https://dx.doi.org/10.22161/ijaers.75.11 *ISSN: 2349-6495(P) | 2456-1908(O)*

Mortality of Coffee Berry Borer, *Hypothenemus hampei* in Field, with Pre and Post Application of Entomopathogenic Fungus *Beauveria bassiana* (Balsamo) Associated to Emulsifiers

Rafael Assis de Souza, Dirceu Pratissoli, Luis Moreira de Araujo Junior, Julia de Assis Pinheiro, Julielson Oliveira Ataíde, Luiza Akemi Gonçalves Tamashiro, Mariana Almeida dos Santos, Alixelhe Pacheco Damascena

Nucleus for Scientific and Technological Development in Phytosanitary Management (NUDEMAFI), Agronomic Sciences Center at the Federal University of Espirito Santo, Rua Alto Universitário, s/n, Caixa Postal 16, CEP: 29500-000, Alegre-ES, Brazil.

Abstract**—** *The coffee berry borer, Hypothenemus hampei (Ferrari, 1867) (Coleoptera: Curculionidae: Scolytinae) presents a cryptic life cycle, which occurs all within the fruit, which makes its control a difficult task. For this reason, one of the methods that has stood out is the biological control through the entomopathogenic fungus Beauveria bassiana (Balsamo) (Hypocreales: Cordycipitaceae). However, the efficiency of this fungus is conditioned to environmental factors, such as ultraviolet radiation, temperature, humidity, concentration, correct application, among others. Therefore, the objective of the present study is to evaluate the mortality of coffee berry borer, pre and post application of the entomopathogenic fungus Beauveria bassiana associated with emulsifiers under field conditions. The experiments were conducted in three farms in different locations. The fungus isolate used was the PL63, and emulsifiers (Gum arabic and X1) evaluated were gum arabic and X1 (product subject to patent). Two modes of application were performed: pre and post the coffee berry borer release. In the pre-application, the fungi were mixed in with the emulsifiers and the pure, and after thirty minutes, the coffee berry borer was released. In the post-application, it released the insects, and after 24 hours, the fungus was applied with the emulsifiers and pure. All treatments tested showed coffee berry borer mortality; the emulsifying agents (Gum and X1) in admixture with the fungus B. bassiana, did not increase the mortality of coffee berry borer; the application of the fungus B. bassiana before the entry of the drill into the coffee fruit, resulted in higher mortality.*

Keywords**— Biological control, Environmental factors, Microbial control, Protection.**

I. INTRODUCTION

The coffee berry borer, *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Curculionide: Scolytinae) is the most important coffee pest in the world (Infante et al., 2012). In Brazil, the losses exceed $ 300 million (Oliveira et al., 2013). Due to its cryptic life cycle, which occurs all within the fruit, the control becomes a difficult task (Damon, 2000; Vega et al., 2015).

Therefore, one of the control methods that has stood out is the biological through entomopathogenic fungus *Beauveria bassiana* (Bals.) (Hypocreales: Cordycipitaceae), which infects the coffee berry borer all around the world (Monzón et al., 2008; Vega et al., 2009; Wraight et al., 2018). This entomopathogen, naturally occurring, with insect infection levels ranging from 1 to 70% in several coffee producing countries like the Brazil (Costa et al., 2002), Colombia (Posada-Flórez et al., 2008), Nicaragua (Monzón et al., 2008), Puerto Rico (Gallardo-Covas et al., 2010) and Cameroon (Mbang et al., 2012). In most cases, the mortalities were attributed to highly favorable environmental conditions (during rainy seasons or extended periods of high humidity). The most favorable environmental conditions for fungus *Beauveria bassiana* activity in general are moderate temperature, high humidity and low sunstroke (Zimmermann, 2007; Jaronski, 2010; Wraight et al., 2018).

https://dx.doi.org/10.22161/ijaers.75.11 *ISSN: 2349-6495(P) | 2456-1908(O)*

In spite of this microbial agent play an important role in the control of *H. hampei*, its efficiency is conditioned to environmental factors, like ultraviolet radiation, temperature, moisture, altitude, product concentration, correct application, among others (Vega et al., 2015; Aristizábal et al., 2016). The ultraviolet radiation and high temperatures reduce field conidia viability, because this affects its metabolism, changing enzyme production processes, toxins, spore germination, germ tube development, penetration, colonization and reproduction (Alves & Lecuona, 1998; Rodrigues et al., 2016). Besides that, the fungus loses its ability to infect when subjected to direct sunlight for three hours (Alves & Lecuona, 1998; Rodrigues et al., 2016). Which makes these factors, the main obstacles to successful use of entomopathogenic fungi in agriculture (Rangel et al., 2008; Castrillo et al., 2010; Rodrigues et al., 2016).

In this context, the use of agents that may act as fungal protectors against adverse environmental conditions emerges as a possible alternative. Among these, the emulsifiers have received attention, which are very important additives in the food industries, and have excelled in encapsulation techniques, in order to increase the viability of microorganisms (Yáñez-Fernández et al., 2008). Some emulsifiers have been tested in admixture with the *Beauveria basssina* fungus. For example, 2% castor oil in admixture with Boveril® formulation in the concentration of 3 x 105 conidia/mL, presented mortality of larvae of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) significantly higher, when compared to using pure fungus (Rondelli et al., 2011). However, few studies evaluate the efficiency of emulsifiers as *B. bassiana* protective agents in *H. hampei* mortality under field conditions.

Therefore, the present study aims to determine the mortality of *H. hampei* in the field, pre and post application of the *B. bassiana* in association with emulsifiers.

II. MATERIAL AND METHODS

2.1 Study sites

The study was conducted in three coffee farms, in the months of May and June 2018.

Assay 1. Córrego Fundo site, located in the municipality of Mimoso do Sul (latitude 20° 95' 26.05'' S and longitude 41° 60' 57.43'' W), Espírito Santo, Brazil, altitude of 850 m, *Coffea arabica* cultivar 'Catuaí amarelo', with 15 years, spacing 2.0 x 1.0 m, average temperature of 25 ± 5 °C and relative humidity (RH) of 70 ± 10%

Assay 2. Experimental area of the Instituto Federal do Espírito Santo, Campus Alegre-ES, located in the Rive district, (latitude 20º 45' 50" S and longitude 41º 27' 25''), Espírito Santo, Brazil, altitude of 290 m, *Coffea arabica* cultivar 'Catuaí amarelo' with 15 years, spacing 2.5 x 1.0 m, average temperature of 26± 5 °C and RH of 60 ± 10%.

Assay 3. Bom Ver site, located in the municipality of Alegre, (latitude 20° 52' 23,07'' S and longitude 40° 29' 8,76'' W), Espírito Santo, Brazil, altitude of 700 m, *Coffea arabica* cultivar 'Catuaí vermelho' with 12 years, spacing 2.5 x 0.6 m, average temperature of 25 ±5 °C and RH of 65 ± 10%.

2.2 Obtaining the fungus

The *B. bassiana* fungus used in this work was the ESALQ-PL63 isolated. This isolated was obtained in acommercial product, Boveril®, provided by the company Koppert Brasil Ltda.

2.3 Emulsifiers

Two emulsifiers were used. The first one was the Arabic gum, polymer of D-glucuronic acid, L-raminose, D-galactose and L-arabinose, with approximately 5% protein. The second emulsifier was the X1 (product subject to patent), a polyalcohol derived from fruits.

2.4 Obtaining the insects

The *H. hampei* adult females were obtained from stock creation at the Nucleus for Scientific and Technological Development in Phytosanitary Management (NUDEMAFI), Agronomic Sciences Center at the Federal University of Espirito Santo, Alegre, ES (CCAE-UFES). These insects were raised in plastic boxes (15 x 30 x 5 cm), containing fruits, occupying only one side of the box, getting the other side free so the newly emerged coffee berry borer, when leaving the grains. So that they could move to the free extremity, for insect collection. The collection was made with small insect suction, adapted to a vacuum pump (Dalvi & Pratissoli, 2012).

2.5 Solution Preparation

500 mL distilled water was added in a Becker® of 1 L. Right after, a 1.0% (w/v) arabic gum solution was prepared, with subsequent incorporation of 1 g of *B. bassiana* fungus with suspension of 1x1012 conidia.mL-1. In a second Becker®, the same procedure was performed with 1.0% X1 (w/v) and 1 g of *B. bassiana* fungus. The concentration of emulsifiers was obtained from preliminary compatibility tests with the *B. bassiana*. The compatibility was calculated with the formula proposed by Alves, et al. (1998) to classify chemicals according to their toxicity to entomopathogenic fungi in vitro. The *B. bassiana*'s

https://dx.doi.org/10.22161/ijaers.75.11 ISSN: 2349-6495(P) | 2456-1908(O)

concentration was used according to the manufacturer's recommendation.

2.6 Product application

In each coffee plant, a rosette with ripe fruits was selected, on both sides, leaving five fruits in each. Two application modes were performed: coffee berry borer pre and post release. In pre-application, the conidia were pulverized, with the emulsifiers and pure, on the fruits and after 30 minutes, period for the solution to dry, the rosette was bagged with organza fabric (Fig. 1B), and then the insects were released. In the post application, five coffee berry borer were released on the bagged rosette, and after a period of 24 hours, with the confirmation of the insects that entered the fruit, the conidia were applied with a pre-compression spray, with 40 lb pressure, on the fruits to the point of dripping (Fig. 1A). After each application, the spray was washed with distilled water.

The experiments were conducted in randomized blocks with five treatments (T1 - Arabic gum + *B. bassiana*, T2 - X1+ *B. bassiana*, T3 - Arabic gum +X1+ *B. bassiana*, T4 - pure *B. bassiana*) and attestant (Water) with two repetitions per treatment. In each experiment five blocks were used.

The field coffee berry borer mortality evaluations were performed 12 days after the applications (12 DAA). The dead insects were taken to the NUDEMAFI Entomology Laboratory and then they were placed in a humid chamber and incubated in a type Biological Oxygen Demand (B.O.D) at 25 ± 2 °C, 80 ± 5% of RH and phototase of 12 h, for 10 days, to confirm mortality through pathogen sporulation.

The data were subjected to analysis of variance and the means were compared by Tukey test at 5% significance level in statistical software R (ExDes.pt package) (R Development Core Team 2009).

For collecting precipitation data (mm) and temperature (°C), weather stations were installed in the areas, where the experiments were conducted.

III. RESULTS AND DISCUSSION

Assay 1

The results obtained in this study showed no interaction between the factors, emulsifiers and application pre and post release of the coffee berry borer, in *H. hampei* mortality.

The coffee berry borer mortality occurred in all treatments. However, the treatments showed no significant difference among themselves (Table 1).

In the different application modes of the fungus, pre-release coffee berry borer application (P1) presented higher mortality with 40.62%, differing statistically (F39:32 = 4.19; p>0.005) of the post-release application (P2), which presented a mortality of 29.99% (Table 2).

Assay 2

The factors presented significant interaction, emulsifiers and application pre and post release of the coffee berry borer, in *H. hampei* mortality (F39:32 = 2.99; p>0.005) (Table 3).

The coffee berry borer mortality occurred in all treatments. However, the treatments in both modes of application did not differ statistically (Table 3).

The pre and post coffee berry borer applications presented mortality of *H. hampei*. However, only at T4 there was a statistical difference. In this treatment, the pre-release application of insects, with mortality of 48.88%, differed statistically from post-release application, with 17.77% (F39:32 = 6.01; p>0.005) (Table 3).

Assay 3

The factors presented significant interaction, emulsifiers and application pre and post release of the coffee berry borer, in *H. hampei* mortality (F39:32 = 33.32; p>0.005) (Table 4).

When comparing treatments, within application modes, the significant difference occurred only in the pre-release application, in which mortality in T1 (4.77±1.25%) differed, significantly, with lower value of T4 (30.43±12.39%) (F39:32 = 38.88; p>0.005) (Table 4).

When comparing application modes, within treatments, the significant difference occurred only in T1, in which the post-release application of insects with mortality of 23.91%, differed, significantly, of the pre-release application, with 4.77% (F39:32 = 0.30; p>0.005) (Table 4).

The emulsifiers (Arabic gum and X1), in mixture with *B. bassiana* fungus, did not show better coffee berry borer mortality rates in relation to the pure *B. bassiana* fungus. These results suggest that the Arabic gum and X1 did not increase the viability of conidia, through adverse environmental factors, mainly UV radiation, since the factors temperature and humidity were in favorable conditions for the development of the fungus. The UV light is one of the determining factors in the viability of conidia (Rodrigues et al., 2016; Wraight et al., 2018). Laboratory studies with pure conidia subjected to different radiation times, showed that in five minutes of exposure to UV light, with an irradiance of 6153.3 mW.m-2 or 22.15 kJ.m-2.h-1, the germination was 52%, while, that in control (without

https://dx.doi.org/10.22161/ijaers.75.11 ISSN: 2349-6495(P) | 2456-1908(O)

radiation) was 94%. At 10 minutes this rate dropped to 11%. At 15 minutes, dropped to 1,0% (Rodrigues et al., 2016).

Unprotected conidia of *Beauveria* are unable to survive a few hours of direct exposure to solar radiation (Edgington et al., 2000). Another factor that may have influenced the survival, without emulsifiers, was the fungal activity. In the case of conidia, this action can be delayed, once, the germ tube needs to break the emulsifier barrier, which leads to slower action (Rodrigues et al., 2016).

The mode of application of the fungus is one of the determining factors for the success in the control of *H. hampei*. This is due to the cryptic behavior of the insect. Because, it spends most of the life cycle inside the fruit; and only mated females go out to seek new fruit (Dalmon, 2000).

The results, obtained in the present study, indicate that in general the application of the fungus pre-release of the coffee berry borer, presented higher mortality when compared to post-release application. In the field, the contamination of the insect by *B. bassiana*, when the body is partially inside the fruit, it must to the presence of conidia in the fruit (Mota et al., 2017). The mortality of *H. hampei* in the post-release application of the insect, may have occurred due to the behavior of the coffee berry borer, that right after making the hole in the fruit, back to the surface to deposit the drilling material (Samuels et al., 2002).

IV. FIGURES AND TABLES

Table 1. Corrected mortality (%) of Hypothenemus hampei in Beauveria bassiana fungus applications associated with emulsifiers and pure. Córrego Fundo Site, municipality of Mimoso do Sul - ES, average temperature of 25 ± 5 °C, RH of 70 ±10 % and altitude of 850 m.

Treatments	Averages
T1 (Arabic gum + *B. bassiana*)	39.58±16.13a
T2 (X1+*B. bassiana*)	36.45±19.91a
T3 (Arabic gum +X1+ *B. bassiana*)	31.87±14.33a
T4 (Pure *B. bassiana*)	33.33±17.15a

*Averages represented by the same lowercase letters do not differ from each other at 5% probability level by Tukey test.

Table 2. Corrected mortality (%) of Hypothenemus hampei in Beauveria bassiana fungus applications associated with emulsifiers and pure, pre (P1) and post (P2) adults release. Córrego Fundo Site, municipality of Mimoso do Sul - ES, average temperature of 25 ± 5 °C and RH of 70 ±10 % and altitude of 850 m.

Application mode	Averages
P1 (coffee berry borer pre-release)	40.62±17.59a
P2 (coffee berry borer pos-release)	29.99±14.05b

*Averages represented by the same lowercase letters do not differ from each other at 5% probability level by Tukey test.

Table 3. Corrected mortality (%) of Hypothenemus hampei in Beauveria bassiana fungus applications associated with emulsifiers and pure, pre (P1) and post (P2) adults release. Experimental area of the Instituto Federal do Espírito Santo, Campus Alegre - ES, average temperature of 26 ± 5 °C, RH of 60 ± 10% and altitude of 290 m.

Treatments	Application mode	
	P1 (pre-release)	P2 (post-release)
T1 (Arabic gum + *B. bassiana*)	37.77±18.59Aa	28.88±9.93Aa
T2 (X1+*B. bassiana*)	28.88±12.66Aa	33.33±7.85Aa
T3 (Arabic gum +X1+ *B. bassiana*)	35.55±12.17Aa	28.88±18.59Aa
T4 (Pure *B. bassiana*)	48.88±9.93Aa	17.77±14.90Ab

*Averages followed by the same letters, uppercase in the column and lowercase in the row, do not differ from each other, at 5% probability level by Tukey test.

Fig.1. A - Beauveria bassiana fungus application associated with emulsifiers on the fruits; B - Coffee berry borer release after product application; C - Bagged fruits with organza fabric.

https://dx.doi.org/10.22161/ijaers.75.11 *ISSN: 2349-6495(P) | 2456-1908(O)*

Table 4. Corrected mortality (%) of Hypothenemus hampei in Beauveria bassiana fungus applications associated with emulsifiers and pure, pre (P1) and post (P2) adults release. Sítio Bom Ver Site, municipality of Alegre - ES, average temperature of 25 ± 5 °C, RH of 65 ± 10 % and altitude of 700 m.

Treatments	Application mode	
	P1 (pre-release)	P2 (post-release)
T1 (Arabic gum + *B. bassiana*)	4.77±1.25Bb	23.91±10.87 Aa
T2 (X1+*B. bassiana*)	15.75±4.70ABa	23.91±13.31Aa
T3 (Arabic gum +X1+ *B. bassiana*)	19.12±10.09ABa	21.51±12.11Aa
T4 (Pure *B. bassiana*)	30.43±12.39Aa	18.18A±10.02Aa

*Averages followed by the same letters, uppercase in the column and lowercase in the row, do not differ from each other, at 5% probability level by Tukey test.

V. CONCLUSION

All treatments tested showed mortality from coffee berry borer.

The emulsifying agents (Arabic gum and X1) in mixture with *Beauveria bassiana* fungus, did not increase coffee berry borer mortality.

The application of the *B. bassiana* fungus, in the pre-release of the coffee berry borer, increased the mortality.

ACKNOWLEDGEMENTS

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES).

REFERENCES

[1] Alves, S.B. & Lecuona, R.E. (1998). Epizootiology Applied to the Microbial Control of Insects. In: ALVES, S.B. (2ªed). Controle Microbiano de Insetos. Piracicaba, FEALOQ, p.97-170.

[2] Alves, S.B., Moino J.R.A. & Almeida J.E.M. (1998). Produtos fitossanitários e entomopatógenos. In: ALVES, S.B., (ed.). Controle microbiano de insetos. Piracicaba, FEALQ, p.217-238.

[3] Aristizábal, L.F., Bustillo, A.E. & Arthurs, S.P. (2016). Integrated Pest Management of Coffee Berry Borer: Strategies from Latin America that Could Be Useful for Coffee Farmers in Hawaii. Insects, 7:1, 11-14. DOI: http://dx.doi.org/10.1653/024.098.0165.

[4] Bustillo, A.E., Bernal, M.G., Benavides, P. & Chaves, B. (1999). Dynamics of *Beauveria bassiana* and *Metarhizium anisopliae* infecting *Hypothenemus hampei* (Coleoptera: Scolytidae) populations emerging from fallen coffee berries. Florida Entomologist, 82:4, 491-498. DOI: 10.2307/3496468.

[5] Costa, J.N.M., Silva, R.B., Ribeiro, P.A. & Garcia, A. (2002). Ocorrência de *Beauveria bassiana* (Bals) Vuill. em broca-do-café (*Hypothenemus hampei*, Ferrari) no estado de Rondônia, Brasil. Acta Amazonica, 32:1, 517-519. DOI: 10.1590/1809-43922002323519.

[6] Cruz, L.P., Gaitan, A.L. & Gongora, C.E. (2006). Exploiting the genetic diversity of *Beauveria bassiana* for improving the biological control of the coffee berry borer through the use of strain mixtures. Applied Microbiology and Biotechnology, 71:6, 918-926. DOI: 10.1007/s00253-005-0218-0.

[7] Dalvi, L.P., Pratissoli, D., Polanczy, R.A. & Andrade, G.S. (2011). Selection of native isolates of *Beauveria bassiana* (Ascomycota, Hypocreales) for the control of the coffee borer beetle *Hypothenemus hampei* (Scolytinae) in Brazil. Biological Letters, 48:1, 39-46. DOI: 10.2478/v10120-011-0006-8.

[8] Dalvi, L.P. & Pratissoli, D. (2012). Técnica de criação de *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Scolytidae). In: PRATISSOLI, D. (Ed.). **Técnicas de criação de pragas de importância agrícola, em dietas naturais**. Vitória: EDUFES. p.297-305.

[9] Damon, A. (2000). A review of the biology and control of the coffee berry borer, *hypothenemus hampei* (Coleoptera: Scolytidae). Bulletin of Entomological Research, 90: 6, 453-465. DOI: https://doi.org/10.1017/S0007485300000584.

[10] De La Rosa, W., Alatorre, R., Barrera, J.F. & Torriello, C. (2000). Effect of *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycetes) upon the coffee Berry Borer (Coleoptera: Scolytidae) Under Field Conditions. Journal of Economic Entomology, 93: 5, 1409-1414. DOI: 10.1603/0022-0493-93.5.1409.

[11] Edgington, S., Segura, H., La Rosa, W. & Williams, T. (2000). Photoprotection of *Beauveria bassiana*: Testing simple formulations for control of the coffee berry borer. International Journal of Pest Management, 46:3, 169-176. DOI: 10.1080/096708700415490.

[12] Gallardo-Covas, F., Hernandez, E. & PAGAN, J. (2010). Presencia natural del hongo *Beauveria bassiana* (Bals.) Vuill. en la broca del fruto del café *Hypothenemus hampei* (Ferrari) en Puerto Rico. The Journal of Agriculture of the University of Puerto Rico, 94:1, 195-198.

[13] Infante, F., Pérez, J. & Vega, F.E. (2012). Redirect research to control coffee pest. Nature, 489:1, 502-502. DOI: 10.1038/489502a.

[14] Jaronski, S.T. (2010). Ecological factors in the inundative use of fungal entomopathogens. BioControl, 55:1, 159-185. DOI: 10.1007/s10526-009-9248-3.

[15] Mbang, J.A.A., Mounjouenpou, P., Mahob, R.J., Mbarga Amougou, M., Mouen Bedimo, J. & Nyasse, S. (2012). Evaluation naturelle de l'impact de *Beauveria bassiana*: champignon entomopathogene dans la dynamique de population de *Hypothenemus hampei*, scolyte de baies des cerises de *Coffea canefora* [sic]. African Crops Science Journal, 20:2, 443-451.

[16] Monzón, A.J., Guharay, F. & Klingen, I. (2008). Natural occurrence of *Beauveria bassiana* in *Hypothenemus hampei* (Coleoptera: Curculionidae) populations in unsprayed coffee fields. Journal of Invertebrate Pathology, 97:2, 134–141. DOI: 10.1016/j.jip.2007.07.008.

[17] Mota, L.H.C., Silva W.D., Sermarini, R.A., Demétrio, C.G.B., Bento, J. M.S. & Delalibera, I.J.R. (2017). Autoinoculation trap for management of *Hypothenemus hampei* (Ferrari) with *Beauveria bassiana* (Bals.) in coffee crops. Biological Control, 111:1, 32-39. DOI: 10.1016/j.biocontrol.2017.05.007.

[18] Neves, P.M.O.J. & Hirose, E. (2005). Seleção de isolados de *Beauveria bassiana* para o controle biológico da broca-do-café *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). Neotropical Entomology, 34:1, 77-82. DOI: 10.1590/S1519-566X2005000100011.

[19] Posada-Flórez, F.J. (2008). Production of *Beauveria bassiana* fungal spores on rice to control the coffee berry borer, *Hypothenemus hampei*, in Colombia. Journal of Insect Science, 8:41, 1-13. DOI: 10.1673/031.008.4101.

[20] Rangel, D.E.N., Anderson, A.J. & Roberts, D.W. (2008). Evaluating Physical and Nutritional Stress during Mycelial Growth as Inducers of Tolerance to Heat and UV-B Radiation in *Metarhizium anisopliae* Conidia. Mycological Research, 112:11, 1362-1372. DOI: 10.1016/j.mycres.2008.04.013.

[21] R DEVELOPMENT CORE TEAM. R. (2009). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

[22] Rodrigues, I. M.W., Filho, A.B.; Giordano, I.B., Denadae, B.E., Fernandes, J.B. & Forim, M.R. (2017). Compatibility of polymers to fungi *Beauveria bassiana* and *Metarhizium anisopliae* and their formulated products stability. Acta Scientiarum. Agronomy, 39:4, 457-464. DOI: 10.4025/actasciagron.v39i4.32903.

[23] Rodrigues, I.M.W., Forim, M.R., Da Silva, M.F.G.F., FERNANDES, J.B. & FILHO, A.B. (2016). Effect of Ultraviolet Radiation on *Fungi Beauveria* bassiana and *Metarhizium anisopliae*, Pure and Encapsulated, and Bio-Insecticide Action on *Diatraea saccharalis*. Advances in Entomology, 4:1, 151-162. DOI: 10.4236/ae.2016.43016.

[24] Rondelli, V.M., Pratissoli, D., Polanczyk, R.A., Marquez, E.J., Sturm, G.M. & Tiburcio, M.O. (2012). Associação do óleo de mamona com *Beauveria bassiana* no controle da traça-das-crucíferas. Pesquisa Agropecuária Brasileira, 46:2, .212-214. DOI: 10.1590/S0100-204X2011000200014.

[25] Samuels, R.I., Pereira, R.C. & Gava, C.A.T. (2002) Infection of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae) by Brazilian isolates of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). Biocontrol Science and Technology, 12:5,.631-635. DOI: 10.1080/0958315021000016298.

[26] Vega, F.E., Infante, F., Castillo, A. & Jaramillo, J. (2009). The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae): a short review, with recent findings and future research directions. Terrestrial Arthropod Reviews, 2:2, 129-147. DOI: 10.1163/187498209X12525675906031.

[27] Vega, F.E., Infante, F.; Johnson, A.J. (2015). The genus *Hypothenemus*, with emphasis on *H. hampei*, the coffee berry borer. In: VEGA. F. E.; HOFSTETTER, R. W. (Eds.). Bark beetles: biology and ecology of native and invasive species. San Diego: Academic Press. p.427-494.

[28] Vera, J.T., Montoya, E.C., Benavides, P. & Góngora, C.E. (2011). Evaluation of *Beauveria bassiana* (Ascomycota: Hypocreales) as a control of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) emerging from fallen, infested coffee berries on the ground. Biocontrol Science and Technology, 21:1, 1-14. DOI: 10.1080/09583157.2010.517605.

[29] Wraight, S.P., Galaini-Wraight, S., Howes, R.L., Castrillo, L.A., Carruthers, R.I., Smith, R.H., Matsumoto, T.K. & Keith, L.M. (2018). Prevalence of naturally-occurring strains of *Beauveria bassiana* in populations of coffee berry borer *Hypothenemus hampei* on Hawai'i Island, with observations on coffee plant-*H. hampei*-*B. bassiana* interactions. Journal of Invertebrate Pathology, 156:1, 54-72. DOI: https://doi.org/10.1016/j.jip.2018.07.008.

[30] Yáñez-Fernández, J., Ramos-Ramírez, E. & Salazar-Montoya, J. (2008). Rheological characterization of dispersions and emulsions used in the preparation of microcapsules obtained by interfacial polymerization containing Lactobacillus Sp. European Food Research And Technology, 226:5, 957-966. DOI: 10.1007/s00217-007-0617-9.

[31] Zimmermann, G. (2007). Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology, 17:1, 553-596. DOI: http://dx.doi.org/10.1080/09583150701309006.

[32] Southey, J.F. (1986). Principles of sampling for nematodes: laboratory methods for work with plant and soil nematodes. London: Ministry of Agriculture, Fisheries and Food.

[33] Taylor, A.L. & Sasser, J.N. (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh: North Caroline State University.

[34] Triantaphyllou, A.C. & Hirschmann, H. (1960). Post infection development of *Meloidogyne incognita* Chitwood 1949 (Nematoda: Heteroderidae). Annales de L'Institut Phytopathologique Benaki, 3:1, 1-11.

[35] Williams, B.D., Schrank, B., Huynh C., ShownkeeN, R. & Waterston, R.H. (1992). A genetic mapping system in Caenorhabditis elegans based on polymorphic sequence-tagged sites. Genetics, 131:1, 609-24.

[36] Zambolim, L. (2015). Manejo de doenças. In: Fonseca, A., Sakiyma, N. & Borém, A. (Eds). Café Conilon do plantio à colheita. Viçosa, Minas Gerais: UFV, pp. 114-137.