Brazilian Journal of Biology
ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

https://doi.org/10.1590/1519-6984.195711
Original Article

Alternative substrates for conidiogenesis of the entomopathogenic fungus *Beauveria bassiana* (Bals) Vuillemin (Deuteromycotina: Hyphomycetes)

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Received: April 20, 2018 – Accepted: August 17, 2018 – Distributed: February 28, 2020
(With 1 figure)

Abstract

Beauveria bassiana is a promising fungus for the biological control of insect pests. The growing costs of conidia production have raised the need to ascertain the efficiency of some low cost substrates. The aim of this study was to analyze the potential use of different raw substrates without nutritional supplement for *B. bassiana* conidiogenesis. Growth and sporulation were evaluated using 30 g of substrate and 0.3 µL of a conidia suspension (1 x 10^6 conidia/mL). After 10 days of incubation (70 ± 10% humidity and temperature (T) = 29 ± 1 °C), rice (2.00 x 10^6 conidia/g substrate), algaroba (2.36 x 10^6 conidia/g), malt A (1.22 x 10^6 conidia/g) and malt B (1.75 x 10^6 conidia/g) showed the highest levels of conidia production. The resulting conidia showed insecticidal activity higher than 80% on coconut termites. These new raw substrates may represent viable alternatives for the production of entomopathogenic fungi for use in the biological control of various insect pests.

Keywords: biological control, raw substrates, *Beauveria bassiana*, entomopathogenic fungus.

Substratos alternativos para conidiogênese do fungo entomopatogênico *Beauveria bassiana* (Bals) Vuillemin (Deuteromycotina: Hyphomycetes)

Resumo

Beauveria bassiana é um fungo promissor no controle biológico de insetos-praga. As crescentes despesas na produção de conídios levantam a necessidade de averiguar a eficiência de alguns substratos de baixo custo. O objetivo deste trabalho foi analisar o potencial de utilização de diferentes substratos brutos para a conidiogênese de *B. bassiana.* O crescimento e esporulação foram realizados utilizando 30 g do substrato e 0,3 µL da suspensão de conídios (1 x 10^6 conídios/mL). Após 10 dias de incubação (umidade 70 ± 10% e temperatura T = 29 ± 1° C), o arroz (2,00 x 10^6 conídios/g de substrato), algaroba (2,36 x 10^6 conídios/g), malte A (1,22 x 10^6 conídios/g) e B (1,75 x 10^6 conídios/g), apresentaram maior produção de conídios. Os conídios produzidos mostraram atividade inseticida sobre o cupim do coqueiro acima de 80% de mortalidade. Estes novos substratos brutos podem representar uma alternativa viável para produção de fungos entomopatogênicos para uso no controle biológico de vários insetos praga.

Palavras-chave: controle biológico, substratos brutos, *Beauveria bassiana*, fungos entomopatogênicos.

1. Introduction

Pest control has been mostly achieved through the use of agrochemicals, which generate toxicity problems for humans and wildlife, leave residues in food, water and soil and result in the appearance of new pests and populations resistant to these products (Chávez et al., 2016; Amatuzzi et al., 2018).

The search for ecologically correct techniques and solutions has arisen largely due to awareness of the misuse of pesticides, and biological pest control using microbial agents has shown great promise. Of the microbial control agents used for economically important pests, fungi appear to be the most promising due to the abundance of fungal genera and species (Zimmermann, 1982; Burges, 2000).

Among the entomopathogenic fungi, *Beauveria bassiana* is considered an important agent for biological pest control. This fungus is commonly found parasitizing insects and has specificity for more than 200 species of insect pests (Dorworth, 1997). Conidia are the application structures produced and marketed for the different entomopathogenic fungal species, and white rice and barley are standard media used for their production because they are rich in carbohydrates and has the ability to conserve moisture (Mascarin et al., 2010). Rice stands out because it has a combination of factors, including its nutritional characteristics, price, wide availability worldwide and physical characteristics, such as the grain size and shape (Mascarin and Quintela, 2013).

However, depending on its use, the scale of production on solid culture medium and the shelf life until use make this technology expensive. One of the main factors needed for the success of biological control using *B. bassiana* is production of a large quantity of conidia at a competitive price. Thus, the production process must be inexpensive while producing viable and virulent conidia (Robl et al., 2009).

In an attempt to reduce costs and obtain large quantities of viable propagules, natural substrates, such as millet, corn kernels, wheat grain (Damir, 2006), tapioca flour, sweet potato, pumpkin and papaya extracts (Hepburn, 1985), mixtures of rice bran and husks, barley (Dorta et al., 1990; Nelson et al., 1996) and other substrates, have yielded promising results for the production of *B. bassiana*. However, these substrates were supplemented to ensure growth and sporulation of the fungus effectively. Considering these aspects, basic studies are needed to evaluate and select new substrates, such as algaroba fiber, malt residue, acerola (seeds and fibers) and sugarcane bagasse, due to their high availability and low cost in the northeastern region of Brazil. An evaluation of the important nutritional properties required for fungal growth is also needed.

Algaroba is a non-oleaginous legume belonging to the genus *Prosopis*. The fruit of the algaroba plant is a legume that contains high protein and carbohydrate levels. This species is used for the production of wood, charcoal, alcohol, molasses, animal feed and human consumption. Algaroba is highly abundant in northeast Brazil and is thus used for various purposes (Mahgoub et al., 2005).

Malt bagasse is the wet residue resulting from beer production. Malt bagasse can be described as the mass resulting from the agglutination of the husk with residues from the malting process. The malt residue has high protein and sugar levels resulting from the hydrolysis reactions of the cereal's starch content, which makes it attractive for reuse in other processes (Jenkins et al., 1998).

The acerola or Antilles cherry belongs to the family *Malpighiaceae* and genus *Malpighia*. This species is widely cultivated in the northeastern and southeastern regions of Brazil. The widespread use of this fruit in the juice pulp industry has led to inadequate residue disposal (bark and seeds). During acerola processing for juice or frozen pulp production, fruit pressing produces a highly fibrous residue. This residue is often discarded, which generates a huge volume of organic waste during the acerola harvest (Hanamura et al., 2005; Yahia, 2011). In the environment, large quantities generate pollution that may be minimized by using this product for the growth of entomopathogenic fungi and the production of enzymes during solid state fermentation (Botella et al., 2007).

Sugarcane is a semi-perennial plant that grows in tropical and subtropical climates and belongs to the family *Poaceae*. It is considered one of the main crops produced in Brazil. For each ton of processed sugarcane, an estimated 250 kg of bagasse is generated. This amount of sugarcane bagasse makes it the most abundant lignocellulosic agroindustrial by-product in Brazil (Hofsetza and Silva, 2012; Lago et al., 2012).

Recently, special attention has been given to minimizing or reusing the solid waste generated by different industrial processes. Waste from the food industry involves significant quantities of bark, seeds and other byproducts. In addition to being a source of organic matter, these residues serve as a source of carbon, energy and protein that can be recovered and exploited. The use of non-conventional raw substrates and agroindustrial residues without additional nutritional supplements for fungal growth will provide new perspectives for the study of *B. bassiana* production and advance studies on biological pest control, growth evaluation, conidial viability and pathogenicity. In this context, the present study analyzed the potential use of different substrates for conidiogenesis of the entomopathogenic fungus *B. bassiana.*

2. Material and Methods

2.1. Fungal lineage

The *B. bassiana* lineage used was provided by the Micoteca URM culture collection at the Federal University of Pernambuco (Accession number - URM 2915).

2.2. Origin of insects

The termites (*Heterotermes* sp.) were collected from a coconut crop in the municipality of Alhandra, Paraíba state (PB), Brazil.

2.3. Origins of substrates

Five substrates were used as specified in Table 1.

The brewer's malt residues were collected at the end of the wort clarification and sparging stages of the artisanal brewing process. Malt B was collected when the wort from the sparging stage reached a specific gravity of 1.010, whereas malt A was collected when the specific gravity of the wort reached 1.005. The density of the wort reflects the concentration of soluble solids contained therein, which are mainly sugars (fermentable and dextrins). For the algaroba substrate, the fibers resulting from a pod pressing process were used. The algaroba pods were pressed with a manual hydraulic press at a pressure of 50 kgf/cm^2 based on the methodology of Silva et al. (2003). The fibrous residue resulting from the pressing of these pods (algaroba fiber) was used as the substrate in the solid state fermentation process.

2.4. Algaroba pressing

The algaroba pods were carefully selected, and pods infected by fungi and insects were discarded. Then, the pods were sanitized by immersion in a 3% sodium hypochlorite solution for 5 minutes and rinsed under running water to remove the sanitizing solution. After this procedure, the pods were hydrated in distilled water at 65 ± 2 °C at a 1:1 (m/v) ratio (1 kg of pods to 1 L of water) for 3 hours. At the end of this process, the hydrated pods were pressed in a manual hydraulic press at a pressure of 50 kgf/cm^2 (Silva et al., 2001).

2.5. Medium for maintaining the fungal lineages

Sabouraud-dextrose agar: 10 g of meat peptone, 40 g of dextrose, 15 g of agar and 1000 mL of distilled water at pH 5.6. The samples were inoculated into test tubes containing Sabouraud-dextrose agar medium, and the cultures were incubated at room temperature for 15 days and then refrigerated at 4 °C.

2.6. Conidia production

The fungus was inoculated onto the culture media for conidiogenesis (sporulation) (complete medium - CM) (Alves et al., 2002). Then, the plates were incubated at 25 °C for 7 to 10 days to allow fungal growth and conidiogenesis. The conidia were collected by scraping the surface of the culture medium, transferring the scrapings to test tubes closed with PVC film and storing them under refrigeration at 4 °C for less than 10 days. To prepare the suspensions, distilled water containing 0.01% Tween-80 was added to the conidia. Next, the conidia concentration was estimated using a Neubauer's chamber, and the suspensions were standardized to 1 x 10^6 conidia/mL.

2.7. Preliminary tests to adjust the moisture content of the culture media to 70%

The test was performed to measure the volume of distilled water in 30 g of each substrate contained in a 250-mL Erlenmeyer flask. The Erlenmeyer flasks were autoclaved and weighed after cooling. The Erlenmeyer flasks were placed in an oven (80 °C) to dry the materials until they reached a constant weight. Subsequently, the material was weighed again, and after subtracting the dry mass, the volume of water required for each substrate to reach approximately 70% moisture after autoclaving was calculated. The volume of liquid added to the medium was based on the equation 1 specified below:

$$m_{H_2O} = \frac{m_S(X_2 - X_1)}{1 - X_2} \quad (1)$$

where:

m_s = mass of the substrate

X_1 = initial moisture of the substrate

X_2 = desired moisture content

2.8. Evaluation of Beauveria bassiana production on different substrates

Five different carbohydrate-rich substrates were used (Table 1). The experiment was performed in 250-mL Erlenmeyer flasks containing 30 g of the substrate at an approximately 70% moisture content. Three flasks were prepared for each substrate. Approximately 1x10^6 conidia/mL were inoculated, and the vials were incubated (T = 29 ± 1 °C) and analyzed for 10 days. After this incubation period, 30 mL of distilled water with 0.01% Tween-80 was added to obtain a final dilution of 1:10, and a 0.1 mL aliquot was collected to count conidia using a Neubauer's chamber under an optical microscope.

2.9. Evaluation of conidia viability

Conidia samples produced on the different media were serially diluted to achieve a concentration of 1 × 10^6 conidia/mL. From this suspension, 100 mL was inoculated onto Petri dishes containing PDA (potato dextrose agar) medium in triplicate and then incubated for 18 hours (T = 29 ± 1 °C). After this incubation period, colony counts were performed for each plate, with 200-300 viable or non-viable conidia counted under a light microscope (400X magnification).

Table 1. Substrate origins.

Substrate	Source
Polished rice (standard substrate)	Commercial product
Malt A and B residues	Applied Organic Chemistry Laboratory, CBiotec - UFPB (João Pessoa, PB)
Sugarcane bagasse	Sugarcane juice stand (João Pessoa, PB)
Acerola - fibers and seeds	Ideal Fruit Pulps Industry (João Pessoa, PB)
Algaroba - fibers	Japi City/Rio Grande do Norte state (RN) (semi-arid region)

2.10. Bioassay - evaluation of insecticidal activity

A total of 45 termites were used. Each experiment included 15 termites per treatment and was performed in triplicate. The termites were immersed for 10 seconds in autoclaved distilled water + Tween-80 (0.01%) containing a conidia suspension (1 x 10^6 conidia/mL). Subsequently, the termites were individually transferred to Petri dishes containing an artificial diet (dry leaves and stem pieces of coconut trees). The plates were maintained at room temperature and evaluated every 24 hours for 10 days. The negative control was performed by immersing the insects in autoclaved distilled water + Tween-80 (0.01%) and maintaining them under the same conditions described above.

2.11. Statistical analysis

The experiments were performed in a fully randomized experimental design. The data were analyzed using analysis of variance (F-test) and comparisons of means (Tukey test) at a 5.0% probability level with the Sisvar computational program (Ferreira, 2003).

3. Results and Discussion

3.1. Evaluation of Beauveria bassiana growth

The mycelial growth of *B. bassiana* was evaluated for 10 days, on the different substrates. Variation in growth was observed (Table 2). The polished rice (standard substrate) (2.00 x 10^6 conidia/g of substrate), malt A (1.22 x 10^6 conidia/g of substrate), malt B (1.75 x 10^6 conidia/g of substrate) and algaroba fiber (2.36 x 10^6 conidia/g of substrate) substrates yielded the highest conidia production. The sugarcane bagasse (0.85 x 10^6 conidia/g of substrate), acerola seed (0.56 x 10^6 conidia/g of substrate) and acerola fiber (0.54 x 10^6 conidia/g of substrate) substrates yielded the lowest conidiogenesis levels.

These different results can be explained by the texture and nutritional support of each substrate. The algaroba fiber showed considerable growth and conidia production most likely because it is a nutrient-rich legume with a variable chemical composition that favors microbial growth and conidia production (Mahgoub et al., 2005; Braga et al., 2009). Malt B, which is produced during the beer brewing process, was sparged fewer times and thus yielded higher conidia production than malt A (more sparging) due to its elevated levels of fermentable sugars. The specific gravities of the wort at the end of the recirculation/sparging step were 1.010 for malt B and 1.005 for malt A. These malt collection conditions help explain the improved growth of *B. bassiana* on malt B.

Despite being a substrate rich in polysaccharides and having fibrous characteristics that favor aeration and growth during cultivation, the sugarcane bagasse lost moisture over the 10 days of incubation. The loss of moisture led to low nutrient availability, which affected fungal growth and sporulation (Table 3).

The acerola seeds and fibers also showed low conidiogenesis compared to the other substrates used (rice, algaroba fibers and malts A and B). This reduction may have been due to the different concentrations of lignin present in the composition of this plant species.

Due to the high rigidity and impermeability conferred by lignin, most fungi are unable to hydrolyze this chemical compound. Lousada Júnior et al. (2006) found an average of 18.4% lignin in acerola by-products. This concentration is considerable and may be related to the decreased germination and growth of *B. bassiana* on these substrates. Plant species have varying lignin concentrations. Thus, the presence of lignin in the cell wall hinders the enzymatic hydrolysis of carbohydrates.

In the present study, raw substrates that were not pretreated or supplemented for fungal development were used, which might explain the decreased growth of the microorganism on these substrates, but specifically in sugarcane bagasse, acerola seeds and fibers.

Some environmental factors, such as temperature and moisture content, can also influence fungal growth and development (Yeo et al., 2003). The variation in the moisture content of the different substrates used to culture *B. bassiana* after 10 days of incubation at room temperature (T = 29 ± 1 °C) is described in Table 3.

Table 2. Average yield and viability of *Beauveria bassiana* conidia (conidia/g) grown on different substrates (T = 29 ± 1°C), after 10 days of incubation.

Substrates	Humidity (volume of distilled water)[0]	Conidia production (x 10^6)[1,2] (±SD)[3]	Viability (%)[1] (±SD)[3]
Polished rice (standard medium)	30 mL	2.00 ± 0.27 a	99.96 ± 0.16 a
Malt A	30 mL	1.22 ± 0.20 ab	90.04 ± 0.23 a
Malt B	30 mL	1.75 ± 0.28 ab	93.17 ±0.25 a
Algaroba fiber	30 mL	2.36 ± 0.31 a	98.21 ± 0.27 a
Sugarcane bagasse	60 mL	0.85 ± 0.08 b	55.10 ± 2.83 b
Acerola seed	30 mL	0.56 ± 0.04 b	68.29 ± 2.03 b
Acerola fiber	20 mL	0.54 ± 0.02 b	71.11 ± 1.09 b
CV%[4]		**21.68**	**7.48**

[0]Volume of distilled water required to achieve 70 ± 10% moisture in 30 g of crude substrate; [1] Means followed by different letters in the columns are significantly different from each other (5% level of significance) based on the *Tukey test*; [2]Data transformed by √x + 0.5; [3] Standard error of the mean; [4] Coefficient of variation.

Table 3. Variation of moisture in the different substrates used for *Beauveria bassiana* cultivation after 10 days of incubation (T = 29 ± 1°C).

Substrates	Initial moisture content (%)	Final moisture content (%)
Polished rice (standard medium)	71.50	67.56
Malt A	76.67	68.99
Malt B	74.49	66.28
Algaroba fiber	76.98	69.03
Sugarcane bagasse	66.87	55.98
Acerola seed	71.78	61.17
Acerola fiber	72.25	63.61
CV%	**6.89**	**5.63**

Table 4. Pathogenic activity of *Beauveria bassiana* produced on different substrates against the adult coconut termite under laboratory conditions at a concentration of 10^6 conidia/mL^{-1}. Incubation period - 10 days.

Substrates	Confirmed mortality rate (%) (± SD)[3]
Rice	91.6 ± 23.60
Malt A	83.4 ± 11.02
Malt B	87.9 ± 13.50
Algaroba fiber	89.9 ± 10.98
Sugarcane bagasse	93.02 ± 12.90
Acerola seed	90.04 ± 9.56
Control	1.22 ± 0.10
CV%	**11.8**

The moisture content of the different substrates tested varied during the 10 days of cultivation. Malt A, malt B and the algaroba fibers yielded higher conidia production and were the substrates that lost the least amount of moisture over the 10-day experiment, with final moisture contents of 68.99%, 66.28% and 69.03%, respectively.

Although the final moisture content of the substrates tested varied from 55.98% to 69.03% and differed from the optimal relative moisture content of 93% that favored mycelial development described by Webster and Gunnell (1992), all of the substrates tested showed fungal growth and sporulation.

Notably, the moisture content of the culture medium is one of the main parameters that influences conidia production. A substrate with an adequate moisture content also has the conditions needed for the transfer of nutrients and oxygen. However, the spaces between the particles must remain free to allow oxygen diffusion and dissipation. Therefore, moisture content control is essential for the optimization of solid-state cultivation processes. Excessive quantities of liquid in the solid matrix result in decreased porosity, lower oxygen diffusion and reduced gas exchange, all of which impair microbial respiration (Sánchez, 2009). However, low levels of moisture content lead to inhibition of fungal growth and consequently influence the final product of interest (or, in this case, the production and viability of the conidia) (Holker et al., 2004).

3.2. Viability of the conidia

Viability of the *B. bassiana* conidia (conidia/g) on different substrates are shown in Table 2.

The viabilities of the conidia produced on the rice, malt A, malt B and algaroba fiber substrates after 72 hours of inoculation at room temperature did not significantly differ from one another, with germination percentages of 99.96%, 90.04%, 93.17% and 98.21%, respectively. Conidia produced from the sugarcane bagasse, acerola seeds and acerola fibers showed significantly lower germination capacities (55.10%, 68.29% and 71.11%, respectively).

The verified significant differences in sporulation and conidia viability suggest that some important nutritional factor is lacking in addition to the inability to maintain the moisture content during the cultivation process for conidiogenesis for the sugarcane bagasse, acerola seed and acerola fiber substrates. This deficiency may be due to the reduction in the morphological differentiation of the vegetative portion of the fungus, in the reproductive structures, with consequent reductions in conidia production and their germination.

B. bassiana is considered a eucarpic fungus (Alexopoulos et al., 1996). Reproductive structures develop in only one part of the somatic structure, whereas the remaining portions remain in the vegetative form. This behavior (growth and sporulation) is a response to the composition and richness of the culture medium. However, the variation in the nutrient concentrations, such as carbon sources, and the reduction of the moisture content can explain the different responses in vegetative in vitro development of the fungus on the different substrates.

In a study on the selection of *B. bassiana* isolates for the control of the coffee borer, yields of approximately 2.5 x 10^6 conidia/mL were achieved on the corpses of coffee borer insects, which was similar to the results achieved in the present study (Neves and Hirose, 2005).

In a study performed by Sene et al. (2010), the percent germination of conidia from the fungus *Metarhizium anisopliae* produced on rice and a rice and brewery residue mixture showed a mean viability of approximately 85%, which was similar to the values found for malt A (90.04%), malt B (93.17%) and algaroba fibers (98.21%). Similar results were also found by Oliveira et al. (2008), who reported that

the viability of the fungi *B. bassiana* and *M. anisopliae* cultivated on potato dextrose agar medium supplemented with the antibiotic streptomycin sulfate and Nujol oil was greater than 95% and had pathogenic activity against insects when the different biological characteristic parameters were evaluated for *Diatraea saccharalis*.

3.3. Evaluation of the pathogenicity of Beauveria bassiana on the coconut termite

The *B. bassiana* conidia produced on polished rice, malts A and B, algaroba fibers, sugarcane bagasse, acerola seeds and acerola fibers had a lethal effect at the concentrations tested (10^6 conidia/mL)) (Figure 1). The results showed termite mortality at 72 hours after infection with conidia produced on all substrates. After 96 hours of infection, sparse hyphae were observed projecting from the surface of the termite cuticle. Beginning at 120 hours of infection, mummification and rupture of the insect cuticle were observed, with the exception of the control group.

During the early stages, the infection caused physiological changes, such as decreased movement, loss of motor coordination, cessation of food intake and death. Vey et al. (2002) found similar results in tick (*Ixodes ricinus* (L.) infected with fungi.

The physiological disturbances generated in the hosts were most likely caused by the production of mycotoxins. Destruxins, which are secondary metabolites produced by fungi, are the major toxins that affect the ion transport channels involved in muscle responses and cell membrane integrity. As reported by Shah and Pell (2003), these factors indicate that mycotoxins are involved in the symptomatology exhibited during the early stages of infection.

Roberts (1981) reported the actions of mycotoxins produced by *B. bassiana* when infecting the coconut tree termite were considered acute based on the complete and rapid elimination of nerve control over the bodily functions of the insect. Similar results were observed for leaf cutter ants (*Atta* sp.) and domestic flies infected with *B. bassiana*, where momentary decreases in movement were observed (Pell et al., 2001).

The mortality rates of the termites after infection with the conidia produced on the different substrates did not significantly differ but surpassed 80% mortality (Table 4) for all groups but the control group. The virulence of *B. bassiana* was not affected by its growth on the different raw substrates without any nutritional supplementation after 10 days of cultivation. The infection capacity, which is initially caused by conidial germination on the insect cuticle, may be associated with different factors, including pathogenicity (conidial dimensions, growth rate and enzymatic activities), virulence, specificity and tolerance of the host (Vargas et al., 2003).

The infected termites were placed on Petri dishes containing paper towels moistened with autoclaved distilled water and maintained at room temperature (T = 29 ± 1 °C). Together, these data suggested that *B. bassiana* did not depend on a nutrient-rich external environment to initiate the infectious process because moistened paper towels without nutrients ensured conidia germination and growth on the termite cuticle. Most entomopathogenic fungi require at least 95% relative humidity at the host surface to initiate the germination, germ tube extension and infection processes. High relative humidity and appropriate temperatures are favorable conditions for fungal growth and the occurrence

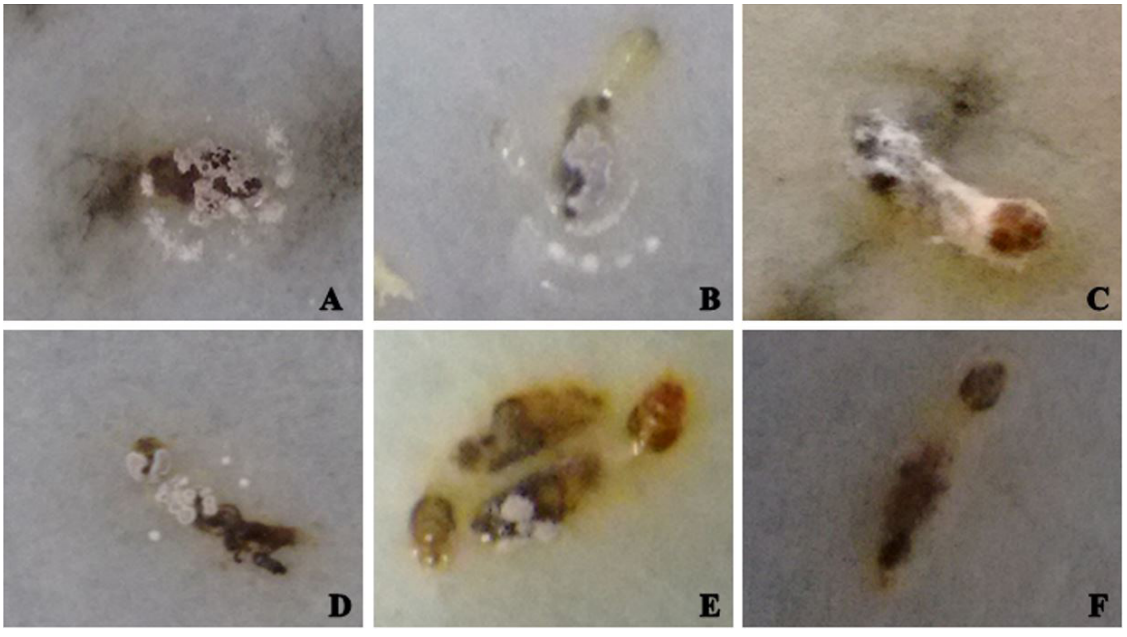

Figure 1. Infection of termites with *Beauveria bassiana*. Bioassay performed in autoclaved distilled water. Mummification of insects after 120 hours of infection. Conidia produced on algaroba fiber (A), malt A (B), malt B (C), acerola seed (D) and sugarcane bagasse (E). Negative control - after 120 hours (F).

of disease. Conversely, high and low temperatures slow fungal growth and consequently the development of disease (Hallsworth and Magan, 1999).

The pathogenicity of *B. bassiana* was not dependent on the medium where the conidia were produced but was dependent on the concentration of the conidial suspension, host specificity and abiotic factors.

B. bassiana conidia are surrounded by a transparent, mucilaginous and apparently viscous substance that, in addition to protecting it against desiccation, facilitates the adhesion of these conidia to the surface of the insect. The mucus that surrounds the conidia has high aminopeptidase levels, which can create favorable conditions for the performance of extracellular enzymes and guarantee the fungal germination and colonization processes on the insect cuticle (Alexopoulos et al., 1996).

The actions of enzymes involved in the pathogenicity of the fungus *B. bassiana* are also notable. The extracellular proteases produced by *B. bassiana* play a crucial role in hydrolysis of the cuticle, which is required for penetration of the fungus throughout the exoskeleton (Bidochka and Khachatourians, 1990). Once in the hemolymph, the fungus may produce toxic metabolites capable of causing paralysis (Shelton et al., 1998), acting on hemocytes (Mazet et al., 1994) and destroying the normal physiological balance of the host system (Sharma et al., 1994; Bechara et al., 2011).

By-products generated by different industrial processes contain many substances with high nutritional value for fungal growth. These by-products can be converted into commercial products or raw materials for secondary processes, thus enhancing the process of producing entomopathogenic fungal conidia used for the biological control of various insect pests.

Among the by-products used in the present study, we highlight the algaroba fiber because it presents similar results to the standard substrate (rice). This substrate may represent an alternative culture medium for the production of *B. bassiana*, since in this substrate the fungus maintained high conidiogenesis and humidity, without the loss of the insecticidal activity. However, other studies with other fungal lineages and bioassays with other insects are necessary.

Acknowledgements

To the Department of Mycology (Micoteca URM) at the Federal University of Pernambuco for providing the fungal strains and the Department of Chemical Engineering at the Federal University of Paraíba for providing the physical facilities to perform part of this work.

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