Amazonian Journal of Plant Research *©2017 Universidade Federal do Pará*
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https://www.ajpr.online - Amaz. Jour. of Plant Resear. 4(1):456-461. 2020

Amazonian Journal of Plant Research

Original Paper

OPEN ACCESS

Effect of isolates of *Beauveria bassiana* pathogenic to leafhopper cacao *Horiola picta* (Hemiptera: Mambracidae)

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Received: 06 January, 2020. Accepted: 13 March, 2020
First published on the web April, 2020
Doi: 10.26545/ajpr.2020.b00054x

Abstract

The leafhopper *Horiola picta* (Membracidae: Smiliinae: Trogopini) is a cocoa secondary pest of Transamazônica and Xingu region, and microbial control is an alternative to keep these populations below the economic injury level. This study aimed to evaluate the virulence of isolates GC 157 and GC 165 of *Beauveria bassiana* under different conidia concentrations in insect adulthood. The following concentrations were used: $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$,$1x10^8$ conidia ml^{-1} and the control to inoculate the insects were fed with cocoa shoots and young fruit, exchanged daily. For the effect of *B. bassiana* on *H. picta* adults performance differences were observed between the concentrations 10^4 a 10^8 conidia ml^{-1}, around 70.31 to 90.31% of total mortality respectively to isolated CG 157, and the isolated CG 165 was obtained 62,50% from the concentration 10^4 conidia mL^{-1}, reaching 82% mortality with a concentration of 10^8 conidia mL^{-1}. However, both the isolated CG 157 as CG 165, show good infective capacity. Confirmed mortality ranges from 27,2% a 72,2% both for isolated as CG157 to CG165 and showed positive linear correlation (R^2 = 0.97) and (R^2 = 0.98) respectively. The lethal time (LT_{50}) for CG157 and CG165 isolated according to Lethal Concentration (LC50) of 10^4 conidia-1 was 1.075 days for the isolated CG157 and 2.30 days for the CG 165 isolated.

Key-words: Biological Control, Microbial Control, Cocoa, Leafhopper

Introduction

Horiola picta (Membracidae: Smiliinae: Trogopini), is one of several species of the Membracidae family, constitutes a secondary plague of cocoa. Sanchez (2011) in studies conducted in Bahia observed that the larvae and adults of these species suck the sap from the new shoots, the flower peduncles, new fruits and branches, causing yellowing and wilting with total drying of leaves and branches and even the death of the cocoa trees, depending on the intensity of colonization and attack.

Although the greatest concerns for cocoa farming today are focused on the management of fungal diseases such as witch broom and brown rot, caused by the phytopathogens Crinipellis perniciosa (Stahel) Singer, Silva and Bastos (2007) and *Phytophtora* spp. respectively, secondary pests represent losses in production. Vegro et al. (2014) state that the production conditions in the Amazon, under the incidence of the witch's broom and the infinity of other diseases and secondary pests that affect the cacao tree, persist as the greatest challenge of research and extension.

The species of the genus Horiola usually inhabit on the fruit. They are brown leafhoppers of about 3 to 4 mm in length and have, in the dorsal hemieliters,

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very characteristic designs in the form of an X (Sanchez, 2011). These insects lay their eggs on the flower stalk, the bobbin, the developed fruit or the new branch, introducing them into the plant tissue in groups of a few dozen. The wounds thus made on the stalk intercept the sap and abort the flowers and bobbins. (Pinho et al., 1977).

A recurrent alternative for the control of these pests is the use of biological microorganisms, and in this case the most conventional is the use of entomopathogenic fungi, used in several species of leafhoppers. According to Alves (1998), entomopathogenic fungi are one of the most promising agents for several agricultural pests, among which the Beauveria bassiana fungus (Ascomycota: Hypocreales), one of the most used in biological control programs, due to its efficiency and easy multiplication, stands out.

The present work aimed to evaluate the virulence of *B. bassiana* isolates GC 157 and GC 165 under different concentrations of conidia in the adult phase of the insect H. picta, cocoa pest.

Material and methods

The experiment was carried out in the Agricultural Plant Pathology and Microbiology and Agricultural Biotechnology Laboratories of the Federal University of Pará - UFPA, Altamira Campus. Isolates of the fungus *B. bassiana* CG 157 and CG 165 from the collection of the Escola Superior de Agricultura Luiz de Queiroz of the University of São Paulo - ESALQ/USP- Piracicaba

Study area

The insects used (Figure 1) were obtained in two cocoa plantations with properties located in the municipality of Brasil Novo under the coordinates S 03º 18'13.5" W 052º 29' 31.7" and S 03º 18'13.7" W 052º 29' 31.6", 40 km from the municipality of Altamira in the Community Cacaulândia, so called according to Silva and Cunha (2014), because of the number of properties that obtain their main source of income from cocoa.

Fig. 1. a) Adults of H. picta on cocoa beanstalk. b) Adults and nymphs on sprouting.

Bioassays

To obtain the fungal suspensions, the isolates were cultivated in the middle of Potato Dextrose-Agar (PDA), in an acclimatized chamber (26+1ºC and photoperiod of 14 h) for 10 days, forming the matrix plates and then 1cm² of each isolate was inoculated in plastic polypropylene bags containing 100 grams of autoclaved rice according to Ferreira (2004).

After reaching the time determined by the methodology of 20 days at 27ºC, the bags were opened in the laminar flow chamber and 10 g of rice + fungus were removed from them for the suspension of conidium, performed in 100 mL of sterile and autoclaved water, Tween® 80 to 0.1% was added. The concentration was determined with the aid of the Newbauer chamber. The following concentrations were used: 1x104, 1x105, 1x106, 1x107.1x108 mL^{-1} coniders.

The bioassays were installed in entirely randomized design, with six repetitions, five concentrations plus the witness. Each repetition consisted of a 1000 mL plastic container, with a transparent lid and a 16 cm² thin screen in the center. In each container, filter paper treated with 0.5 mL of distilled and autoclaved water was placed at the bottom.

The fungi were inoculated through a hand sprinkler over the insects in a volume of 0.5 mL of conidium suspension. For the control, 0.5 mL of autoclaved distilled water + Tween® 80 to 0.1% was used. Six adult insects (male and female) were placed per container, with six containers for each concentration. Using *B. bassiana* CG 157 and CG 165 isolates, a total of 432 adult insects were used, fed with cocoa sprouts and new fruits, exchanged daily.

The evaluations were made daily, for six consecutive days, removing from each container the dead insects, which were transferred to Petri dishes treated with filter paper moistened with autoclaved distilled water for the confirmation of fungus mortality.

The data collected were previously transformed into (x + 0.5)1/2 and to analyze the effect of *B. bassiana* on the mortality of H. picta, they were submitted by ASSISTAT software version 7.7 beta to analysis of variance (ANOVA). The total and confirmed mortality rates were submitted to the Skott-Knott test at 1% and 5% significance, the analysis of polynomial and linear regression. The lethal time was determined by polynomial regression.

Results and Discussion

There was no significant difference in performance between concentrations of 104 to 108 mL-1 levels, although these were higher for CG 157 isolated. However, they were highly significant for insect mortality with a percentage above 50% (Table 1).

Andaló et al. (2004) analyzing 9 isolates of *B. bassiana* and one isolate of Metarhizium anisopliae also found significant difference between mortality percentages, equal to or higher than 50% for the Dysmicoccus texensis cochineal from the coffee tree root.

Therefore, the isolates CG 157 and CG 165 of *B. bassiana* showed good infective capacity on H. picta. For the CG157 isolate, according to the analysis by polynomial regression, the total mortality varied increasingly between 104 and 108 mL^{-1} coniders, around 70.31 to 90.31%, respectively (Figure 2).

Table 1. Effect of *Beauveria bassiana* on adults of *H. picta*, after the application of suspensions of conides, in various concentrations.

Evaluated parameter	Concentration (conides mL^{-1})	Isolated CG157	Isolated CG165
Mortality (%) CV (%): 15,18	10^4	71,31 a**	62,50 a
	10^5	81,50 a	67,17 a
	10^6	87,47 a	71,28 a
	10^7	87,47 a	76,83 a
	10^8	90,31 a	82,39 a
	Control	37,03 b	34,25 b

(**) The averages followed by the same letter do not differ statistically from each other by the Scott-Knott Test at the 5% probability level. CV: Coefficient of Variation.

For the CG165 isolate, the total mortality also varied among all concentrations; however, in relation to the previous isolate, it obtained 62.50% from the 106 mL^{-1} coniders concentration, reaching 82.39% of mortality with the 108 mL^{-1} coniders concentration (Figure 3). Svedese et al. (2012) also found mortality above 50% with the isolate of *B. bassiana* URM2916 on adults of *Zaprionus indianus*, (Diptera: Drosophilidae), ranging from 52 to 98.66%.

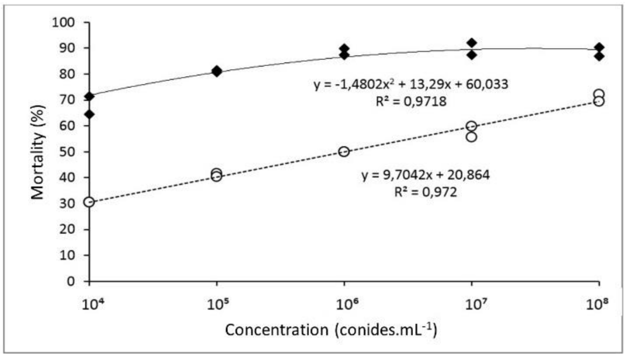

Fig. 2. Total mortality curve ( ) an d confirmed (---) of H. picta submitted to different concentrations of *Beauveria bassiana* CG157.

The high total mortality rate achieved and the variation between them is probably due to the methodology used, which was manual spraying. Barboza et al. (2011) using contact inoculation, containers distributed on filter paper in bioassay with Collaria scenia (Hemiptera: Miridae), observed that there was no variation between the concentrations of 105 and 108 mL^{-1} containers.

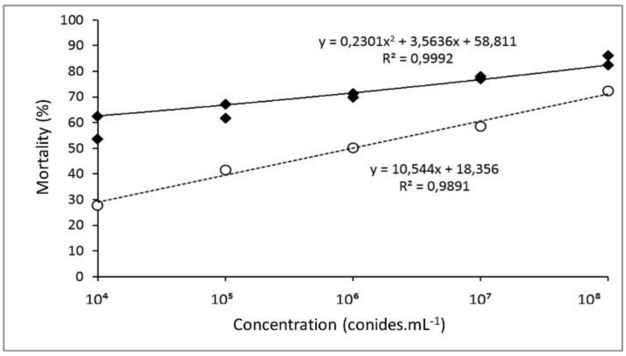

Fig. 3. Total mortality curve ( ) and confirmed (---) of *H. picta* subjected to different concentrations of *Beauveria bassiana* CG165.

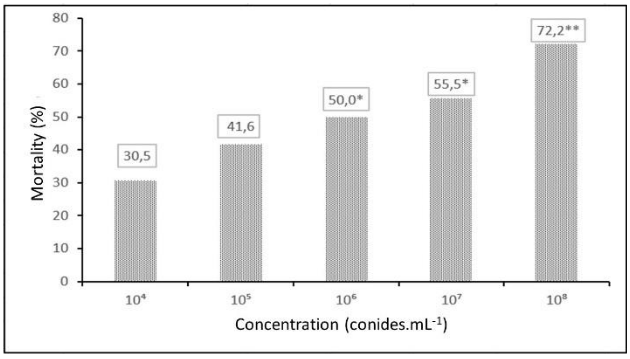

Fig. 4. Conidiogenesis rate (confirmed mortality) in H. picta submitted to different concentrations of the CG157 isolate from *Beauveria bassiana*. Presence of (**) indicates significant difference at the level of 1% deprobability and (*) significant at the level of 5% of probability between concentrations by the Skott Knott test.

In the methods of spraying and immersion, a greater number of conidiospore come into contact with the entire body of the insect, with the possibility of germination and penetration, which increases mortality and decreases the Lethal Concentration (CL50) (Santoro et al., 2007). Another factor, probably cooperative for the mortality rate is the susceptibility of the insect to entomopathogenic action.

The confirmed mortality, where there is extrusion of the pathogen on the insect (Lima et al., 2012), varied from 27.7% to 72.2% for both CG157 and CG165 isolates and showed a linear positive correlation of (R^2 = 0.97) and (R^2 = 0.98), respectively. According to Barboza et al. (2011) prove the lethal action of fungus (Figures 2 and 3).

Analyzing the significance of confirmed mortality for the CG157 isolate in concentrations 104 and 105 was around 30% to 41%, significantly lower than the higher concentrations (106 to 108). Among these, there was a significant difference, with concentration 108 having the highest rate, 72.2% (Figure 4).

For the isolated CG165 in concentrations 104, 105 and 106 the confirmed mortality rate was around 27.7%, 41.6% and 50%, respectively. They were significantly lower than the other concentrations, with a rate of 107 significant and 108 highly significant with 72.2% (Figure 5). Rohde et al., (2006) analyzing the confirmed mortality in cascade Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae) with *B. bassiana* found higher confirmed adult mortality, ranging from 0 to 86.7%.

Another factor to be considered is the speed that a given control agent is able to act on its target (Barboza et al., 2011). In this work the lethal time (TL50) for CG157 and CG165 isolates according to the Lethal Concentration (CL50), of 104 Conidium was 1.075 days for CG157 isolate and 2.30 days for CG 165 isolate (Figures 6 and 7). Studies by Castilho et al., (2010) with M. anisopliae isolate ANS04 found higher virulence against Atta bisphaerica soldiers, spending less time (1.15 days) to kill 50% of the population while *B. bassiana* isolate ANS07 was less virulent (5.15 days).

CL50 is given by the lower conides mL^{-1} concentration capable of causing mortality greater than or equal to 50% in the insect population. The knowledge of lethal concentration leads to conclusions about pathogen potency, insect susceptibility to the disease, and even pest control predictions using microbial insecticides (Haddad, 1998; Santoro, et al., 2007).

Neves and Hirose (2005) analyzing the CL50 in isolates from *B. bassiana* to Coffee borer, Hypothenemus hampei (Ferrari) (Coleoptera: Scolytidae) found that the concentration 104 conidium/ml was not considered in the data analysis, since the percentage of burs mortality obtained at this concentration was very low, adopting the concentration 106 as CL50.

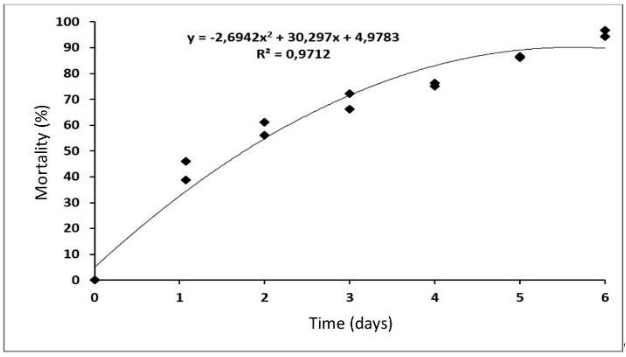

Fig. 6. Lethal Time Curve of *H. picta* submitted to concentration of 104 conidiums.mL^{-1} of the isolated CG157 of *Beauveria bassiana*.

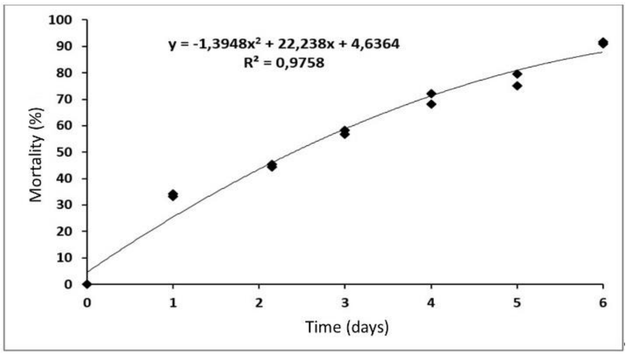

Fig. 7. Lethal Time Curve of H. picta submitted to concentration of 104 conidiums.mL^{-1} of the isolated CG165 of *Beauveria bassiana*.

Conclusion

The isolates CG 157 and CG 165 of *B. bassiana* negatively affect the development of *H. picta*, a secondary cocoa pest. The strains analyzed are potentially virulent, causing high mortality rates, according to the increase in concentrations, especially GC 157 which was more efficient, because besides the high mortality rate in the lowest concentration, 104 mL^{-1} coniders, presented the lowest TL50.

Once the mortality capacity of these entomopathogens has been proven, there is a need to promote research around actions to test other isolates and other species of fungi, bacteria and entomopathogenic nematoids, in addition to other bioassay methodologies, including field trials, which help to control pests in the region.

Acknowledgements

The Fundação Amazônia Paraense de Amparo à Pesquisa - FAPESPA for the Scientific Initiation Scholarship granted, of the utmost importance, as it introduced this work. Dr. Simone Maria Costa de Oliveira Moreira, my supervisor and the team of the Agricultural Plant Pathology Laboratory and Agricultural Microbiology and Biotechnology Laboratory, has provided space for the research experiments of both the grant and this concluding work.

Conflict of interest: All authors declare no conflict of interest

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Cite this article as:

Da Silva, A.F.C.; Oliveira Moreira, S.M.C.; Da Silva Cunha, R.S.; Moreira, D.A.; Teodoro, A.; Do Nascimento, A.H.C.; Júnior, W.S.E. 2020. Effec of isolates of *Beauveria bassiana* pathogenic to leafhopper cacao *Horiola picta* (Hemiptera: Mambracidae).. **Amaz. Jour. of Plant Resear.** 4(1): 456-461.

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