Effect of isolates of entomopathogenic fungi in the coconut eye borer

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Abstract

The objective of this study was to evaluate the effect of entomopathogenic fungi on adults of Rhynchophorus palmarum (Coleoptera: Curculionidae). The experimental design was completely randomized in a factorial design (5x3) + control, composed of five treatments (isolated IBCB 66, CPATC 032, CPATC 057 and T9, and the commercial product Boveril®) and three concentrations of each fungus (10^7, 10^8 and 10^9 conidia.mL^-1). The data of confirmed mortality were submitted to analysis of variance (ANOVA) using the Proc ANOVA of SAS, and the means compared by Tukey test at 5% probability. To determine percentage survival, confirmed mortality data were subjected to Log-Rank test using the Kaplan-Meyer method. Subsequently the values of LC50 and LT50 were estimated submitting mortality confirmed the Probit analysis. For the concentration 10^8 conidia.mL^-1, the isolates CPATC 032 and CPATC 057 caused confirmed mortality of 52 and 44% of the adults of R. palmarum, respectively. At the concentration 10^9 conidia.mL^-1, the isolates CPATC 032 and CPATC 057 caused mortality of 64 and 52% of the insects, respectively. For the CPATC 032 isolate, in the three concentrations tested, the insects had an average survival of 11 to 12 days. The TL50 of isolate CPATC 032 at concentrations 10^8 and 10^9 conidia.mL^-1 was approximately 17 days. All isolates tested and the Boveril® product are pathogenic to R. palmarum.

Keywords: Cocos nucifera, Rhynchophorus palmarum, Beauveria bassiana, Trichoderma harzianum

Introduction

The coconut beetle Rhynchophorus palmarum (Linnaeus, 1758) (Coleoptera: Curculionidae), known in Brazil as coconut eye borer, is a hey-pest of the coconut crop (Cocos nucifera L.) in the Ocidental Indian and in South America (Takada et al., 2014).

The coconut eye borer is responsible to cause direct damage in function of larva feed, and indirect damage, specially by the adult insects, being the principal vectors of the nematode Bursaphelenchus cocophilus (Cobb, 1919) Baujard, 1989 (Nematoda: Aphelenchoidea), which can cause the death of the plant (Cysne et al., 2013).

Knowing that the adult insects of R. palmarum are not permanent in the plants, only feed and oviposites inside the coconut stipe, the application of entomopathogenic fungi directly on the insects is non efficient.

Thus, an viable alternative in field is to inocule the fungi in food attractants and make it disponibile to the insects by the pitfalls of autoinoculation, where the insect may come in and out freely of the putfalls, causing direct mortality inside the pitfalls or spreading the disease in the field.

Considering that there is a great genetic variability in the species of fungi, the selection of isolates is essential in studies of biological control of pests with entomopathogens, in order to establish a natural tool and an ecological and viable alternative to overcome the problems caused by pest insects.

The entomopathogenic fungus Beauveria bassiana (Balsamo) Vuillemin presents a development process in the environment that begins with the adhesion and germination of the conidia to the host integument, in which it involves enzyme production and cell differentiation to ensure colonization and dispersal.
Effect of isolates of entomopathogenic fungi on the H. hampei adult survival

Entomopathogenic fungi viability assay

The viability of the conidia of all isolates and the commercial product was determined by the germination method, by inoculating 0.1mL of suspension of each isolate into two Petri dishes containing agar-agar and spread with the aid of a Drigalsky handle. Then, Petri dishes were incubated in BOD chambers at 25±1ºC for 24h, and light optical microscope readings were performed to find conidia germination and non-germinated conidia (Alves & Moraes, 1998). For this purpose, the Petri dishes were divided into four quadrants, on which coverslips were placed for reading under a microscope.

Preparation of entomopathogenic fungal suspensions

Sugarcane stalks were plunged into the standard spore suspensions of isolates IBC 66, CPATC 032, CPATC 057 and T9, and the Boveril® (commercial product) at concentrations 10⁷, 10⁸ and 10⁹ conidia.mL⁻¹ suspension + Will Fix® adhesive spreader (0.1%) for 30 min. In the control treatment, the stems were immersed only in distilled water + Will Fix® adhesive spreader (0.1%), during the same period of the other treatments. The adults of R. palmarum were separated into a group of 25 insects, transferring them to sealed containers and perforated lids containing the sugarcane stalks previously treated by the fungus, where they remained in contact with the inoculum for 3 h, according to the methodology described by Mendonça (2007).

Fungus inoculation bioassays

Insects were individualized in plastic containers, and the food (sugarcane stalks), not treated by fungi, replaced every three days. Mortality was evaluated daily for a period of 20 days.

Dead insects were washed with Sodium Hypochlorite (2%) and subsequently with distilled water to...
clean the surface of the insects and then individualized in plastic containers containing sterile cotton and moistened with sterile distilled water. BOD incubator (temperature 25±2°C, relative humidity 70±10% and photophase of 12 h), aiming to confirm the mortality through sporulation of the fungi.

Statistical Analysis

The experimental analysis was completely randomized in factorial scheme (5x3) and the control treatment, composed by five treatments (strains IBCB 66, CPATC 032, CPATC 057, T9, and the comercia formulation Boveril®), and three concentrations to each fungi (10⁷, 10⁸ e 10⁹ conidia.mL⁻¹). The treatments were constituted of five replications composed by five insects each, totaling 25 insects/fungi concentration and 25 insects used in control treatment.

Data of confirmed mortality was transformed in arcseno(√(x/1000)), and the data from conidia viability of B. Bassiana and T. harzianum and the Boveril® were subject to analysis of variance (ANOVA), by the use of Proc ANOVA of SAS 9.0. The means were compared by the Tukey test (p<0.05).

Upon confirmed mortality data of adults from R. palmarum, determined the mean live percentage being the data subjected to Log-Rank test by the method of Kaplan-Meyer by pairs of isolates, usein the Proc Lifetest. In determination of the Letal time (LT₅₀) the isolates IBCB 66, CPATC 032, CPATC 057 and T9 and Boveril, subjected the confirmed mortality of adults from R. palmarum to Probit analysis. To all analysis were used the software SAS 9.0 (SAS Institute, 2002).

Results and Discussion

The conidia viability of the isolates IBCB 66, CPATC 032, CPATC 057, and T9, and the commercial product Boveril® showed up suitable, being that the isolates CPATC 057 and IBCB 66 showed, respectively, higher and lower viability percentage, do not differing from the other treatments (F=3.026; df=4; P=0.051) (Table 2).

| Treatment       | Viability (%) |
|-----------------|---------------|
| IBCB 66         | 97.00 ± 0.82b |
| CPATC 032       | 98.00 ± 0.82ab|
| CPATC 057       | 99.25 ± 0.96a |
| Boveril®        | 98.75 ± 1.26ab|
| T9              | 98.95 ± 0.96ab|
| CV (%)          | 0.99          |

Means followed by the same letter do not differ statistically each other by Tukey test (p<0.05).

All the isolates, in three concentrations tested (10⁷, 10⁸, and 10⁹ conidia.mL⁻¹), showed pathogenic to adults of R. palmarum (Table 3). Despite the isolate T9 (T. harzianum) have been pathogenic to adults of R. palmarum, penetrating your cuticle, which is composed principally of chitin (Shakeri & Foster, 2007), the used concentrations of the fungus do not influence in mortality percentage.

In concentration 10⁷ conidia.mL⁻¹, the mortality percentage confirmed of Boveril® and the isolates T9, IBCB 66, CPATC 032, and CPATC 057 do not differ each other (F=4.16; df=5; P=0.007) (Table 3). Meanwhile Pires et al. (2010) found confirmed mortality of 13% of caterpillar Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) when feeding them with tomato leaflets sprayed with the suspension of the isolate CPATC 057 in concentration of 10⁷ conidia.mL⁻¹.

Rondelli et al. (2012) found that the commercial formulation Boveril®, in concentration of 10⁷ conidia.mL⁻¹, was responsible by the confirmed mortality of 86.4% of caterpillars from traça-das-cruçíferas Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae), showing higher results in comparison to found in the present study. This high difference in the percentage of mortality probably occurred due to the strong sclerosis of the exoskeleton and the elites of insects belonging to the order Coleoptera (Silva, 2011), making it difficult for the fungus to penetrate, which does not occur in the order Lepidoptera.

For the concentration 10⁸ conidia.mL⁻¹, the isolate CPATC 032 caused high mortality, but did not differ significantly from the isolate CPATC 057. The confirmed mortality by Boveril® and the isolates T9 and IBCB 66 also did not differ from each other (F=5.02; df=5; P=0.003) (Table 3). In contrast, Giametti et al. (2010) found confirmed mortality of 70% treating adults of bicoudo-do-agodoedeiros Anthonomus grandis (Boheman, 1843) (Coleoptera: Curculionidae) with the isolate IBCB 66 in concentration of 10⁸ conidia.mL⁻¹. Perhaps, this difference may be associated with the method of application of the fungus directly inside, with a higher mortality rate applicable.

Differences found in confirmed mortality rates may be related to the virulence of the isolates of B. bassiana or even by the method of application of the fungus. Lo Verde et al. (2015), treating adults of Rhynchophorus ferrugineus (Olivier, 1790) (Coleoptera: Curculionidae) with isolate L1 of B. bassiana by 30 s in concentration of 10⁸ conidia.mL⁻¹, verify 20% of confirmed mortality. On the other hand, Nussenbaum & Lecuona (2012) verify that B. bassiana Bb 23, Bb 286, Bb 301, and
Bb 302 occasioned mortality of 90-92% under adults of A. grandis, when treated by 15 seconds with concentration of 5x10^3 conidia.mL\(^{-1}\).

In concentration of 10^6 conidia.mL\(^{-1}\), do not occur significant statistics of Boveril\(^\circ\) and the isolates IBCB 66, CPATC 032, and CPATC 057 (F = 6.83; df=5; P=0.004) (Table 3). The isolates T9 from T. harzianum occasioned lower mortality in adults of R. palmarum.

Table 3. Mean ± EP\(^1\) of confirmed mortality (%) of adults from Rhynchophorus palmarum fed with sugarcane treated with entomopathogenic fungi in concentration of 10^7, 10^8, and 10^9 conidia.mL\(^{-1}\).

| Treatments       | Concentration [conidia.mL\(^{-1}\)] |
|------------------|-------------------------------------|
|                  | 0                                  |
|                  | 10^7                               |
|                  | 10^8                               |
|                  | 10^9                               |
| IBCB 66          | 8.00 ± 4.29 cB                      |
| CPATC 032        | 8.00 ± 4.29 cA                      |
| CPATC 057        | 8.00 ± 4.29 cA                      |
| Boveril\(^\circ\) | 8.00 ± 4.29 cA                      |
| T9               | 8.00 ± 4.29 cA                      |

Means followed in columns by the same lowercase letter do not differ each other by Log-Rank test by pair of isolates after survivor analysis by method of Kaplan-Meier. *Commercial formulation Boveril\(^\circ\) PM PL63/Koppert Biological Systems, from the fungus B. bassiana.

Although the isolate CPATC 032 differed statistically only from the isolate T9, it is likely that the highest mortality percentage of R. palmarum caused by this treatment is related to the fact that it is a fungus isolated from the insect under study, providing greater virulence. A similar fact, but with greater efficiency, occurred with Yasin et al. (2019) treating adults of R. ferrugineus with the isolate WG 41 of B. bassiana from the same species by 90 seconds in concentrations 10^7 and 10^8 conidia.mL\(^{-1}\), causing mortality of 41.90% and 75.95%, respectively, in adults.

Still in relation to isolate CPATC 032, a close value was found by Mendonça (2007) that, when studying the efficiency of this isolate on adults of R. palmarum, fed with sugarcane orevious treated by the fungi in concentration of 10^6 conidia.mL\(^{-1}\) during 3h, found 61.4% of insect mortality.

Close result was verified by Nowakonski (2019), who analyzed the pathogenicity of the IBCB 66 isolate from B. bassiana on citrus black fly nymphs Aleurocanthus woglum (Ashby, 1915) (Hemiptera: Aleyrodidae) in the laboratory at concentration 10^8 conidia.mL\(^{-1}\), showing virulent, presenting a mortality of 60.58% on nymphs on the 4th day of evaluation.

In all treatments, except for the isolated T9, the percentage of mortality observed when the insects were subjected to the concentration 10^7 conidia.mL\(^{-1}\) differed significantly only in relation to the confirmed mortality caused in the control and in the concentration of 10^9 conidia.mL\(^{-1}\) (F=1.68; df=19; P=0.001) (Table 3).

Regarding the average survival, that is, the lifetime of adults from R. palmarum after being fed with sugarcane treated with entomopathogenic fungi, in the concentration 10^6 conidios.mL\(^{-1}\) was possible to verify that has significative difference between the isolates CPATC 032, T9, Boveril\(^\circ\) and the control (\(\chi^2 = 11.50; df=5; P=0.042\)) (Table 4).

Table 4. Means ± EP\(^1\) of survivor (days) in adults of Rhynchophorus palmarum fed sugarcane treated with entomopathogenic fungi in concentrations of 10^7, 10^8, and 10^9 conidia.mL\(^{-1}\).

| Treatments       | Concentration [conidia.mL\(^{-1}\)] |
|------------------|-------------------------------------|
|                  | 10^7                               |
|                  | 10^8                               |
|                  | 10^9                               |
| Control          | 19.50 ± 0.50 a                      |
| IBCB 66          | 13.00 ± 0.00 abc                    |
| CPATC 032        | 12.25 ± 1.81 c                      |
| CPATC 057        | 13.33 ± 0.56 abc                    |
| Boveril\(^\circ\) | 14.50 ± 0.50 ab                     |
| T9               | 16.00 ± 0.00 ab                     |

\(\chi^2 = 11.50\) \(\chi^2 = 17.50\ \) \(\chi^2 = 14.93\)  

Means followed by the same letter in columns do not differ each other by Log-Rank test by pair of isolates after survivor analysis by method of Kaplan-Meier. *Commercial formulation Boveril\(^\circ\) PM PL63/Koppert Biological Systems, from the fungus Beauveria bassiana.* Significative of 5% of probability.

It can be seen that insects fed with sugarcane treated with isolate CPATC 032, in the concentration 10^8 conidia.mL\(^{-1}\) not only differed from the product Boveril\(^\circ\) (\(\chi^2=17.60; \ df=5; P=0.001\)) (Table 4). These values differ from the results found by other authors, possibly due to the insects not being in direct contact with the fungal solution. For example, Nussenbaum & Leucuna (2012) verify that adults of A. grandis treated during 15 s with the isolates of B. bassiana Bb 23, Bb 286, Bb 301, and Bb 302, showed middle survivor of 7 to 9 days.

On the other hand, Lo Verde et al. (2015) when treating adults of R. ferrugineus with the isolate L1 of B. bassiana for 30 s at the concentration 10^9 conidia.mL\(^{-1}\), found an approximate survival of 23 days.
The average survival of adults of *R. palmarum* adults exposed to the food treated with the concentration 10⁷ conidia.mL⁻¹ of the product Boveril® and of the isolates IBCB 66, CPATC 032 and CPATC 057 did not differ among themselves (χ²=14.83; df=5; P=0.011) (Table 4). The results of this study proved to be more efficient than those found by Dembilio et al. (2010), who found that the average survival of adults of *R. ferrugineus* immersed in the fungal suspension of isolate EABb 07/06-Rf from *B. bassiana*, for 90 s, was 16 days.

By the Probit analysis it was verified that in the concentration 10⁸ the isolates CPATC 032 and CPATC 057 showed the best performance during the 20 days of the assay evaluation (17.61 days, 23.11 days, respectively) (Table 5).

| Isolate         | TL₅₀ (days) (IC 95%) | χ²(3) | Value P(4) | β(4) |
|-----------------|----------------------|-------|-------------|-------|
| IBCB 66         | 24.86 (21.66 – 42.67) | 1.53  | 1.00        | 9.61  |
| CPATC 032       | 17.61 (15.89 – 20.31) | 4.99  | 0.99        | 3.53  |
| CPATC 057       | 23.11 (20.28 – 29.29) | 5.24  | 0.99        | 4.70  |
| Boveril®        | 25.61 (21.84 – 36.37) | 6.96  | 0.99        | 5.11  |
| T9              | 55.79 (30.83 – 13.94) | 2.97  | 1.00        | 3.25  |

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Francardi et al. (2012), when testing the efficiency of *B. bassiana* Bba 01/T02 and Bba 09/101 isolates in the concentration of 7x10⁶ conidia.mL⁻¹ under adults of *R. ferrugineus*, could not determine the LT₅₀. Although the concentration used by the authors is higher than in the present study, the efficiency of the fungal concentrations used in the control of insect pests is related to the variability that exists between the isolates of *B. bassiana*.

In concentration 10⁸, the isolates CPATC 032 e CPATC 057 also presented the best results for the period evaluated (17.25 days, and 21.92 days, respectively) (Table 6).

| Isolate         | LT₅₀ [days] (CI 95%) | χ²(3) | P value(6) | β(6) |
|-----------------|----------------------|-------|-------------|-------|
| IBCB 66         | 32.31 (24.89 – 54.15) | 9.81  | 0.94        | 2.69  |
| CPATC 032       | 17.25 (15.40 – 20.13) | 7.91  | 0.98        | 3.07  |
| CPATC 057       | 21.92 (19.44 – 26.84) | 5.81  | 0.99        | 4.49  |
| Boveril®        | 38.51 (26.77 – 80.16) | 4.44  | 0.99        | 1.67  |
| T9              | 42.19 (27.67 – 64.05) | 2.93  | 1.00        | 3.98  |

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