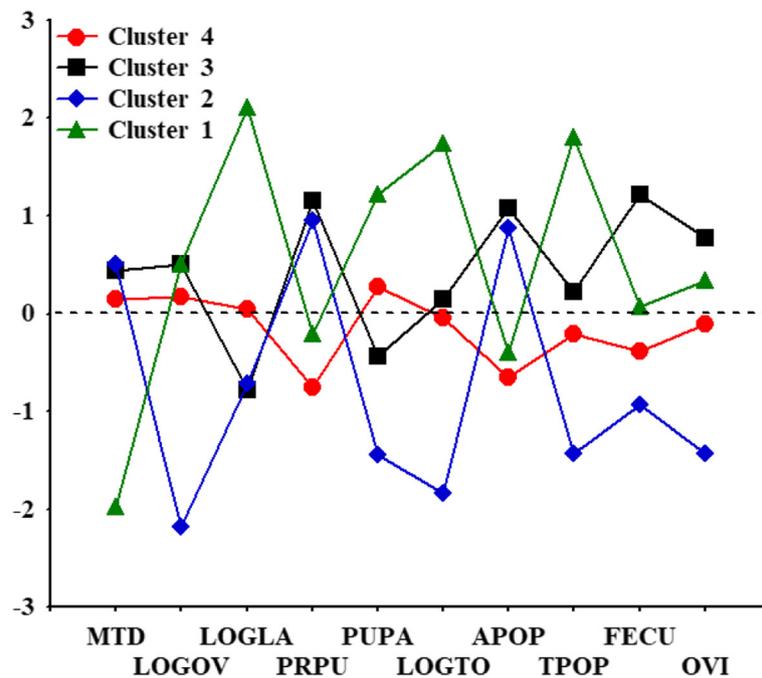


Fig. 3 Non-hierarchical cluster analysis (K-means) for the ordering of fungal isolates. Mortality (MTD), egg stage duration (LOGOV), caterpillar longevity (LOGLA), pre-pupa longevity (PRPU), pupa stage duration (PUPA), total longevity (LOGTO), adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), fertility (FECU), oviposition (OVI)

with the isolates when compared to the control (Rohlf and Churchill 2011).

Survival decreased considerably at the pupal stage, i.e., surviving caterpillars reached the pupal stage with a decrease weight and morphological malformations that caused their death (Fig. 5). These

morphological deformations are explained by the insect defense system, which is composed of structural barriers and active responses against external elements that reach the hemocoel, such as EPF (Renwanz 1986). The sex ratio is not affected by any of the treatments (Table 5).

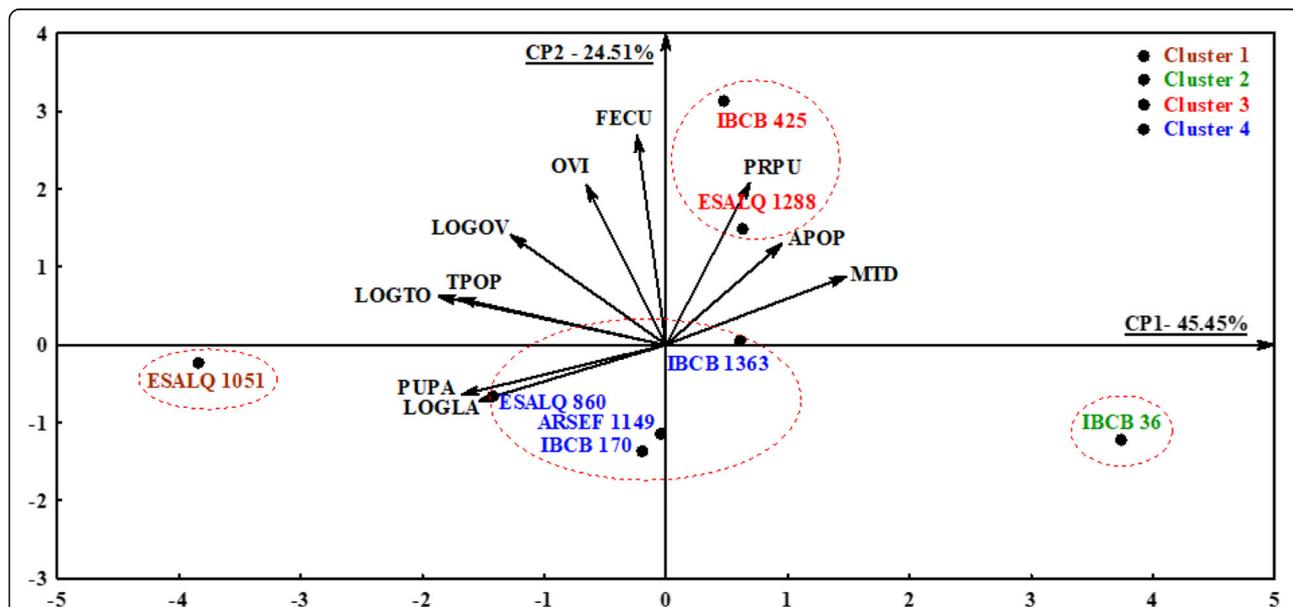


Fig. 4 Biplot graph for distribution of fungal isolates and variables in the first two main components



Fig. 5 Morphological abnormalities in *Helicoverpa armigera* treated with LC₅₀ of the fungus *B. bassiana* (isolate IBCB1363). **a** Interference in the development of caterpillars shown by the difference in size. **b** Early pupal phase. **c** Low pupal weight and size. **d** Pupal and adult deformations

Effect of the *M. anisopliae* on biological parameters of *H. armigera*

The caterpillar weight after 10 days of application of the treatments was lower than that in the control for all treatments. The pre-pupal and pupal weights of the

ESALQ 860 and ESALQ 1051 isolates differed than the control, but were similar to each other. The IBCB 425 isolate did not differ from the control, but differed from the other isolates. The effect of the treatments on the sex ratio was non-significant (Table 5).

Table 5 Sex ratio, number of eggs per female, egg viability, caterpillar weight, pre-pup weight (g), and pupa weight (g) of *Helicoverpa armigera* treated with *Beauveria bassiana* and *Metarhizium anisopliae*

Treatments	Sex ratio	Egg viability (%)	10th day weight	Pre-pupal weight	Pupa weight
<i>Beauveria bassiana</i>					
ARSEF 1149	0.55	95.87 ± 0.95 a	0.10 ± 0.01 b	0.36 ± 0.01 a	0.31 ± 0.00 a
ESALQ 1288	0.52	92.23 ± 0.86 a	0.09 ± 0.01 b	0.36 ± 0.01 a	0.30 ± 0.00 a
IBCB 36	0.50	95.16 ± 0.98 a	0.07 ± 0.00 b	0.31 ± 0.01 a	0.32 ± 0.01 a
IBCB 170	0.41	83.09 ± 0.78 b	0.09 ± 0.01 b	0.36 ± 0.01 a	0.33 ± 0.00 a
IBCB 1363	0.40	75.24 ± 0.72 c	0.07 ± 0.00 b	0.21 ± 0.01 b	0.20 ± 0.01 b
Control	0.50	96.36 ± 0.65 a	0.26 ± 0.01 a	0.33 ± 0.02 a	0.32 ± 0.01 a
<i>Metarhizium anisopliae</i>					
IBCB 425	0.50	60.14 ± 0.69 c	0.07 ± 0.01 bc	0.31 ± 0.02 a	0.32 ± 0.01 a
ESALQ 860	0.43	86.83 ± 0.41 b	0.08 ± 0.01 bd	0.22 ± 0.01 b	0.24 ± 0.01 b
ESALQ 1051	0.41	93.56 ± 0.58 a	0.09 ± 0.00 bd	0.22 ± 0.02 b	0.25 ± 0.01 b
Control	0.50	97.39 ± 0.65 a	0.21 ± 0.01 a	0.36 ± 0.01 a	0.35 ± 0.00 a

Means (± standard error) followed by the same letter in the columns are not significantly different by Tukey's test at 5% significance level ($p < 0.05$)

Table 6 Mean parameters (\pm SE) of the *Helicoverpa armigera* life table subjected to different isolates of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* over one generation at 25 ± 1 °C and $70 \pm 10\%$ RH

Population parameters					
Treatments	<i>Beauveria bassiana</i>				
	<i>T</i> (daya)	R_0	<i>r</i> (day ⁻¹)	λ (day ⁻¹)	GRR
ARSEF 1149	40.03 \pm 0.15 b	97.34 \pm 15.88 b	0.114 \pm 0.00 b	1.12 \pm 0.00 a	97.34 \pm 15.88 c
ESALQ 1288	51.07 \pm 0.32 a	83.12 \pm 16.41 c	0.086 \pm 0.00 c	1.09 \pm 0.00 a	87.05 \pm 18.01 d
IBCB 36	31.93 \pm 0.11 c	70.14 \pm 17.24 d	0.133 \pm 0.00 a	1.14 \pm 0.00 a	78.39 \pm 19.01 e
IBCB 170	42.76 \pm 0.14 b	63.58 \pm 19.39 e	0.097 \pm 0.00 c	1.10 \pm 0.00 a	146.44 \pm 38.82 b
IBCB 1363	40.15 \pm 0.24 b	98.48 \pm 24.85 b	0.114 \pm 0.00 b	1.12 \pm 0.00 a	148.94 \pm 36.55 b
Control	41.29 \pm 0.16 b	216.58 \pm 35.01 a	0.130 \pm 0.00 a	1.11 \pm 0.00 a	248.61 \pm 44.39 a
Treatments	<i>Metarhizium anisopliae</i>				
	<i>T</i> (day)	R_0	<i>r</i> (day ⁻¹)	λ (day ⁻¹)	GRR
IBCB 425	43.63 \pm 0.28 c	172.84 \pm 34.50 b	0.118 \pm 0.00 b	1.13 \pm 0.00 a	205.23 \pm 42.71 b
ESALQ 860	51.05 \pm 0.28 b	127.24 \pm 22.23 c	0.094 \pm 0.00 c	1.09 \pm 0.00 a	136.45 \pm 25.41 c
ESALQ 1051	61.05 \pm 0.33 a	92.48 \pm 21.09 d	0.074 \pm 0.00 d	1.07 \pm 0.00 a	113.35 \pm 26.16 d
Control	41.29 \pm 0.16 c	216.58 \pm 35.01 a	0.130 \pm 0.00 a	1.11 \pm 0.00 a	248.61 \pm 44.39 a

The data of each parameter were calculated using 100,000 bootstraps. Means followed by the same letters are not different by the paired bootstrap test ($p < 0.05$). *T* average duration of one generation (days); R_0 net reproduction rate, *r* intrinsic rate of increase (day⁻¹), λ finite rate of increase (day⁻¹)

Life table

The average generation time (*T*) of *H. armigera* treated with the *B. bassiana* isolates ARSEF 1149, reached 40.03 days, and ESALQ 1288 (51.07 days) had a significant difference than the control (41.29 days). The net reproduction rate (R_0) in the control was 216.58. It was 2.5% higher than those in the treatments (Table 6).

The intrinsic rate of increase (*r*) in the control (0.130 day⁻¹) was significantly higher than those at 4 isolates. Only the isolate IBCB 36 showed no difference than the control. The finite rate of increase (λ) in the treatments showed a non-significant difference. The gross reproduction rate (GRR) of *H. armigera* in the control (248.61) was higher than those of all isolates tested, showing a significant difference between females at all treatments (Table 6). All *M. anisopliae* isolates affected the average generation time (*T*), which was significantly higher than in the control. The net reproduction rate (R_0) of the treatments was lower than that in the control. The intrinsic rate of increase (*r*) in the control (0.130) was higher than that in the treatments, but the finite rate of increase (λ) showed no difference for any isolate tested. The gross reproduction rate (GRR) in the control (248.61) was higher than that in the treatments IBCB 425 (205.23), ESALQ 860 (136.45), and ESALQ 1051 (113.35) (Table 6).

Beauveria bassiana and *M. anisopliae* affected the biological parameters *T*, R_0 , *r*, λ , and GRR of *H. armigera*, but with lower intensity than the control, and depending on the isolate. The net reproduction rate (R_0) was significantly lower than in the control, promoting effects

that will compromise the subsequent generation, acting effectively in the control of the species.

Conclusion

The use of sublethal concentrations of the fungi isolates of *M. anisopliae* and *B. bassiana* resulted in an adverse effect on the biology of *H. armigera*. The high number of promising fungus isolates increases the probability of an effective control of these species by these fungi and reduction in the development of resistance in *H. armigera* populations.

Abbreviations

Bt: *Bacillus thuringiensis*; ARSEF: Collection of Entomopathogenic Fungal Cultures; ESALQ: School of Agriculture Luiz de Queiroz; 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

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Authors' contributions

TDS: 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. RAP: Project supervisor; guided the data collection and reviewed the manuscript. All authors have read and approved the 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.

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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