Fungi from the body surface of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and bioassay of insecticidal activity

Fungos da superfície corporal de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) e bioensaio de atividade inseticida

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**Bruna Novais Silva**
Master's Degree Program in Agricultural Microbiology
Institution: Universidade Federal de Viçosa (UFV)
Address: Av. Peter Henry Rolfs, s/n, Campus Universitário, Viçosa - MG, Brazil,
CEP: 36570-900
E-mail: bruna.n.silva@ufv.br

**Thiago Fernandes Sousa**
Doctorate of the Post-Graduate Program in Biotechnology
Institution: Universidade Federal do Amazonas (UFAM)
Address: Av. General Rodrigo Octavio Jordão Ramos, 1200, Coroado I, Bloco M,
Manaus - AM, Brazil, CEP: 69067-005
E-mail: thiago-fernandes2@hotmail.com

**Mateus Marques Maciel**
Graduate of Veterinary Medicine
Institution: Universidade Federal do Norte do Tocantins (UFNT)
Address: Centro de Ciências Agrárias, Rodovida BR 153, Km 112,
Araguaína - TO, Brazil, CEP: 77804-970
E-mail: marques.maciel@mail.uft.edu.br

**Maykon Jhuly Martins de Paiva**
PhD from the Graduate Program in Pharmaceutical Sciences
Institution: Universidade de Brasília (UNB)
Address: Faculdade de Ciências da Saúde, Asa Norte,
Brasília - DF, Brazil, CEP: 70910-900
E-mail: maykonjhuly@hotmail.com
Gilvan Ferreira da Silva  
Doctor of Agricultural Microbiology  
Institution: Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) - Amazônia Ocidental  
Address: Laboratório de Biologia Molecular e Genômica, Rodovia AM 010, Km 29, Manaus - AM, Brazil, CEP: 69010-970  
E-mail: gilvan.silva@embrapa.br

Bruna Alexandrino  
PhD in Veterinary Medicine  
Institution: Universidade Federal do Norte do Tocantins (UFNT)  
Address: Centro de Ciências Agrárias, Rodovida BR 153, Km 112, Araguaína -TO, Brazil, CEP: 77804-970  
E-mail: bruna.alexandrino@ufnt.edu.br

Taides Tavares dos Santos  
PhD in Biodiversity and Biotechnology  
Institution: Universidade Federal do Oeste da Bahia (UFOB)  
Address: Centro Multidisciplinar de Luís Eduardo Magalhães, Rua Itabuna, 1278, Luís Eduardo Magalhães, BA, Brazil, CEP: 47855-218  
E-mail: taidests@gmail.com

ABSTRACT
Spodoptera frugiperda (Lepidoptera: Noctuidae) is an important insect pest in Brazil and worldwide, and its control is based mainly on chemical methods that are harmful to the environment and human and animal health. We investigated the fungal community associated with the body surface of S. frugiperda and evaluated the potential of members of this fungal community to act as biocontrol agents. The caterpillars were collected in the field, reared in the laboratory on a natural diet, evaluated for association with fungi on their surface and the environment, and subjected to a bioassay for insecticidal activity. Our results suggest that fungi are often associated with the body of S. frugiperda, as it was possible to obtain these microorganisms from all insects sampled, with a higher frequency of filamentous fungi than yeast. Fusarium verticillioides was the most frequent filamentous fungus species (F3 = 42.1%), followed by Aspergillus aureolus (F2 = 36.8%). Candida pseudointermedia was the most frequent yeast species (L1 = 57.1%). Aspergillus aureolus F2 was the only strain that exhibited toxicity against S. frugiperda and can be considered a good candidate to be explored from the perspective of obtaining biotechnological products of interest for agriculture.

Keywords: bioinsecticide, corn, fungus-insect interaction, insect pest, yeast.

RESUMO
Spodoptera frugiperda (Lepidoptera: Noctuidae) é um importante inseto-praga no Brasil e no mundo, e seu controle é baseado principalmente em métodos químicos nocivos ao meio ambiente e à saúde humana e animal. Nesse estudo, investigou-se a comunidade...
fúngica associada à superfície corporal de *S. frugiperda* e avaliou-se o potencial dos membros dessa comunidade fúngica atuarem como agentes de biocontrole. As lagartas foram coletadas no campo, criadas em laboratório com dieta natural, avaliadas quanto à associação com fungos na superfície e no ambiente e submetidas a um bioensaio para atividade inseticida. Nossos resultados sugerem que os fungos estão frequentemente associados ao corpo de *S. frugiperda*, pois foi possível obter esses microrganismos de todos os insetos amostrados, com maior frequência de fungos filamentosos do que de leveduras. *Fusarium verticillioides* foi a espécie de fungo filamentoso mais frequente (F3 = 42,1%), seguido por *Aspergillus aureolus* (F2 = 36,8%). *Candida pseudointermedia* foi a espécie de levedura mais frequente (L1 = 57,1%). *Aspergillus aureolus* F2 foi a única cepa que apresentou toxicidade contra *S. frugiperda* e pode ser considerada uma boa candidata a ser explorada na perspectiva de obtenção de produtos biotecnológicos de interesse para a agricultura.

**Palavras-chave:** bioinseticida, milho, interação fungo-inseto, insetos-praga, levedura.

## 1 INTRODUCTION

Decreases in agricultural productivity and consequent economic losses in Brazil and other tropical countries are strongly associated with the performance of insect pests (Ramos *et al.* 2020; Horikoshi *et al.* 2022). Among these insects, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), also known as fall armyworm, stands out. It is the main pest of corn crops in the Americas, and despite its preference for grasses, it can also be present in bean, cotton, and sorghum crops (Jaraleño-Teniente 2020).

The proliferation of *S. frugiperda* can be favored by factors such as climate, sowing time, and irrigation systems, with the use of chemical methods prevailing for its control. In these methods, the product is sprayed over a broad spectrum, eliminating pests and any other natural enemies that may be present in the environment. As is already known, chemical control can cause environmental and human health problems, which can cause negative effects on beneficial organisms in the crop, in addition to selecting populations of caterpillars resistant to the pesticides used (Dos Santos *et al.* 2021). In addition, the indiscriminate use of insecticides causes damage to the soil, environmental balance, and human and animal health (Meena *et al.* 2020; Martins and Oliveira 2022).

In view of the environmental problems mentioned above, owing to the use of pesticides, the need to search for solutions for the control of insect pests that are less
aggressive to the environment and, at the same time, efficient, becomes evident. From this perspective, crop management and pest control have been studied for various agricultural pests. Several predators act to control *S. frugiperda* in corn, and compounds obtained from microorganisms have been shown to act as bioinsecticides (Figueiredo et al. 1999; Bateman et al. 2018), which may change the perspective of insect pest control, which is currently based on chemical control.

Several studies have suggested that insects and other arthropods, such as ticks, generally harbor multiple microbial taxa (Douglas 2015; Santos et al. 2021). For example, yeasts and filamentous fungi have been recovered from the external surfaces of gorse-associated insect species, including lepidoptera *Cydia ulicetana* (Lepidoptera: Tortricidae) (Yamoah et al. 2008). However, studies are still scarce, and the role played by the microbiota associated with the external surface of insects is still not clear, and it may be symbiotic (beneficial association) (Biedermann and Vega 2020) or harmful to the insect, acting as a natural enemy (a potential agent of biocontrol) (Ruiz-Nájera et al. 2013; Firake and Behere 2020).

In terms of the use of microorganisms and/or their metabolites in biological control strategies, the use of fungi with insecticidal activity has been highlighted, which can be used in agriculture to partially or fully replace chemical insecticides (Bai et al. 2019; Yuan et al. 2020; Berestetskiy and Hu 2021). Approximately 700 of the 100,000 described species of fungi are pathogenic to insects, consequently controlling their population levels in a given agricultural crop; therefore, they are defined as biological products or bioinsecticides (Khan et al. 2012). Despite this, there are few reports on the use of bioinsecticides that are currently used to control *S. frugiperda* and are related to the use of viruses of the Baculoviridae family (Baculoviruses) and bacteria of the species *Bacillus thuringiensis* (Cruz et al. 2002; Dos Santos et al. 2021; Abbas et al. 2022).

Therefore, the importance of the present study is evident. The objective of this study was to analyze the fungal community associated with the body surface of *S. frugiperda* and screen the potential of members of this community that act as biocontrol agents with insecticidal potential. We hypothesized that during the rearing of insects in the laboratory, with a natural diet, fungi associated with the body surface, present in the
rearing environment or in the food items of these insects, can act as natural enemies and exhibit biocontroller/bioinsecticide action.

2 MATERIALS AND METHODS

2.1 CHARACTERIZATION OF THE STUDY AREA

The study was conducted in the western region of Bahia State, Brazil. The region has a tropical climate (Aw/Köppen-Geiger) (Peel 2007), with a dry winter that lasts from May to September, and a rainy and hot summer that extends from October to April (Batistella et al. 2002). The predominant vegetation in the region is the Cerrado (Sano et al. 2009) and annual rainfall varies from 800 to 1,600 mm with average maximum and minimum temperatures of 26 °C and 20 °C, respectively (Batistella et al. 2002).

*Spodoptera frugiperda* was collected in the larval stage at the Centro de Pesquisa e Tecnologia do Oeste da Bahia (CPTO), Fundação Bahia, located on highway BR 020/242, km 53, in the municipality of Luís Eduardo Magalhães, Bahia State, Brazil (Figure 1).
Figure 1 - Map showing the place where the corn and caterpillar collections were performed, in Luís Eduardo Magalhães, Bahia State, Brazil.

Source: IBGE, 2022; Authors.

2.2 COLLECTION AND BREEDING OF CATERPILLARS (*S. frugiperda*)

The caterpillars were collected in the field, manually removed from the region called the "cartridge" of the corn plant (Figure 2), and identified by Dr. Lucas Souza Arruda, using the appropriate taxonomic key (Smith and Abbot 1797). The caterpillars were stored in a bowl containing corn leaves and were sent to the laboratory.
Figure 2 - Corn plantation (Zea mays L.) used in the study and indication of the part of the plant called "cartridge."

Source: Authors.

In the laboratory, the caterpillars were fed corn leaves (Zea mays L.) and were accompanied during the creation cycles. The corn plants used in this study were obtained from the IPR64 variety. The cultivation of this variety began in May 2021 in the same experimental area where the caterpillars were collected and was undertaken exclusively to feed the caterpillars in the laboratory and perform the other tests described in this study. This corn variety is called conventional, that is, without Bt technology, and was cared for and maintained without chemical pesticides or any other species to prevent damage to the growth and development of caterpillars as well as interference with the development of the experimental trials.

Corn leaves were randomly chosen for collection and the minimum distance from one plant to another was determined. Leaves were collected at the same stage of development as the region called the "cartridge." All plants had a healthy appearance without any symptoms of disease or deformation. Leaves that were very close to the ground or aged were excluded. Immediately after collection, the leaves were stored in first-use plastic bags, packed in isothermal boxes, and sent to the laboratory for disinfection and caterpillar feeding.
2.3 MONITORING THE GROWTH OF FUNGI ON SUBSTRATES AND SURFACES SURROUNDING THE INSECT DURING A LABORATORY BREEDING CYCLE

Daily monitoring of fungal growth on the surface of the body of *S. frugiperda*, on substrates used for food (corn leaves) and surfaces surrounding the larval-rearing environment during a laboratory rearing cycle was performed. Furthermore, sterile swabs were performed on the surface of the breeding environment and used to inoculate 90 mm diameter Petri dishes containing Sabouraud Dextrose Agar (SDA) culture medium supplemented with antibiotics (cephalothin at 175 µg/µL). The plates were incubated at 25 ± 2 °C and monitored for up to 15 days.

2.4 ISOLATION, PURIFICATION, AND CONSERVATION OF FUNGI ASSOCIATED WITH THE BODY OF *S. frugiperda*

For the isolation of the fungal microbiota from the surface of *S. frugiperda*, 10 caterpillars that were 20 days old were sampled; all caterpillars were healthy, active, and had body measurements visually compatible with their growth time. Individually and under aseptic conditions, a sterilized swab was gently placed on the entire surface of the insect and then used to inoculate Petri dishes (90 mm in diameter) containing SDA culture medium with cephalothin at 175 µg/µL. The plates were incubated at 25 ± 2 °C and monitored for up to 15 days. As filamentous or yeast-like fungi grew, the subcultures, morphological characterizations, and colony-forming units (CFUs) were counted.

All fungal colonies that grew in the culture medium were morphologically characterized and grouped into morphological types (morphotypes). Morphotype determination was performed according to the criteria proposed by Lacap *et al.* (2003) and Ibrahim *et al.* (2017), which included the colony growth rate, shape, and color. To avoid problems of subjectivity in the determination of colors, photographic records were made, and the obverse and reverse colors were defined using the RGB color scales, an abbreviation for the color system formed by Red (red), Green (green), and Blue (blue), used in color reproduction in electronic devices. The RGB system defines a color using a code composed of three values, attributing the corresponding proportion of each color with a minimum value of 0 and a maximum of 255. In the characterization (https://www.
site24x7.com/pt/tools/seletor-de-codigo-cor.html), colonies that had the same or very close codes were considered to have the same color.

The filamentous fungi were microscopically characterized using the microculture technique (Riddell 1950), which consists of cultivating the fungus in small pieces of the culture medium between a slide and a coverslip, where the fungus growth expands and fixes on the lower portion of the coverslip, which when carefully removed keeps the important structures for taxonomy intact, allowing its visualization under a microscope.

Filamentous fungi were preserved using the Castellani method (Castellani 1939) in triplicate. We used GYP medium (2.0% glucose, 1.0% yeast extract, and 0.5% peptone) and glycerol (15%) (Spencer and Spencer 1996) for yeast preservation. Under aseptic conditions, 1.0 mL of GYP medium was transferred to 1.5-mL microtubes used for yeast storage immediately afterwards, with the aid of a platinum loop. Each isolate was transferred to a microtube. The process was performed in triplicate, and the yeast isolates were stored under refrigeration in a cryopreservation box.

2.5 DNA EXTRACTION AND MOLECULAR IDENTIFICATION

Cell/spore suspensions were prepared at a concentration of $10^6$ and inoculated into BD medium (200 g/L potato; 20 g/L dextrose) for 2 days at 28 °C with orbital shaking at 150 rpm. The cell mass was filtered and lysed with liquid nitrogen. The DNA extraction followed the protocol for extraction with 2% CTAB cationic detergent by Doyle and Doyle (1987). The amount of DNA obtained was estimated by spectrophotometry (ND-2000, NanoDrop Technologies, Wilmington, DE), and its integrity was verified by electrophoresis on a 0.8% weight per volume (w/v) agarose gel. The DNA was diluted to a concentration of 75 ng/µL.

Each PCR reaction mixture reached a final volume of 25 µL, containing 2.5 µL of 10× Easytaq® buffer, 10 nM dNTP, 1U Easytaq® DNA Polymerase, 0.5 pM of each primer, 1 µL of total DNA (75 ng), and ultrapure distilled water to reach final volume. The amplification reactions for the ITS region using ITS1 and ITS4 primers were as follows: initial denaturation at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 60 s. The final extension step
was performed at 72 °C for 5 min. The PCR products were resolved on 1.5% (w/v) agarose gel stained with ethidium bromide; the photos were obtained using the Molecular Imaging System (Locus Biotecnologic L-Pix. Chemi) and the amplicons were compared with a 1 kb plus marker (Invitrogen, Catalog number: 10787018).

Prior to sequencing, the PCR products were cleaned with ExoSAP-IT (Applied Biosystems, product code:15819906). For this purpose, 5 µL of PCR products was mixed with 2 µL of EXOSAP and incubated at 37 °C for 15 min, followed by the ExoSAP-IT inactivation step at 80 °C for 15 min. The cleaned PCR products were then subjected to sequencing using the BigDye terminator protocol using a 10 µL bulk reaction containing 2 µL of ultrapure water, 1.5 µL of 5× BigDye buffer, 0.5 µL of BigDye terminator. v3.1 (Thermo Fisher Scientific), 1 µL of each primer, and 5 µL of clean PCR products. The thermocycling conditions consisted of denaturation at 96 °C for 60 s, followed by 35 cycles at 96 °C for 15 s, 50 °C for 15 s, and 60 °C for 4 min. The reactions were resolved by capillary electrophoresis on a 3500 Genetic Analyzer sequencer (Thermo Fisher Scientific).

Consensus sequences were obtained based on forward and reverse sequence alignments using DNA baser assembly software (http://www.dnabaser.com/). The new sequences obtained were deposited in GenBank (http://www.ncbi.nlm.nih) under accession numbers OP454868, OP454869, and OP454912. Gender-level identification was performed based on the consensus tape using the BLASTn tool.

2.6 STATISTICAL ANALYSIS OF THE FUNGAL COMMUNITY ASSOCIATED WITH *S. frugiperda*

The statistical analysis of the fungal community associated with *S. frugiperda* was performed according to the methods of Krebs (1978) and Ludwig and Reynolds (1988). The occurrence of filamentous fungi and yeasts associated with *S. frugiperda* is expressed as the number of CFUs per host. The CFUs of each host were morphologically characterized and grouped into morphospecies. Morphospecies from the different hosts were grouped into morphotypes. The frequency of occurrence (Fo) of the morphotypes was calculated as the percentage of caterpillars in which a given morphotype was found.
in relation to the total number of caterpillars sampled. The constancy of any morphotype (x) was based on the Fo data and corresponded to the percentage of samples in which morphotype x was present. Morphotypes were classified as constant when present in >50% of samples, accessory when present in 25–50% of samples, and accidental when present in less than 25% of samples.

2.7 SCREENING FOR INSECTICIDAL ACTIVITY AGAINST *S. frugiperda*

Representatives of the three morphotypes associated with the body of caterpillars with the highest frequency of occurrence (Fo) were used in the screening assay for insecticidal activity against *S. frugiperda*. The liquid containing fungal metabolites was obtained following the methodology proposed by Souza *et al.* (2004) with slight modifications. After eight days of culture, colony disks (filamentous fungi or yeast) measuring 6 × 6 mm were obtained for fermentation in 20 mL of GYP. The culture media inoculated with the fungi were incubated on a shaker platform (Luca – 222), at 25 °C and 120 rpm. The incubation lasted five days for the yeast isolates and eight days for the filamentous fungi. After this period, the cell mass was separated from the metabolic medium by centrifugation in a refrigerated centrifuge (SL - 706) for 20 min at 3,500 rpm, followed by the vacuum filtration of the metabolic liquid on 80 g of qualitative filter paper. Under sterile conditions, the liquid was filtered again through a syringe filter with 0.45 μm pores and stored at 4 °C for later testing with the caterpillars.

The caterpillars used in the bioassay were reared in the laboratory and used in the assay after three days of hatching. The metabolic liquids obtained were used for the immersion of three corn leaves (repeat), previously disinfected by immersion in 70% ethanol solution (100 mL) for 1 min, followed by a solution of sodium hypochlorite containing 2.0–2.5% of active chlorine (100 mL) for 4 min, and immersion to a 70% ethanol solution for 30 s (100 mL). Finally, the fragments were washed three times in sterile distilled water for 2 min. After disinfection, each leaf was submerged in each metabolic liquid (treatment) for 30 s. The leaves were removed and dried at room temperature. Subsequently, the treated corn leaves were transferred to sterile Petri dishes and then 10 caterpillars were transferred to each dish. As a negative control, the
caterpillars were exposed to corn leaves immersed in a culture medium without inoculated fungi.

The assay was monitored daily and the mortality rate was determined 72 h after exposure to the metabolites. The criterion for the evaluation was the caterpillar's motor coordination, and the caterpillar was considered dead when no movement was observed. A brush with soft bristles was used to encourage the caterpillar to move, lightly touching its posterior portion (Oliveira and Nunes 2017).

In order to determine the toxicity (insecticidal action of the metabolic liquids), the following qualitative classification was used, proposed by Rabelo et al. (2021), which is related to the mortality rate of the caterpillars: mortality rate >50% = toxicity; mortality rate between 10 and 50% = with signs of toxicity; mortality rate <10% = no evidence of toxicity.

3 RESULTS

In terms of the daily monitoring of the growing caterpillars, the substrates used to feed them (corn leaves), and the surrounding surfaces, the growth of fungi was not observed macroscopically. However, when swabbing the surfaces surrounding the caterpillar, the growth of filamentous fungi (2.0 CFU/sampled point) and yeast (countless CFUs) was observed.

On the other hand, fungi were obtained quite frequently from the surface of *S. frugiperda*, as 100.0% (*n* = 10/10) of the sampled caterpillars had at least one CFU of filamentous fungus or yeast, 90.0% (*n* = 9/10) had at least one CFU of filamentous fungus, 90.0% (*n* = 9/10) had at least one CFU of yeast, and 80.0% (*n* = 8/10) had simultaneous CFUs of filamentous fungi and yeasts (Table 1).
Table 1 - Colony-forming Units (CFUs) of filamentous fungi per sampled caterpillar, CFUs of yeast per sampled caterpillar and fungal morphospecies (filamentous or yeast) by sampled caterpillar unit.

<table>
<thead>
<tr>
<th>Host</th>
<th>CFUs of filamentous fungi</th>
<th>CFUs of yeasts</th>
<th>Morphospecies of filamentous fungi</th>
<th>Morphospecies of yeasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caterpillar 01</td>
<td>4.6 x 10^1</td>
<td>1.1 x 10^1</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Caterpillar 02</td>
<td>2.0 x 10^0</td>
<td>1.0 x 10^0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Caterpillar 03</td>
<td>1.4 x 10^1</td>
<td>2.0 x 10^0</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Caterpillar 04</td>
<td>-</td>
<td>4.0 x 10^0</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>Caterpillar 05</td>
<td>1.1 x 10^1</td>
<td>4.0 x 10^0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Caterpillar 06</td>
<td>5.2 x 10^1</td>
<td>2.0 x 10^0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Caterpillar 07</td>
<td>1.4 x 10^1</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Caterpillar 08</td>
<td>3.9 x 10^1</td>
<td>1.0 x 10^0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Caterpillar 09</td>
<td>9.4 x 10^1</td>
<td>1.4 x 10^1</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Caterpillar 10</td>
<td>7.8 x 10^1</td>
<td>1.1 x 10^1</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3.5 x 10^2</td>
<td>5.0 x 10^1</td>
<td>19.0</td>
<td>14.0</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>3.5 x 10^1</td>
<td>5.0 x 10^0</td>
<td>1.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Source: Authors.

In terms of the population size, expressed as CFU/caterpillar, there was a higher occurrence of filamentous fungi (mean ± standard deviation = 3.5 x 10^1 ± 3.2 x 10^1 CFU/caterpillar) than yeast (5.0 x 10^0 ± 5.1 x 10^0 CFU/caterpillar). A total of 33 morphospecies of fungi (filamentous or yeast) were obtained from the sampled caterpillars. Of these, 19 were filamentous fungi (mean ± standard deviation per caterpillar = 1.9 ± 0.9; median = 2.0), and 14 were yeasts (mean ± standard deviation per caterpillar = 1.4 ± 0.7; median = 1.5). After characterizing and comparing the morphospecies, it was possible to group them into four filamentous fungus morphotypes (F1–F4) and four yeast morphotypes (L1–L4) (Table 2).

Table 2 - Morphological characterization of filamentous fungi and yeast from the body surface of S. frugiperda (Lepidoptera: Noctuidae). *Total isolates equals the total of morphospecies; †The frequency of occurrence (Fo) was calculated as the relative occurrence of the filamentous fungi or yeast in relation to the total occurrence; ‡Percentage was calculated by multiplying Fo by 100. Abbreviations: F - Filamentous fungus; L – yeast.

<table>
<thead>
<tr>
<th>Morphotype</th>
<th>Macromorphological characterization</th>
<th>Total isolates (a)</th>
<th>Fo (b)</th>
<th>% (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>(208, 179, 109) / (216, 204, 162) / irregular / high air part</td>
<td>3</td>
<td>0.158</td>
<td>15.8</td>
</tr>
<tr>
<td>F2</td>
<td>(039, 042, 049) / (089, 083, 067) / regular / velvety aerial part</td>
<td>7</td>
<td>0.368</td>
<td>36.8</td>
</tr>
<tr>
<td>F3</td>
<td>(188, 109, 37) / (175, 156, 155) / regular / high cotonosa aerial part</td>
<td>8</td>
<td>0.421</td>
<td>42.1</td>
</tr>
</tbody>
</table>
The filamentous fungus morphotype with the highest Fo was F3 (42.1%), followed by F2 (36.8%). The most frequent yeast morphotype was L1 (57.1%), followed by L2. In terms of constancy, morphotypes F2 and F3 can be classified as accessory, whereas F1 and F4 are classified as accidental. In terms of yeast morphotypes, L1 can be classified as constant and all others as accidental.

A representative of each morphotype of the most frequent filamentous fungus (F2 and F3) and one of the most frequent yeast (L1) were identified using classical and molecular methods. Identity analysis using sequence data corresponding to the ITS region revealed that F2, F3, and L1 isolates corresponded respectively to the Aspergillus, Fusarium, and Candida genera. Isolate F2 (OP454869) was 99.19% identical with the type species of A. aureolus NRRL 2244, isolate F3 (OP454912) was 100% identical with F. verticillioides JCP2002, and isolate L1 (OP454868) was 99.59% identical with the type species of C. pseudointermedia CBS 6918.

A representative of each of the most frequent morphotypes [A. aureolus (F2), F. verticillioides (F3), and C. pseudointermedia (L1)], mentioned above, was used in the insecticidal activity assay, and the results of the action of each fungus can be seen in Table 3. Based on the classification criteria used, F. verticillioides (F3) and C. pseudointermedia (L1) were considered to have no signs of toxicity, whereas A. aureolus (F2) was considered to be little toxic.

<table>
<thead>
<tr>
<th>Morphotype</th>
<th>Fo (%)</th>
<th>Description</th>
<th>Identity Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2</td>
<td>36.8%</td>
<td>Irregular / High aerial part; center of the colony is greenish</td>
<td>99.19% identical with A. aureolus NRRL 2244</td>
</tr>
<tr>
<td>F3</td>
<td>42.1%</td>
<td>Irregular / Opaque and creamy aspect</td>
<td>100% identical with F. verticillioides JCP2002</td>
</tr>
<tr>
<td>L1</td>
<td>57.1%</td>
<td>Irregular / Opaque appearance, rough texture (recalls snowflakes)</td>
<td>99.59% identical with C. pseudointermedia CBS 6918</td>
</tr>
<tr>
<td>L2</td>
<td>21.4%</td>
<td>Irregular / Bright and creamy appearance</td>
<td></td>
</tr>
<tr>
<td>L3</td>
<td>14.3%</td>
<td>Regular / Glossy and Viscous Aspect</td>
<td></td>
</tr>
<tr>
<td>L4</td>
<td>7.1%</td>
<td>Regular</td>
<td></td>
</tr>
</tbody>
</table>

Source: Authors.
Table 3 - Bioassay of insecticide activity against *S. frugiperda* (Lepidoptera: Noctuidae). T - Total caterpillars on the Petri plate; M - Number of dead caterpillars.

<table>
<thead>
<tr>
<th>Treatment/ Repetition</th>
<th>Control</th>
<th>Aspergillus aureolus (F2)</th>
<th>Fusarium verticilloides (F3)</th>
<th>Candida pseudointermedia (L1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>M</td>
<td>T</td>
<td>M</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>1</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>1</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>2</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>7.7 %</td>
<td>29.6 %</td>
<td>4.3 %</td>
<td>-</td>
</tr>
</tbody>
</table>

Source: Authors.

4 DISCUSSION

The study of microorganisms associated with insects is important in terms of their impact on ecology and in the possible contributions that mediate the discovery and recognition of these organisms in the biotechnological field. In this study, the interaction between *S. frugiperda* and fungi associated with its body surface was investigated.

The fungal community associated with the body surface of caterpillars could be grouped into eight morphotypes, four filamentous fungi and four yeasts. Although a morphotype is not perfectly related to taxonomic species, a high morphotype richness indicates a potentially high richness of host-associated species. In the case of the fungal microbiota associated with the body surface of *S. frugiperda*, it is not a very rich but is quite an abundant community, which can and should be investigated regarding the ecological roles played, which potentially brings benefits to the health of the host, as reported in the literature on fungi associated with insects (Douglas 2015; Santos *et al.* 2018; Biedermann and Vega 2020). Such studies can provide insights into the development of products and/or processes of biotechnological interest that can go beyond insecticidal activity, such as the production of enzymes, antimicrobials, and other compounds of interest to humanity.

The morphotype with the highest Fo (F3 = 42.1%) in association with the body surface of *S. frugiperda* was identified as *F. verticilloides*. This species has been associated with corn diseases at all stages of development (Sartori *et al.* 2004), can infect seeds and seedlings, and is an economically important pathogen for cereals, given that it can cause substantial losses in productivity and seed quality (Broders *et al.* 2007). Corn
grains with color changes, ranging from pink to dark brown in the upper part, are characteristic of pathogen symptoms (Ramos et al. 2014).

Although *F. verticillioides* is a seed-associated pathogen, its transmission to other maize seedling organs (the remaining seed coat, primary root, subcoronal internode, coleoptile, and leaf base) has been reported (Sartori et al. 2004). In the present study, the isolate representing the F3 morphotype and other members of the group were isolated from the body of healthy *S. frugiperda*, which was fed on healthy corn leaves, indicating that this isolate could be in a state of latency of its pathogenicity, as a saprophytic in corn plants, or even being carried by the caterpillar and living symbiotically with it.

Pamphile and Azevedo (2002) reported the occurrence of *F. verticillioides* as an endophytic associated with maize plants and performed molecular characterization of the detected strains using a random amplified polymorphic DNA (RAPD) technique. Their analysis enabled them to separate endophytic strains of *F. verticillioides* by different seed genotypes and demonstrate a strong association among them, which may be specific for the genotype. According to the authors, the results obtained can be explained by a coevolutionary process involving endophytic isolates of *F. verticillioides* and corn lines, which is corroborated by the studies of Leslie (1996), who considered it possible that some of the mating populations of *F. verticillioides* have evolved with the host.

The second morphotype with the highest Fo was F2 (36.8%), which was identified as *A. aureolus*. Strains of this species have already been detected as endophytes in aerial plant tissues from Aizoaceae in South Africa (Pieterse et al. 2018). In addition, this species was also detected as an endophyte in upland rice (*Oryza sativa* L.) roots in China, with positive effects on the growth of seedlings of this plant (Pang et al. 2020). Fungi of the genus *Aspergillus* are part of the endophytic microbiota of *Z. mays*, with non-specific occurrence in plant tissues (Potshangbam et al. 2017), which suggests the existence of a symbiotic relationship between *A. aureolus* and *Z. mays*.

Although the relationship between *A. aureolus* and *Z. mays* appears to be clear and possibly beneficial, the relationship between this fungus and *S. frugiperda* requires clarification. Lubis et al. (2021) carried out a study to verify fungi in the soil rhizosphere of corn and *S. frugiperda* larvae that were attacked by fungi in the field in Indonesia. In
a preliminary identification, based only on morphology, they detected representatives of the genus *Aspergillus* both from the rhizosphere soil and fungus-infested *S. frugiperda* larvae. To the best of our knowledge, the present study is the first to accurately identify fungi of the genus *Aspergillus* from the body surface of healthy *S. frugiperda*, as well as *A. aureolus* in association with the body of an insect.

The representative of the most frequent yeast morphotype, L1 (57.1%), was identified as *C. pseudointermedia*. This species was isolated and described from “Kamaboko,” a traditional fish paste product in Japan (Nakase et al. 1976). Strains of this species have shown beneficial associations with maize, either as a nutritional enhancer in hydroponic crops when added by irrigation (Bedolla-Torres et al. 2015) or as an endophyte in leaf tissues (Khunnamwong et al. 2018). Although *C. pseudointermedia* has also been detected in other substrates, such as *Coffea arabica* during fermentation (Masoud et al. 2004) and in rotten wood (Barrilli et al. 2020), this is the first report of its association with the body of an insect.

Knowledge of the interactions between yeast and insects can be explored in several ways. Knight and Witzgall (2013) proposed the use of yeasts as a biological control for the moth *Cydia pomonella*, a known pest of apple trees. They combined a pathogenic granulovirus with yeasts isolated from *C. pomonella* larvae and, as a result of this combination, a significant increase in neonatal insect mortality was observed when compared to the use of the virus alone.

With regard to the interaction between yeasts and insects, the association of yeasts and *Drosophila* was studied by Murphy et al. (2016) using RNA interference. *Saccharomyces cerevisiae* cells genetically modified to express dsDNA were able to alter locomotion and survival in *Drosophila* larvae, reducing the insects’ fitness. This approach highlights the potential of yeasts as biocontrol agents, which can be exploited to control diverse insect pests, including *S. frugiperda*.

Screening insecticidal activities against *S. frugiperda* using fungal extracts is scarce in the literature. Most studies are based on the use of essential oils or plant extracts, as proposed by Niculau et al. (2013) and Tagliari et al. (2010), respectively, which makes
the results obtained here of great value not only for research aimed at controlling insect pests at the local level, but also for other pests, both nationally and worldwide.

The test showed that *F. verticillioides* (F3) and *C. pseudointermedia* (L1) did not show signs of toxicity as the mortality rate was very low. Additionally, considering that up to 10% mortality was acceptable in the caterpillars of the control group, this percentage is insignificant with regard to the insecticidal capacity of these isolates. However, *A. aureolus* (F2) showed signs of toxicity, which makes it significant from a biotechnological point of view, as it is a screening test that aims to identify strains with potential for biotechnological use.

The concentrations and exposure times used in this study may have influenced our results. Zhang *et al.* (2015) reported that the doses used in the bioassays performed by them directly influenced mortality rates, which suggests the need to optimize the methodology adopted in the screening. New assays can be established, and concentrated crude extracts of *A. aureolus* (F2) metabolites can be obtained with the aim of reassessing toxicity through the methodology used in the present study. Additionally, acute toxicity can be assessed as well, as proposed in the bioassay performed by Lima *et al.* (2009), in which the assessment of toxicity was performed at an interval of 96 h after application, in addition to observing the symptoms of neurotoxicity through the knockdown effect (immediate effect of the extracts on the caterpillars).

5 CONCLUSIONS

Despite its low toxicity, the isolate *A. aureolus* (F2) can be considered a good candidate for exploration from the perspective of obtaining biotechnological products of interest for agriculture. Future complementary studies should be conducted to quantitatively characterize the insecticidal activity of the metabolites of this fungus against *S. frugiperda*, as well as to elucidate the compound(s) responsible for this activity.
ACKNOWLEDGMENTS

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