

In vivo compatibility between *Beauveria bassiana* (Bals.) Vuillemin and castor oil on *Hypothenemus hampei* (Ferrari)

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ABSTRACT

The degree of *in vivo* compatibility between the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuillemin (Ascomycota: Hypocreales) and castor oil is important, as the interaction can be additive, synergistic or antagonistic. Thus, the objective of this study was to evaluate the *in vivo* compatibility between castor oil and *B. bassiana* on the coffee berry borer. The CCA-UFES/Bb-4 isolate of *B. bassiana* was used in concentrations of 0.0 (control), 1×10^4 , 1×10^5 , 1×10^6 and 1×10^7 conidia mL⁻¹. The concentrations of castor oil were: 0.0 (control), 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% v v⁻¹. The total and confirmed mortality were evaluated, and data submitted to variance analysis. To verify the effect of *B. bassiana* and castor oil concentrations, the data were submitted to regression analysis at 5% probability. Castor oil had an antagonistic effect on *B. bassiana* reducing the mortality of coffee berry borer. The mortality of *H. hampei* reduced caused by interaction between *B. bassiana* and castor oil, due to increasing concentration of castor oil. Lower interference of castor oil was observed for high concentrations of *B. bassiana*. Based on the results, management of *H. hampei* cannot be normally performed using the association between castor bean oil and *B. bassiana*, except if the economic viability of the higher concentrations justified.

Key words: *Ricinus communis*; Botanical insecticides; Biological control; Phytosanitary pest management; Coffee crop.

1 INTRODUCTION

Coffee (*Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner) is the tropical agricultural product most exported in the world and in 2015 the global coffee industry generated approximately 200 billion dollars (International Coffee Organization - OIC, 2019 Samper; Giovannucci; Vieira, 2020). In addition, coffee is also one of the most important commodities for Brazilian economy (Ministério da Agricultura, Pecuária e Abastecimento -MAPA, 2020a). However, the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), is one of the most important biotic factors that negatively affects coffee planting (Vega et al., 2014). This pest destructs fruits, reduces weight of beans, changes coffee type, classification and beverage taste (Vega et al., 2009). Thus, it is estimated that in Brazil, losses around 215 and 358 million dollars per year occur, just by *H. hampei* direct damages (Oliveira et al., 2013).

To reduce losses caused by coffee berry borer, effective management methods are necessary, due to the difficulties to control it inside coffee beans. Chemical method is the main used by farmers, however it presents several problems, such as an insufficient number of active ingredients, leading to the selection of resistant strains, and consequently to low efficiency (Campos et al., 2019; MAPA, 2020b). In addition, it can also cause environmental problems, such as elimination of beneficial fauna and increasing contamination of soil, water, atmosphere and living beings (Molina et al., 2014; Campos et al., 2019).

Such problems have driven research into new management methods, emphasizing the use of botanical insecticides and biological control (Dara, 2019). In the last decades, promising results have been obtained using compounds derived from aromatic plants to control agricultural pests (Isman; Grieneisen, 2014; Campos et al., 2019). Among these plants, castor bean (*Ricinus communis* L.) has shown toxicity to several pests, among them the coffee berry borer, either through the use of oil and/or extracts or by isolated compounds such as ricinin, ricinoleic acid and albumins 2S, Ric c 1 and Ric c 3 (Bigi et al., 2004; Ramos-López et al., 2010; Arnosti et al., 2011; Tounou et al., 2011; Nascimento et al., 2011; Celestino et al., 2015; 2016a; Machado et al., 2020).

Beauveria bassiana (Bals.) Vuillemin (Ascomycota: Hypocreales) stands out as one of the most important biological pest control agents in the biological control context (Vega et al., 2009; Celestino et al., 2018). Some factors that justify the interest in this fungi include its mass production “*in vitro*”, its wide genetic variability, possibility of use in organic crops, as well as, reduction in the use of synthetic chemical insecticides (Dalvi et al., 2011; Islam; Omar, 2012). In addition, it is noteworthy that *B. bassiana* is a registered biopesticide for a wide range of hosts, approximately 700 species of insect-pests, including *H. hampei* (Swathi; Visalakshy; Das, 2018).

In Colombia, since the arrival of the coffee berry borer in 1988, several researches were initiated by the National Coffee Research Center (Cenicafé), aiming at the biological

control of this pest. There, the success of the use of *B. bassiana* is due, among other reasons, to the shaded cultivation of the coffee tree, which provides sufficient protection against solar radiation and optimal levels of relative humidity at certain times of the day (Correal et al., 2018). This fact differs from Brazil, where most of the coffee plantations are carried out in full sun, being important the development of researches that aim to make compatible the association of methods that can confer protection against solar radiation and, also, help in the maintenance of humidity.

Although entomopathogenic fungi allow association with other management methods, it is necessary to study the degree of compatibility between them, since interaction can be additive, synergistic, or antagonistic (Islam; Omar, 2012; Celestino et al., 2018). Compatibility between vegetable oils and entomopathogenic fungi has some advantages, as they can be certified and used in organic crops; they are generally more viscous and, therefore, give greater adhesion to the surface of insects and plants; they are not flammable and safer; they provide some protection to spores against ultraviolet radiation and evaporate less than mineral oils (Alves; Faria, 2010). Therefore, in addition to *in vitro* compatibility testing, the *in vivo* testing is also important, since during insect infection process, numerous factors may be involved and affect the interaction between methods (Fang et al., 2005; Mohan et al., 2007; Islam; Olleka; Ren, 2010; Pedrini et al., 2010; Rondelli et al., 2011; Zhang et al., 2012). Thus, the objective of this study was to evaluate *in vivo* compatibility between castor oil and *B. bassiana* on coffee berry borer.

2 MATERIAL AND METHODS

The experiment was carried out in the sector of Entomology of the Nucleus of Scientific and Technological Development in Phytosanitary Management (NUDEMAFI) from Agricultural Sciences Center at the Federal University of Espírito Santo (CCA-UFES), in Alegre - ES.

2.1 Creation and maintenance of *H. hampei*

Creation and maintenance of coffee berry borer was carried out according to Celestino et al. (2016b), in an air-conditioned room at 25 ± 2 °C, relative humidity (RH) of $60 \pm 10\%$ and photophase of 12h. The brocaded coffee fruits of *Coffea canephora* Pierre ex Froehner were collected in the field, in the municipality of Jerônimo Monteiro - ES (latitude: $-20^{\circ} 47' 22''$ and longitude: $-41^{\circ} 23' 42''$).

2.2 Acquisition and production of *B. bassiana*

The CCA-UFES/Bb-4 isolate from collection of the Bank of Entomopathogens of the CCA-UFES was used, which was previously evaluated in previous studies that proved its

virulence to coffee berry borer (Dalvi et al., 2011). To be sure of pathogenicity and virulence, the isolate was previously reinvigorated in adults of coffee berry borer. During the bioassays, the fungi were grown in a PDA culture medium (potato-dextrose-agar) plus yeast and, after approximately ten days, they were multiplied in Petri dishes containing PDA plus yeast. They were incubated in an air-conditioned chamber at 25 ± 1 °C, RH of $70 \pm 10\%$ and photophase of 12h, where they remained for ten days.

2.3 Extraction and characterization of castor oil

The seeds of IAC 80 variety of *R. communis* plant were purchased from the Agronomic Institute of Campinas (IAC). The oil was extracted using the cold pressing method. Castor oil was stored in a container covered with aluminum foil and hermetically sealed. The castor oil used showed physical-chemical characteristics according to Celestino et al. (2016a) (Table 1).

Table 1: Physico-chemical characteristics of vegetable oils.

Chemical composition (%) ⁽¹⁾	Castor bean
Palmitic acid (C _{16:0})	0.70
Stearic acid (C _{18:0})	0.90
Oleic acid (C _{18:1})	2.80
Ricinoleic Acid (C _{18:1})	90.20
Linoleic acid (C _{18:2})	4.40
Linolenic Acid (C _{18:3})	0.20
Chemical constant	
Saponification (mg/KOHg)	182.90
Iodine (g I ₂ /100g)	84.50
Physical constant	
Refractive index (40°C)	1.479
Viscosity (cP) (30°C)	332.00

⁽¹⁾Number of carbon atoms: number of double bonds; (2) Composition of the oil used in the experiment according to Celestino et al. (2016a).

2.4 Compatibility between castor oil and *B. bassiana*

The experiments were carried out in an air-conditioned chamber at 25 ± 1 °C, RH of $70 \pm 10\%$ (a condition necessary for experiments with entomopathogenic fungi that require high humidity) and photophase of 12h. Suspensions of the *B. bassiana* isolate were adjusted to the following concentrations: 0.0 (control), 1×10^4 , 1×10^5 , 1×10^6 and 1×10^7 conidia mL⁻¹ in order to evaluate the compatibility. Castor oil concentrations were as follows: 0.0 (control), 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% v v⁻¹. Thus, association of fungi and castor oil concentrations corresponded to 35 treatments. In the

preparation of suspensions, an adhesive spreader (Tween® 80 PS; Company: Dinâmica Química Contemporânea LTDA, Diadema, SP) was used at 0.05% v v⁻¹.

The experimental units consisted of an acrylic box (gerbox®) (6 cm in diameter x 2 cm in height) lined with filter paper and containing 15 adult females of *H. hampei*. The spraying on the females of coffee berry borer was performed with Potter's Tower® at a pressure of 15 pounds in⁻², applying a volume of 5.5 mL per repetition. Thus, with the pressure and volume used in the Potter's Tower, an average volume of 1.78 mg cm⁻² was deposited, varying from 1.43 to 2.08 mg cm⁻² according to the recommendation of IOBC/WPRS ("International Organization for Biological and Integrated Control of Noxious Animals and Plants/West Palearctic Regional Section"), which is a deposit of 1.5 to 2.0 mg cm⁻² for glass or sheet surfaces (Overmeer; Van Zon, 1982). After spraying, 0.15 grams of ground coffee/gerbox® was offered as food.

The experimental design was completely randomized, with 5 repetitions, in a factorial arrangement 5 x 7 (*B. bassiana* concentrations x castor oil concentrations). Mortality evaluations were performed daily until the seventh day and the dead individuals were transferred to moist chambers in order to confirm the etiological agent. The wet chamber consisted of an acrylic box (gerbox®) (6 cm diameter x 2 cm height), in which a cotton pad moistened in the lid was placed and identified according to the treatment and repetition. After 10 days the cadavers were examined to check presence or absence of fungi.

The data of total mortality and confirmed mortality were submitted to variance analysis and to verify the effect of *B. bassiana* and castor oil concentrations, they were submitted to regression analysis, at 5% probability. Maximum mortality points were determined for the results that fitted to hyperbolic model. From these data, the concentration was determined to obtain 90% of maximum mortality point, according to the equations below:

$$X = (\beta_1 * (0.9 * PMM)) / (PMM - (0.9 * PMM))$$

β_1 = estimated parameter; PMM = point of maximum mortality.

Regression analysis was also performed, using the response surface methodology based on hyperbolic model with two independent variables, given by:

$$Z = (\beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \beta_3 X_1 Y_1) / (\beta_4 + X_1)$$

Z = Parameter analyzed (total mortality); X₁ = *B. bassiana* concentration; Y₁ = Castor oil concentration; β_i , with i ranging from 0 to 4 = estimated parameters.

The model presented above was chosen based on equation that best fitted to the data, based on coefficient of determination (R²) and betas significance (β) and regression by F test, at 5% probability.

3 RESULTS

The results obtained in this bioassay showed interaction between *B. bassiana* concentration and castor oil concentration on total and confirmed mortality of coffee berry borer (Figure 1, Figure 2).

Any model fitted to total and confirmed mortalities of coffee berry borer with 0.0 and 1 x 10⁴ conidia mL⁻¹ of *B. bassiana*, remaining constant in response to castor oil concentrations (Figure 1). However, for 1 x 10⁵ and 1 x 10⁶ conidia mL⁻¹, the total and confirmed mortality of *H. hampei* fitted to linear model, where mortality reduced due to increasing castor oil concentration (Figure 1). Although *B. bassiana* concentrations show the same behavior for total and confirmed mortality of *H. hampei* due to castor oil concentrations, a higher intercept value is observed in 1 x 10⁶ conidia mL⁻¹ i.e., higher mortality (Figure 1). Unlike previous concentrations, for 1 x 10⁷ conidia mL⁻¹ of *B. bassiana*, any model fitted to total mortality of coffee berry borer, remaining constant according to castor oil concentrations, with total average mortality of 90.49% (Figure 1). However, the confirmed mortality for this concentration fitted to linear model, reducing mortality due to the increase in castor oil concentration (Figure 1). When this confirmed mortality is compared to that presented by 1 x 10⁵ and 1 x 10⁶ conidia mL⁻¹, a much lower slope is observed, which shows lower influence of castor oil on mortality of *H. hampei* in high fungi concentrations (Figure 1).

Analyzing the total and confirmed mortality of coffee berry borer from concentrations of castor oil in response to concentrations of *B. bassiana*, it was observed that 0.0, 0.5, 1.0, 1, 5 and 2.0% v v⁻¹ of castor oil, the mortalities fitted to a hyperbolic model, in which, the mortality tends to increase in response to *B. bassiana* concentrations until reaching a certain point and from then on, increases on mortality is insignificant and practically constant (Figure 2). However, in high castor oil concentrations, 2.5 and 3.0% v v⁻¹, the total and confirmed mortality fitted to a linear model, presenting an increase in *H. hampei* mortality due to the increase in *B. bassiana* concentration (Figure 2).

For concentrations that fitted to hyperbolic model, it is possible to determine the oil concentration that gives maximum coffee berry borer mortality (Table 2). For statistical determination it was observed that 0.0, 0.5, 1.0, 1.5 and 2.0% v v⁻¹ of castor oil and 1 x 10⁷ conidia mL⁻¹ of *B. bassiana* was the one that conferred maximum total mortality and confirmed mortality (Table 2). In addition to the previous inference, *B.*

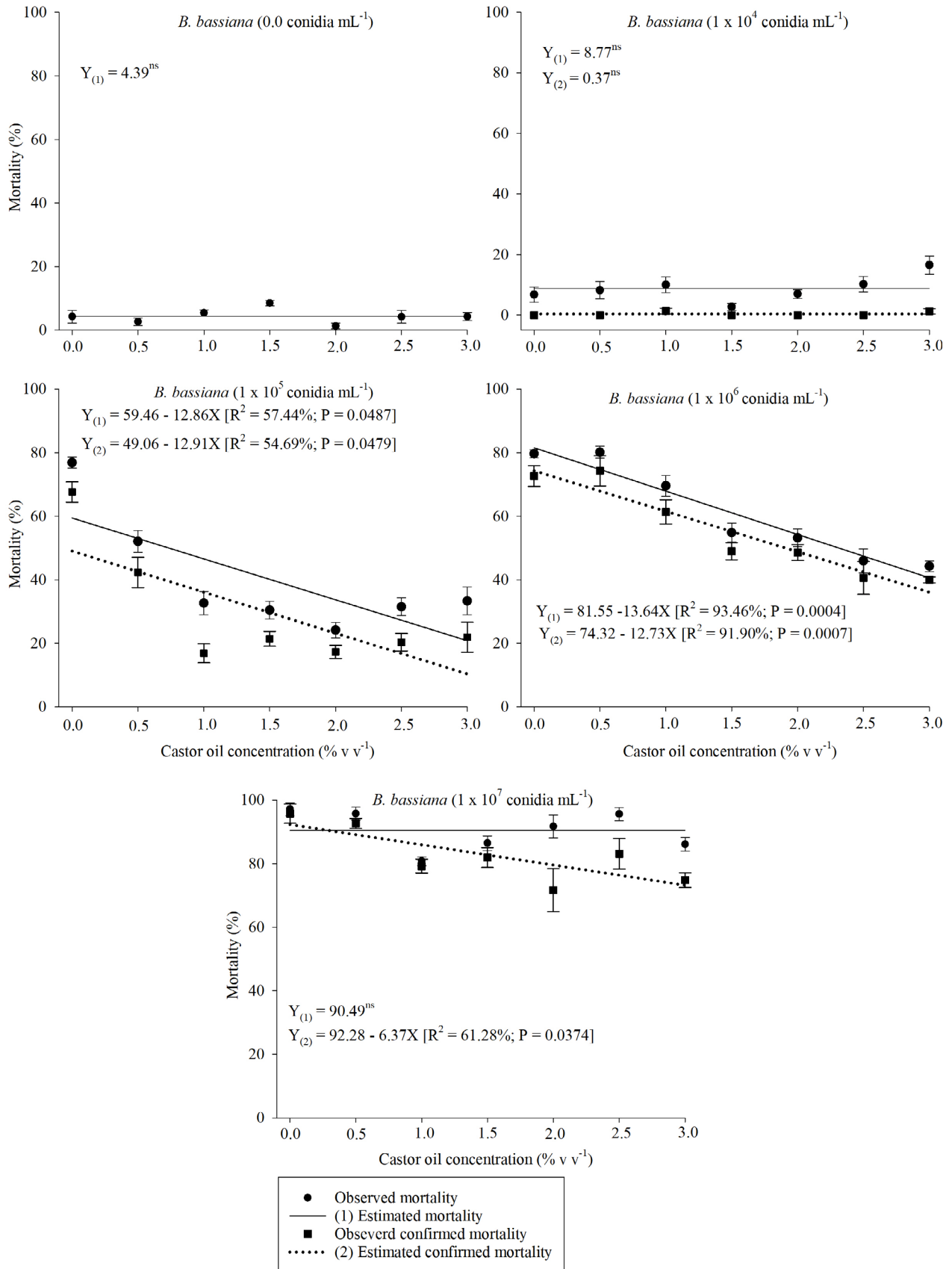


Figure 1: Mortality of *Hypothenemus hampei* caused by interaction between *Beauveria bassiana* and castor oil in different concentrations, at 25 ± 1 °C, RH of 70 ± 10% and 12h photophase.

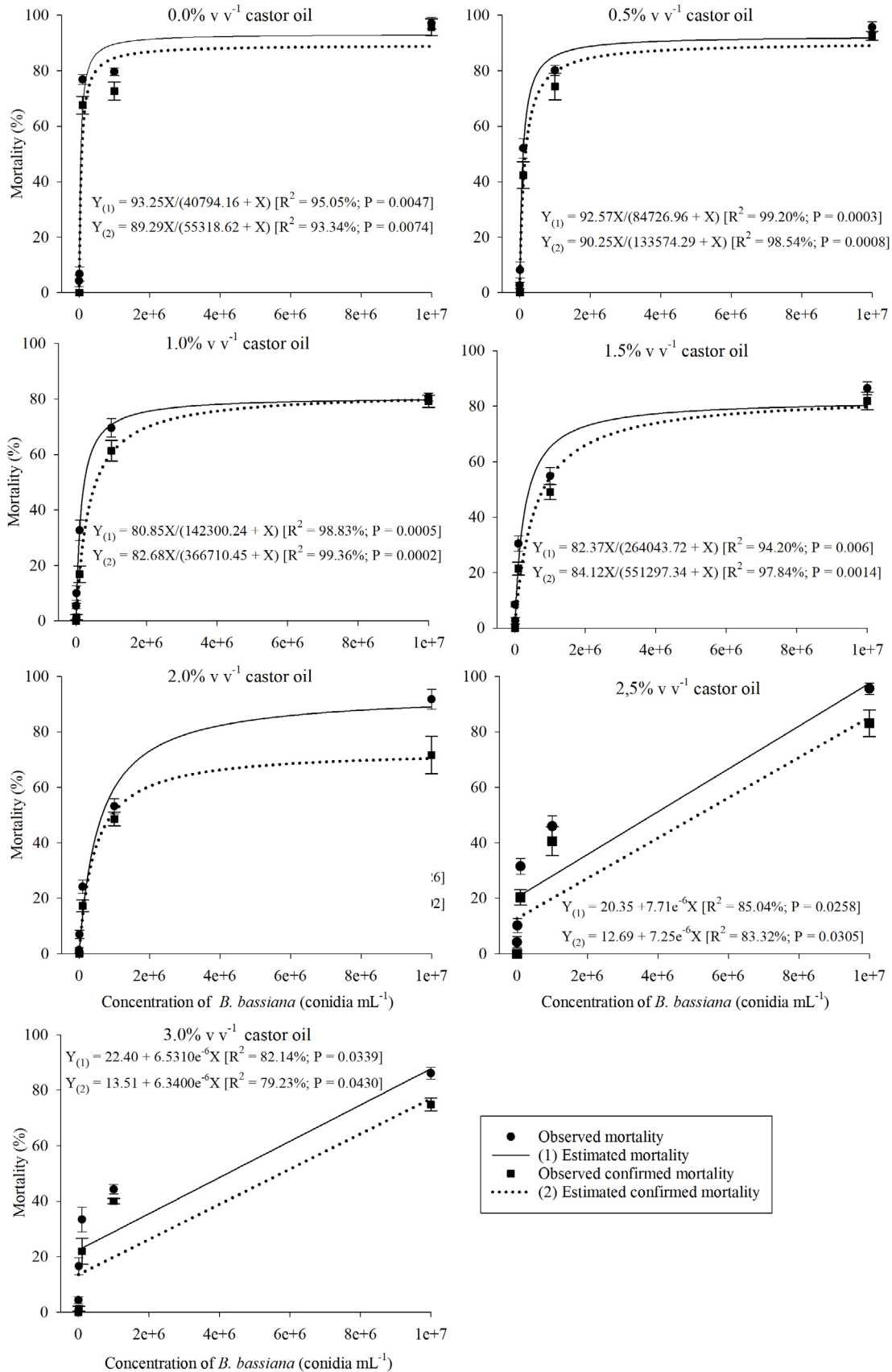


Figure 2: Mortality of *Hypothenemus hampei* caused by interaction between castor oil and *Beauveria bassiana* in different concentrations, at 25 ± 1 °C, RH of 70 ± 10% and 12h photophase.

bassiana concentration necessary to reach 90% of maximum mortality of coffee berry borer was determined, where it was observed reductions in concentration of up to 27.24 times for total mortality and 20.09 times for confirmed mortality in absence of castor oil (Table 2). From the results obtained, it is possible to determine the economic viability of a given concentration in response to reduction in mortality and reduction in *B. bassiana* concentration (Table 2). Based on the level of economic damage, the results can be worked out to obtain the best economic response for management of coffee berry borer.

Table 2: Determination of maximum points and 90% of maximum mortality and respective concentrations, for hyperbolic equations (Figure 2).

COM ¹	PMM ²	CBb ³	90% PMM ⁴	CBb ³	RM ⁵	RC ⁶
Total Mortality						
0.0	93.25	1 x 10 ⁷	83.93	3.67 x 10 ⁵	9.32	27.24
0.5	92.57	1 x 10 ⁷	83.13	7.62 x 10 ⁵	9.44	13.11
1.0	80.85	1 x 10 ⁷	72.77	1.28 x 10 ⁶	8.08	7.81
1.5	82.37	1 x 10 ⁷	74.13	2.38 x 10 ⁶	8.24	4.21
2.0	94.13	1 x 10 ⁷	84.72	5.18 x 10 ⁶	9.41	1.93
Confirmed Mortality						
0.0	89.29	1 x 10 ⁷	80.36	4.98 x 10 ⁵	8.93	20.09
0.5	90.25	1 x 10 ⁷	81.23	1.20 x 10 ⁶	9.02	8.32
1.0	82.68	1 x 10 ⁷	74.41	3.30 x 10 ⁶	8.27	3.03
1.5	84.12	1 x 10 ⁷	75.71	4.96 x 10 ⁶	8.41	2.02
2.0	73.47	1 x 10 ⁷	66.12	3.90 x 10 ⁶	7.35	2.56

¹Castor oil concentration; ²Point of maximum mortality; ³*Beauveria bassiana* concentration (conidia mL⁻¹); ⁴90% of maximum mortality; ⁵Reduction in mortality; ⁶Reduction in concentration (conidia mL⁻¹).

The data were also analyzed by response surface to obtain a function where, from concentrations of castor oil and concentrations of *B. bassiana*, the coffee berry borer mortality caused by interaction could be estimated (Figure 3). The proposed model was $Z = (128697.57 + 75.87X + 2.9721e^{-6}X^2 - 9.71XY)/(57998.92 + X)$, in which the linear, quadratic, interaction and hyperbolic effects are observed (Figure 3). Therefore, it is possible to verify that increasing castor oil concentration, the mortality of coffee berry borer reduced regardless *B. bassiana* concentration (Figure 3). Therefore, it was found that there was no effect of castor oil on *H. hampei* (Figure 3). Castor oil had an antagonistic effect on *B. bassiana* (Figure 3).

4 DISCUSSION

Low mortality observed with castor oil in absence of *B. bassiana* is probably correlated to the high relative humidity required to set up an experiment with entomopathogenic

fungi. Unlike the results obtained in this research, castor oil, at a concentration of 3.0% (v v⁻¹) of the cultivar IAC80 and Paraguaçu, caused 57.29 and 44.03% of coffee berry borer mortality, respectively (Celestino et al., 2015). In research comparing the mortality of the *H. hampei* caused by vegetable and mineral oils, it was found that in the concentration of 3.0% (v v⁻¹) castor oil and neem oil, caused 53.7 and 40.8% mortality, respectively (Celestino et al., 2016a). It may contribute to increase formation of droplets, preventing the coating of coffee berry borer female by oil. Also, the probable action of castor oil is due to spiracles coating and/or tracheas causing insect death by asphyxia (Celestino et al., 2016a), the relative humidity may have directly influenced the action of this oil on coffee berry borer mortality.

Although castor oil derivatives have insecticidal action on some pest-insects (Bigi et al., 2004; Ramos-López et al., 2010; Arnosti et al., 2011; Tounou et al., 2011; Nascimento et al., 2011; Celestino et al., 2015; 2016a; Machado et al., 2020), there are few studies that evaluate the interaction between castor oil derivatives and entomopathogenic fungi. However, Rondelli et al. (2011) found that when castor oil at 2% v v⁻¹ was added, suspensions of the ESALQ-447 isolate and the formulated Boveril[®] PM (ESALQ-PL63 isolate) (Company: KOPPERT, Itapetinga, SP) at 3 x 10⁵ conidia mL⁻¹, the mortality of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) were significantly higher compared to the use of these methods in isolation, a result opposite to that observed in the present study, probably due to the feeding habits.

Despite the increase in mortality presented by ESALQ-447 isolate, previously reported in an *in vitro* compatibility test, castor oil in concentrations of 0,5, 1,0, 1,5, 2,0, 2,5 and 3,0% v v⁻¹ was moderately toxic to this isolate (Celestino et al., 2018). Conversely, *B. bassiana* CCA-UFES/Bb-4 isolate, whose castor oil was moderately toxic only at 3.0% v v⁻¹ (Celestino et al., 2018), in the *in vivo* test this oil negatively affected the fungi performance on *H. hampei* mortality, in concentrations below 1 x 10⁷ conidia mL⁻¹ (Figure 1). However, when fungi concentration was increased, i.e., inoculum amount, there was no interference in the total mortality of coffee berry borer; and in the confirmed mortality, although there was a reduction, it was less significant than that presented in lower fungi concentrations. Such fact makes possible to use these management methods in association if both concentrations are observed. Therefore, a field evaluation is necessary to determine the efficiency of these methods depending on local climatic conditions, which will enable a better analysis of the relationship between increased fungi concentration, interaction with castor oil and efficiency on coffee berry borer control.

Unlike castor oil that demonstrated antagonism to *B. bassiana* on coffee berry borer control, neem oil, despite reports *in vitro* fungitoxic effects to *B. bassiana* (Depieri; Martinez;

Menezes, 2005; Moran et al., 2007; Araujo JR; Marques; Oliveira, 2009; Islam; Olleka; Ren, 2010), when applied in association with this fungi, showed greater ovicidal action on *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) eggs and reduced the lethal time (TL₅₀) of nymph mortality (Islam; Castle; Ren, 2010). However, genetic variability of *B. bassiana* may influence the interaction of this fungi with neem oil (Mohan et al., 2007). Since, when Margoside® formulated at 0,3% v v⁻¹ (Active ingredient: 0.15% azadiractina; Company: M/s Monofix Agroproducts Ltd, Hubli, India) was associated with *B. bassiana*, additive and antagonistic effects were observed on mortality of caterpillars of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) in response to fungi isolate used (Mohan et al., 2007). Thus, *in vivo* compatibility studies with other *B. bassiana* isolates should be performed, as it is possible to exist isolates less sensitive to castor oil action.

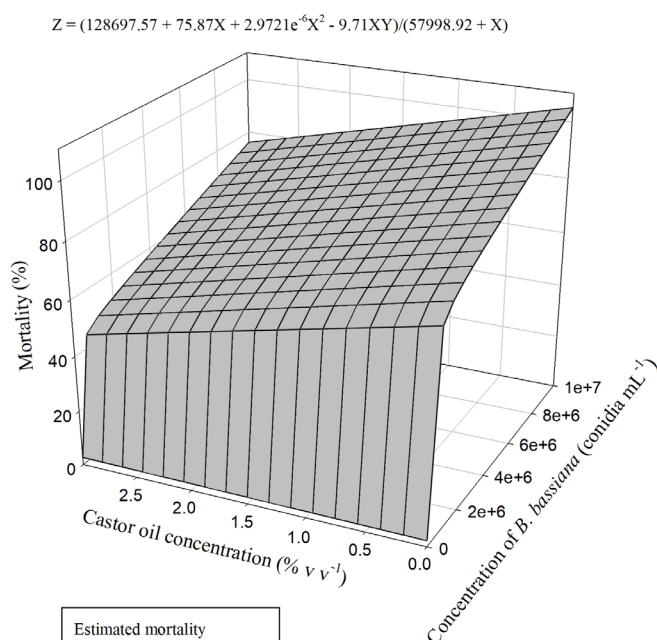


Figure 3: Response surface model for *Hypothenemus hampei* mortality caused by the interaction between castor oil and *Beauveria bassiana*, at 25 ± 1 °C, RH of 70 ± 10% and 12h photophase.

5 CONCLUSIONS

Castor oil presented an antagonistic effect on *B. bassiana*, reducing the coffee berry borer mortality. Mortality of *H. hampei* caused by interaction between *B. bassiana* and castor oil, decreased in response to increasing concentration of castor oil. Less interference of castor oil was observed for highest *B. bassiana* concentrations. Based on the results, management of *H. hampei* cannot be normally performed using the association between castor bean oil and *B. bassiana*, except if the economic viability of the higher concentrations justified.

6 ACKNOWLEDGEMENTS

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