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Applying different infective structures of *Beauveria bassiana* to *Coffea arabica* infested with *Hypothenemus hampei* (Coleoptera: Curculionidae) under field

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ABSTRACT
Coffee is the most appreciated beverage in worldwide; Brazil is the largest producer and exporter of this commodity. Organochlorine endosulfan was banned from the country in 2013 due to its teratogenic agent-related features. Since then, coffee plantations have experienced increased *Hypothenemus hampei* infestation rates. The aim of the current study is to assess variations in the rates of *Coffea arabica* fruits brocaded by *H. hampei* after the application of entomopathogenic fungal species *Beauveria bassiana* IBCB66. Experiments were carried out with 'Catuaí' and 'Mundo Novo' cultivars between 2018 and 2020, during the borer transit period. Three experiments were carried out based on the application of the aforementioned fungal species on the investigated coffee plant species, both by spraying and sprinkling, at 30-day intervals; 10 fruits were collected per face of each useful plant in each repetition. The experiment has followed a randomized blocks design with five treatments, including the control, and five repetitions, each. *Beauveria bassiana* Ecobass (IBCB66) wettable powder spray, at the concentration of $2 \times 10^{13} \cdot ha^{-1}$, was used in experiments I and II. On the other hand, the mix used in experiment III was prepared with blastospores at concentration of $5 \times 10^{12} \cdot ha^{-1}$ blastospores + 0.1% Silwet. The sprinkling process in all three experiments has used dry aerial conidia at concentration of $2 \times 10^{13} \cdot ha^{-1}$. Collected data were subjected to analysis of variance (ANOVA), which was followed by Fisher's test at 5% probability level, in the SISVAR software. More than 35,000 fruits were assessed. In addition to variations between experiments, results have evidenced that the rate of brocaded fruits remained high.

Keywords: catuaí; ibcb66; blastospores; aerial conidia; spraying.

INTRODUCTION

Coffee is a shrub species belonging to the genus *Coffea*, family Rubiaceae. Its culture has important and prominent history in Brazil, which is the world's largest producer of this commodity (D'AGOSTINI et al., 2008; AGNOLETTI et al., 2019; BARBOSA et al., 2021). Beetle species *Hypothenemus hampei*, also known as coffee borer, stands out among the main pests affecting coffee crops; it is widely distributed in virtually all territories holding coffee plantations (COSTA et al., 2002; SANTORO et al., 2007; JARAMILLO et al., 2010; VEGA et al., 2015; BRITO et al., 2020; PEREIRA et al., 2021). The origin of *H. hampei* remains unknown, although it is likely African, given its incidence in the same coffee-origin regions (JARAMILLO et al., 2011).

Endosulfan use in Brazil was banned by the National Health Surveillance Agency (ANVISA) in 2013; since then, coffee plantations have been experiencing high coffee berry borer infestation rates, countrywide (MOTA et al., 2017; PEREIRA et al., 2021).

Coffee borer is not an easy-to-handle insect, since it is quite resistant and adapts well to different environmental conditions. These features enable it to attack, and to survive in, both fruits, yet in the plant, and seeds, in the soil, at the most varied maturation stages (FERREIRA et al., 2003; MOTA et al., 2017; FOLLETT, 2018). *Hypothenemus hampei*

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has cryptic habit and endogamous behavior; moreover, this multivoltine and monophagous species presents fully intrafruit cycle—only adult fertilized females leave the fruit of origin for proliferation purposes (JARAMILLO et al., 2006; PARDEY, 2015). In coffee culture, climatic factors, such as increased humidity and shading rates, higher density, difficulty to carry out mechanized harvesting, lack of transfer and post-harvest grain collection, favor the development and proliferation of this pest (LAURENTINO; COSTA, 2004; VEGA et al., 2015).

Biological control is the alternative adopted by producers of different cultures given the need of using products accounting for less risk to human health and to natural enemies of different pests, as well as for less negative environmental impacts (DALZOTO; UHRY, 2009; PARRA; REIS, 2013; GEREMIAS, 2018; MEDEIROS; RODRIGUES, 2017; PEREIRA et al., 2021).

Fungal species *Beauveria bassiana* is widely used for the biological control of coffee berry borer species *H. hampei*—several formulations and products based on it are available in the market. Despite the significant use of this entomopathogenic fungus, studies focused on investigating blastospore-based formulations, as well as their application in the field, remain scarce. Moreover, further studies focused on investigating its mechanisms of action in large-scale production systems to enable its effective and low-cost trading and application are necessary (FARIA; MAGALHÃES, 2001; LEITE et al., 2003; OTTATI-DE-LIMA et al., 2010; J. ALMEIDA, 2020; CHAGAS-JUNIOR et al., 2020).

Thus, the aim of the current study was to assess the effectiveness of *B. bassiana* against coffee berry borer insects, based on the variation rate recorded for *Coffea arabica* fruits brocaded by *H. hampei*, after the application of aerial conidia and blastospores deriving from the IBCB66 entomopathogen.

MATERIAL AND METHODS

The *B. bassiana* IBCB66 used in the experiments had origin in "Oldemar Cardim Abreu" Entomopathogenic Fungi Collection, Biological Institute in Campinas, São Paulo, Brazil. This strain was previously selected of *H. hampei* (ALMEIDA et al., 2009).

The coffee-growing site used in the herein conducted experiments is approximately 25 years old; it grows two *C. arabica* cultivar types, namely: 'Mundo Novo' and 'Catuaí'. The assessed coffee plants were grown in close, although different, plots. The investigated site was not subjected to the application of any chemical pesticide in the last 13 years or to any pest and disease control in the last three years.

Viability tests were applied to the strains used in each formulation adopted for the herein implemented treatments, before the experiments were carried out. Only formulas showing viability higher than 90% were used in the current study. Previous collection was carried out to confirm infestation by *H. hampei* in the study site. The experiment has followed a randomized blocks design, with five plots for each cultivar and five treatments, namely: control, soil sprinkling, plant sprinkling, soil spraying and plant spraying; with five repetitions, each, including the control. Five useful plants per plot were used for 'Mundo Novo', whereas three useful plants per plot were used for 'Catuaí'. Two experiments were carried out between December 2018 and March 2019; they were called experiments I and II. Experiment III was carried out between November 2019 and March 2020.

All three experiments—i.e., experiments I, II and III—adopted the same sprinkling application methodology. Fungal suspension concentration was standardized at 2×10^{13} CFU·ha^{-1}. Applications were carried out with the aid of manual, sterile and single-use duster. The fungal suspension sprayed in experiments I and II also presented standardized concentration of 2×10^{13} CFU·ha^{-1}; it was applied with the aid of backpack sprayer STIHL SG20. Three fungal suspension applications were carried out at 30-day intervals; coffee fruit collections were performed at 7, 14, 21, and 28 days after each application. Ten fruits were randomly collected per plant, five in each side of it. This procedure was performed by taking into consideration plants' conditions in the field; it aimed at assessing infection caused by the applied fungus in *H. hampei* individuals.

Experiment III was carried out in the site planted with 'Catuaí', between November 2019 and March 2020. The adopted experimental design was the same one used in experiments I and II. The mix applied through the spray application method was made of blastospores at concentration of 5×10^{12} CFU·ha^{-1}, added with 0.1% Silwet spreader. It was applied with the aid of Guarany backpack sprayer. Rice powder + fungus was used for sprinkling application purposes; the application was performed with the aid of manual, sterile and single-use duster, at standardized concentration of 2×10^{13} CFU·ha^{-1}. Three applications were carried out at 30-day intervals, each. Two coffee fruit collections were performed fortnightly, after each application—10 fruits were randomly collected from each useful plant by taking into consideration plants' fructification conditions. The total number of fruits in each treatment and the number of brocaded fruits were evaluated at each collection time.

The study site used for all three experiments was demarcated with numbered stakes, by respecting the row and edge plants; fungal suspension applications were always carried out between 4:00 pm and 6:30 pm to favor fungal adhesion and germination, and to increase the likelihood of spraying coffee berry borers during flight. In total, more than 35,000 fruits were evaluated under Stemi DV4 Zeiss stereomicroscope, at 12- to 16-fold magnitude increase, depending on image clarity necessary to assess fruits' condition.

Data collected in experiments I and II were subjected to statistical analysis. Brocaded fruit indices were checked through analysis of variance (ANOVA); means were compared to each other through Fisher's least significant difference test at 5% probability level—original data were transformed through the square root of x + 0.5 in the analysis conducted in the SISVAR software (FERREIRA, 2011). ANOVA was applied in experiment III; means were compared to each other through Duncan's test (VIEIRA, 2011) at 5% probability level, in SPSS Statistics software version 12.1.

RESULTS AND DISCUSSION

Viability tests applied to products based on aerial conidia deriving from *B. bassiana* IBCB66 recorded viable aerial conidia rates ranging from 85.5% to 90%, whereas mean colony forming units at blastospores' concentration of 2.33×10^6 was in compliance with indices indicating microorganism efficiency, based on OTTATI-DE-LIMA et al. (2010).

Tolerable indices indicated to monitor *H. hampei* infestation correspond to 3% of infected fruits (MOTA et al., 2017). Based on previous collections carried out in the experimental site, registered indices were much above the indicated one, since they reached approximately 19% for 'Mundo Novo' (experiment area I) and 32%, for 'Catuaí'.

There was small variation in bored fruit rates 30 days after the first application of fungal species *B. bassiana*, although there was not decrease in them. There was statistical difference among plant spraying, plant sprinkling and soil sprinkling treatments, 28 days after fungal solution application. These treatments recorded smaller number of bored fruits than both the control and the soil sprinkling treatments (Fig. 1).

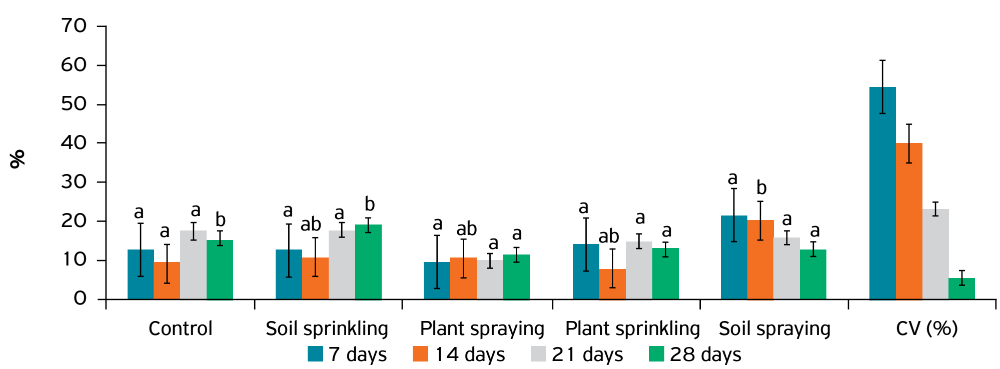

Figure 1. Bored fruit rates (%) after the first application of treatments conducted with conidia deriving from *Beauveria bassiana* IBCB66, from December 2018 to January 2019. Means followed by the same letter did not significantly differ from each other in Fisher's test, at 5% probability level.

OLIVEIRA et al. (2014) have exposed conidia deriving from *Metarhizium anisopliae* and *B. bassiana* to UV radiation variations and observed high sensitivity of both species to UV rays. Their research has evidenced germination capacity decrease by 38% and 65% in the investigated entomopathogens, respectively. These results have indicated that the high temperature observed on the day of the first application may have affected the efficiency of the applied strain.

The bored fruit rate observed after the second application was higher than the previous ones. However, it is worth emphasizing that it was not possible performing the collections planned for the 7th and 14th experimental days. Moreover, significant differences between mean bore fruit rates were not recorded for the herein performed collections (Fig. 2).

Mean rate (%) ranged from 18.24% (7 days) to 16.15% (14 days) after the third application. The other variations in it were not statistically significant. Mean fruit infestation remained higher than the economic loss threshold (3%) (Fig. 3).

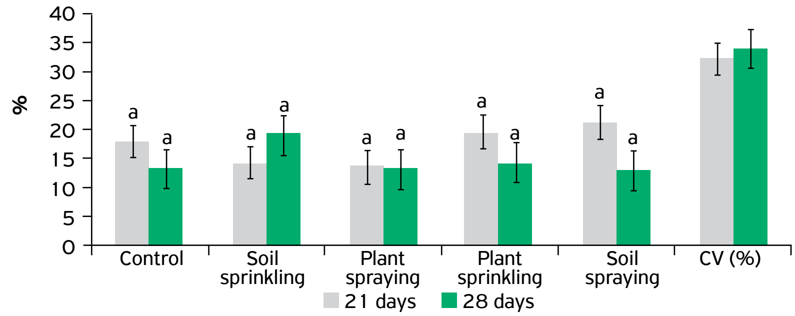

Figure 2. Bored fruit rates (%) after the second application of treatments conducted with conidia deriving from *Beauveria bassiana* IBCB66, January–February 2019. Means followed by the same letter did not significantly differ from each other in Fisher's test, at 5% probability level.

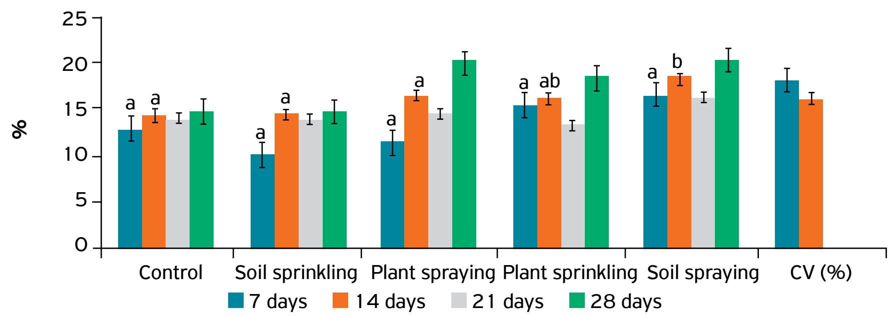

Figure 3. Bored fruit rates (%) after the third application of treatments conducted with conidia deriving from *Beauveria bassiana* IBCB66, February–March 2019. Data recorded for the 21st and 28th experimental days were not significant. Means followed by the same letter did not significantly differ from each other between applications; data recorded for the 21st and 28th experimental days were not significant, based on Fisher's test, at 5% probability level.

The previous collection performed in experiment II—which was conducted with 'Catuaí'—has shown bored fruit rates ranging from 21.19% to 34.25%. This outcome has evidenced that this cultivar is more susceptible to attacks by *H. hampei* than 'Mundo Novo'. The soil spraying treatment accounted for 15.99% of bored grains at the first application conducted at the 7th experimental day and for 11.33% of bored grains at 21st experimental day. The 21st experimental day recorded the lowest bored fruit rate for all treatments, with emphasis on the soil spraying method, which was more efficient than the others (Fig. 4).

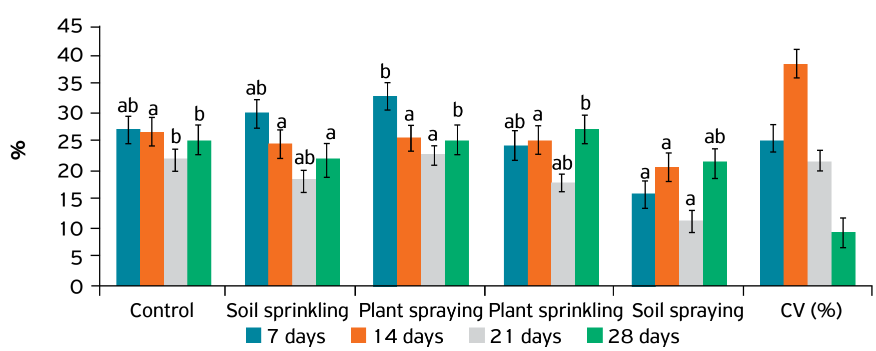

Figure 4. Bored fruit rates (%) after the first application of treatments conducted with conidia deriving from *Beauveria bassiana* IBCB66, from December 2018 to January 2019. Means followed by the same letter did not significantly differ from each other in Fisher's test, at 5% probability level.

Similar to experiment I, assessments performed at the 21st and 28th experimental days did not show decreased number of bored fruits after the second application, in comparison to the previous collection, or statistically significant differences between them (Fig. 5).

The lowest mean number of bored grains was recorded after the third application of the soil sprinkling treatment - the lowest bored fruit rate (11.99%) was recorded at the last collection (28 days). Bored grain rate recorded for this treatment remained the lowest one during the evaluation of the third application (Fig. 6).

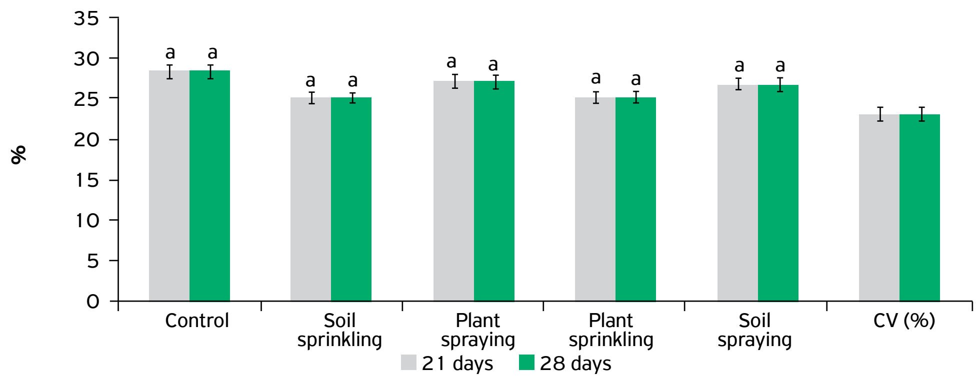

Figure 5. Bored fruit rates (%) after the second application of treatments conducted with conidia deriving from *Beauveria bassiana* IBCB66, January/February 2019. Means followed by the same letter did not significantly differ from each other in Fisher's test, at 5% probability level.

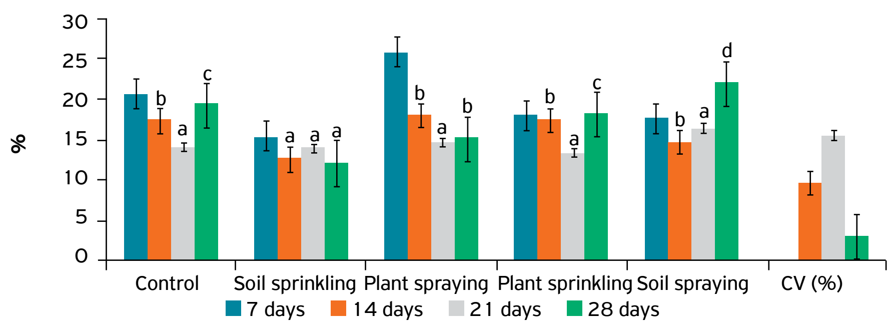

Figure 6. Bored fruit rates (%) after the third application of treatments conducted with conidia deriving from *Beauveria bassiana* IBCB66, February–March 2019. Means followed by the same letter did not differ from each other, and columns without letter were not significant, in Fisher's test, at 5% probability level.

The lowest bored fruit rate recorded for experiment III was observed for collection 1, after the first application; the control treatment accounted for 28.0% of it. The lowest bored fruit rate recorded after the second application was observed for the plant spraying treatment with blastospores, which accounted for 38.8% of bored fruits. Soil spraying with blastospores was the treatment presenting the lowest bored fruit rate after the third application; it accounted for 34.7% of bored fruits (Table 1).

Table 1. Fruits attacked (%) by coffee berry borer species *Hypothenemus hampei*, treated with IBCB66 blastospore suspension spraying and with *Beauveria bassiana* conidia sprinkling, in site planted with 'Catuaí' coffee (São Paulo, SP).

(n=5)[1]	1st application Nov. 2019		2nd application Dec. 2019		3rd application Jan. 2020	
Treatments	**Collection 1**	**Collection 2**	**Collection 3**	**Collection 4**	**Collection 5**	**Collection 6**
Control	28.0 ± 4.9 a	31.0 ± 2.6 a	42.8 ± 2.7 a	52.3 ± 2.5 a	42.0 ± 1.3 b	45.3 ± 2.0 a
Spray. Bl. Pl.	44.0 ± 9.3 a	41.3 ± 4.9 a	38.8 ± 2.9 a	45.7 ± 1.7 a	40.8 ± 1.3 ab	44.6 ± 2.3 a
Spray. Bl. So.	30.6 ± 4.5 a	33.2 ± 4.5 a	42.6 ± 3.9 a	50.0 ± 4.0 a	34.7 ± 2.0 a	40.0 ± 1.0 a
Sprin. Co. Pl.	36.7 ± 4.9 a	34.0 ± 3.1 a	43.6 ± 2.7 a	50.6 ± 2.5 a	39.7 ± 3.0 ab	45.3 ± 2.2 a
Sprin. Co. So.	38.0 ± 4.6 a	38.6 ± 4.4 a	41.5 ± 2.8 a	44.2 ± 1.3 a	41.7 ± 1.9 b	42.0 ± 2.2 a
CV (%)	38	25.2	14.8	14.4	12.3	10.5

[1]Means followed by the same letter, in the columns, did not significantly differ from each other in the Duncan test, at 5% probability level.
CV: coefficient of variance.

The blastospore suspension treatment recorded decreased bored fruit rates in comparison to treatments with rice conidia + fungus powder. Results recorded by OTTATI-DE-LIMA et al. (2010) have indicated that blastospores have caused *Diatraea saccharalis* mortality rate ranging from 26% to 29% for *M. anisopliae* and *B. bassiana* in the assessed media, respectively. Blastospores' resistance in the environment is quite low due to the sensitivity of its hyphal structure. However, these structures are more virulent and infectious than aerial conidia; besides, their mass production process through liquid fermentation is more profitable (LEITE et al., 2003). The study by OKUMURA et al. (2003) based on the spraying application of B. bassiana conidia to control coffee berry borers in coffee drying terraces, has evidenced insect mortality rate of 49.72% at concentration of 1×10^{11} conidia·ha^{-1}. However, mortality rate of 44.63% was also recorded for other reasons, such as handling the borer during the experiment and incidence of saprophytic and/or opportunistic fungi. Although the concentration used by OKUMURA et al. (2003) to be lower than that used in this study, results too did not show satisfactory decrease in *H. hampei's* attack to coffee fruits.

Although infestation rates remained high after fungal applications with *B. bassiana*, there was *H. hampei* infection in plants subjected to Experiment III (Fig. 7), which was carried out from November 2019 to March 2020. This outcome shows that blastospores are infectious agents in the field, just like air conidia.

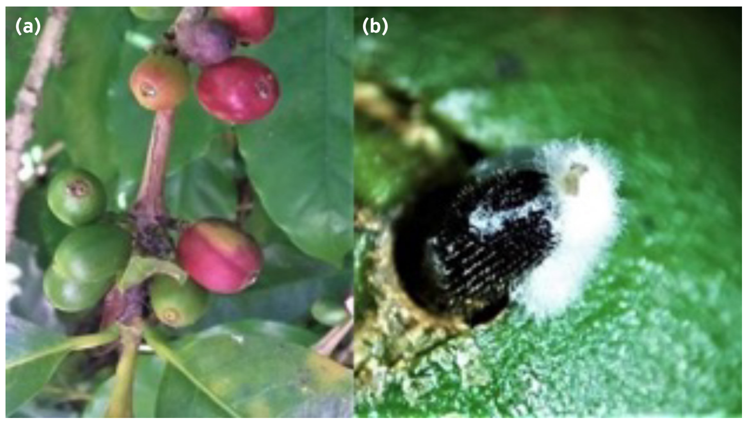

Figure 7. 'Catuaí' coffee fruits with *H. hampei*: (a) Infected by *B. bassiana* in the field; (b) Infected borer in fruit observed under Stemi DV4 Zeiss stereomicroscope, at magnification ranging from 12 to 16 times during laboratory analyses
Source: Elaborated by authors (2020).

CONCLUSIONS

Blastospores belonging to the IBCB66 strain are as infectious as their aerial conidia.

Biological control based on entomopathogenic fungi in areas affected by high *H. hampei* infestation is not enough to solve the problem.

It is necessary to adopt integrated management based on using other products, such as botanical extracts or even chemical products.

AUTHORS' CONTRIBUTIONS

Conceptualization: R. M. C. Pereira. **Data curation:** R. M. C. Pereira and J. E. M. Almeida. **Formal analysis:** R. M. C. Pereira and J. E. M. Almeida. **Investigation:** R. M. C. Pereira and J. E. M. Almeida. **Methodology:** R. M. C. Pereira, J. E. M. Almeida and A. Batista Filho. **Project administration:** R. M. C. Pereira and J. E. M. Almeida. **Supervision:** J. E. M. Almeida and A. Batista Filho. **Visualization:** R. M. C. Pereira and J. E. M. Almeida. **Writing – original draft:** R. M. C. Pereira. **Writing – review & editing:** R. M. C. Pereira, J. E. M. Almeida and A. Batista Filho.

AVAILABILITY OF DATA AND MATERIAL

If needed, contact the author.

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CONFLICTS OF INTEREST

Not applicable.

ETHICAL APPROVAL

Not applicable.

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