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Molecular characterization and virulence of *Beauveria bassiana* and *Metarhizium anisopliae* against *Galleria mellonella* (Lepidoptera: Pyralidae) and *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae

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Entomopathogenic fungi considerably vary in their action and virulence mode. The contamination mainly depends on the adhesion and penetration of the fungus in the host integument. Four isolates from *Beauveria bassiana* and *Metarhizium anisopliae* were molecularly characterized by rDNA-ITS sequencing. Their virulence against last instar larvae of *Galleria mellonella* and *Tenebrio molitor* at four conidia concentrations: 1×10^6, 1×10^7, 1×10^8 and 1×10^9 conidia.ml^{-1} were *in vitro* evaluated and the lethal concentrations (LC_{50} and LC_{90}) as lethal time (LT_{50} and LT_{90}) were determined. Sequencing of rDNA-ITS 500 bp fragments allowed the identification of Ma10MI, Ma12MI, Ma58MI and Ma11MI isolates as *M. anisopliae* and Bb11MI, Bb79MI, Bb53MI and Bb27MI isolates as *B. bassiana* by sequence comparison to GenBank. According to the pathogenicity test, *B. bassiana* strains with higher performance for *G. mellonella* were Bb53MI and Bb79MI with $LC_{50}= 3.98\times10^7$ and 1.04×10^7 conidia.ml^{-1} and $LT_{50}= 5.46$ and 5.27 days, respectively, as well as to *T. molitor* was Bb79MI with $LC_{50}= 1.03\times10^7$ conidia.ml^{-1} and $LT_{50}=$ 5.57 days. The *M. anisopliae* strains with the best performance were Ma58MI and Ma10MI both *T. molitor* ($LC_{50}= 1.0\times10^6$ and 1.0×10^7 conidia.ml^{-1} and $LT_{50}= 4.06$ and 5.22 days, respectively) and *G. mellonella* ($LC_{50}= 6.3\times10^7$ and 1.1×10^8 conidia.ml^{-1} and $LT_{50}= 5.18$ and 6.37 days, respectively), these isolates might be considered as new promising candidates for the microbial pest control.

Key words: Pathogenicity, molecular taxonomy, entomopathogenic fungi, microbial control.

INTRODUCTION

It is well known that synthetic insecticides have deleterious effects on the worldwide environment and consequently, attention has addressed to biocontrol agents as suitable alternatives (Safavi et al., 2007). Entomopathogenic fungi are natural common enemies of arthropods, particularly insects, and they might be used in the management of pest populations in agroforestry and urban ecosystems (Lacey and Kaya, 2007). The

most important species, *Beauveria bassiana* (Vuill.) Balsam and *Metarhizium anisopliae* (Metch) Sorok. are associated with the Phylum *Ascomycota*, Order Hypocreales, Families Clavicipitaceae and Cordycipitaceae, respectively (Hibbett et al., 2007). Insect pathogenic fungi have to meet several host challenges producing enough new infectious spores in each generation maintaining viable populations. First, successful transmission often requires the release of massive spore numbers and/or sticky spore surfaces or substances maximizing adhesion in other ways (Vega et al., 2012). Second, spores should germinate and initiate penetration of the solid insect exoskeleton relatively quickly (Cole and Hoch, 1991). Third, the fungal cells must proliferate inside the hemocell, muscles, or other host body tissues collapsing the host immune system in order to the host dies shortly after (Vega et al., 2012). Fourth, the fungal pathogen should manage the host cadaver optimizing spore production and dispersal under prevailing environmental conditions (Roy et al., 2006).

Entomopathogenic fungi act by contact and are able to infect different live stages of insects with piercing-sucking mouthparts. They present horizontal and vertical transmissions, viz., inside the population (among individuals) and over time (across the progenies), respectively. Despite these advantages, a number of factors, such as the difficulty for environmental adaptability, resistance to chemicals, and lower ready-effect compared to the chemicals may limit the use of these fungi for the pest management in greenhouse, and especially, in field (Shah and Pell, 2003; Quesada Moraga et al., 2006a; Wraight et al., 2007). Nevertheless, a wide variability within and among species has been detected in pathogenicity, virulence and ecological features in many entomopathogenic fungi. This variability has been considered and analyzed for selecting candidates for myco-insecticides production (Quesada Moraga et al., 2006a; Wraight et al., 2007).

This study aimed at characterizing and evaluating the pathogenicity of *B. bassiana* and *M. anisopliae* strains isolated from Atlantic Forest soil, Rio de Janeiro, Brazil, against two insect hosts models: *Galleria mellonella* and *Tenebrio molitor,* also comparing the effect of entomopathogenic fungi against Lepidoptera and Coleoptera orders.

MATERIALS AND METHODS

Source of insects and fungal isolates

Four *B. bassiana* (Bb27MI, Bb11MI, Bb79MI and Bb53MI) strains and four *M. anisopliae* (Ma58MI, Ma10MI, Ma11MI and Ma12MI) from Atlantic forest soils in the Rio de Janeiro state, Brazil, were used in the experiment. The fungi were isolated according to the methodology described by Esparza Mora et al. (2016). The strains were maintained at 4°C on potato dextrose agar (PDA). Last instar larvae of *G. mellonella* and *T. molitor* were obtained from the Biological Institute in Campinas - SP. *G. mellonella* was fed by an artificial diet containing 472 g wax pure bee, 96 g skimmed milk powder, 188 g powdered yeast, 385 g maize flour, 160 g soya, 416 g of glycerin, 300 ml water distilled, while *T. molitor* was reared on bread.

Molecular identification of strains

After cultivation, DNA from the strains on PDA media for 7 days at 25 ± 1°C, DNA was extracted according to the CTAB method (Doyle and Doyle, 1987). The polymerase chain reaction (PCR) for amplification of fragment Internal Transcribed spacers (ITS) region was performed by a pair of universal primers ITS1 (5'-TCGGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990), generating estimated size of 500 bp product. The amplification reaction conditions consisted of 2 min at 94°C followed by 40 cycles of 15 s at 94°C, 30 s at 56°C and 2 min at 72°C with a final extension of 4 min at 72°C. The products were visualized under UV light in 1% agarose gels stained by ethidium bromide. The PCR products were purified by precipitation with polyethylene glycol according to the protocol described by Schmitz and Riesner (2006). The sequencing was performed by chain termination method with 3.1 BigDye (Applied Biosystems) reagent and ABI3500 automatic sequencer (Applied Biosystems). After sequencing, phylogenetic tree with similar sequences of the ITS1 and ITS4 strains regions was completed and sequences of different fungal species were obtained from the GenBank/NCBI, using the Maximum Likelihood method (ML), evaluating the strength of the topology, the tree was assessed by the Jukes-Cantor method with 1000 bootstrap replicates, and phylogenetic analysis was carried out in the Molecular Evolutionary Genetics analysis-MEGA 6.0.

Preparation of inoculum

Each strain was cultivated in Petri dishes containing sterile parboiled rice, maintained at 25 ± 1°C for 8 days. The conidia were collected with by sterile loop and suspended in sterile distilled water containing 0.1% Tween 80, and then agitated in vortex for 3 min. Four *M. anisopliae* and *B. bassiana* conidia concentrations: 1×10^9, 1×10^8, 1×10^7 and 1×10^6 conidia.ml^{-1} were prepared. The treatment control received only sterile distilled water with 0.1% Tween 80. The conidial counts in each suspension with an improved Neubauer Hemacytometer (Marienfeld, Germany) were determined.

Virulence of conidia

Ten (10) *G. mellonella* and *T. molitor* larvae were immersed in 10 ml of fungal suspension for 30 s. Thereafter, the treated insects were placed on filter paper (Whatman No. 1) inside a plastic box (18 × 8.5 × 4.5 cm) with ventilation holes (2 cm^2) on three sides. Food was introduced into the box and the ventilation holes were covered with a metal screen. The set-up was placed inside a climatic chamber at 25 ± 1°C. Three replicates per treatment with 30 species of each insect per repetition were accomplished.

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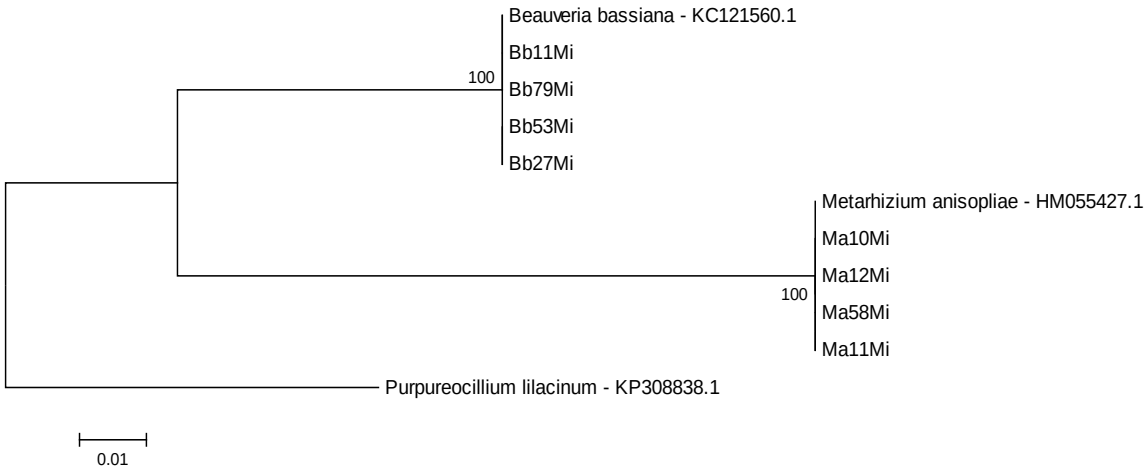

Figure 1. Phylogenetic tree constructed by ML method, Jukes-Cantor showing the relation between the strains of entomopathogenic fungi obtained from Atlantic Forest, and other related species deposited in the GenBank-NCBI. The sequences used are of the ITS regions of rDNA. Built tree in MEGA 6.0 program. The "bootstrap" values for 1,000 repetitions are indicated next to the tree branches. The *Purourеocillium lilacinum* ITS sequences was designated as the outgroup for rooting the tree.

Mortality was daily recorded for 10 days after inoculation. The fungi were re-isolated from dead insect samples selected randomly from each replicate box.

Statistical analysis

The results of *G. mellonella* and *T. molitor* insects mortality according to completely randomized design with three replications in a factorial 4 × 8 corresponding to four concentrations (1×10^6, 1×10^7, 1×10^8 and 1×10^9 conidia.ml^{-1}) and 8 species of fungi (Bb11MI, Bb27MI, Bb53MI, Bb79MI, Ma10MI, Ma11MI, Ma12MI and Ma58MI) were analyzed. After the analysis of variance, average between factor levels at 192 h of evaluation by Scott Kant test at 5% probability was compared. Statistical analyses by the SISVAR program (Ferreira, 2011) were performed.

RESULTS

Molecular characterization

The ITS1 – 5.8 – ITS4 rDNA sequences were compared with those available in the GenBank data base for *M. anisopliae* (accession numbers HM055427.1) and *B. bassiana* (KC121560.1). The amplification of the ITS region resulted in a single product for all isolates. The size of the product was about 500 bp.

The alignments and phylogenetic analysis confirmed the taxonomic identity of the strains used in our study. *M. anisopliae* Ma10MI, Ma12MI, Ma58MI and Ma11MI strains grouped from the same species strain sequence from the database, at 100% similarity. Among the *B. bassiana* species, it was observed that the strains Bb11MI, Bb79MI, Bb53MI and Bb27MI composed a grouping with *B. bassiana* strain from GenBank, at 100% similarity (Figure 1).

Evaluation of virulence

According to the Scott Knott test (α = 0.05), the pathogenic strains to *G. mellonella* were: Bb79MI, Bb27MI, Ma10MI and Ma58MI in three (1×10^7, 1×10^8 and 1×10^9 conidia.ml^{-1}) concentrations and Bb53MI (1×10^8 and 1×10^9 conidia.ml^{-1}) moderately; comparing the middle and lower values of larvae mortality at different concentrations, it was observed that Bb11MI, Ma11MI and Ma12MI strains were less pathogenic in 1×10^6 1×10^7 and 1×10^8 conidia.ml^{-1} concentrations (Table 1). Bb27MI and Bb79MI *B. bassiana* strains were more pathogenic than *T. molitor* in 1×10^7, 1×10^8 and 1×10^9 conidia.ml^{-1} concentrations; as well as Ma10MI and Ma58MI *M. anisopliae* strains were more pathogenic than *T. molitor* in 1×10^9, 1×10^7 and 1×10^8 conidia.ml^{-1} concentrations (Table 2).

Bb79MI *B. bassiana*, strain presented the highest pathogenicity on *G. mellonella* larvae with an 80.66% average mortality, $LC_{50} = 1.04\times10^7$ conidia.ml^{-1} and LT_{50}= 5.27 days (Table 3). Ma58MI *M. anisopliae* strain was the most virulent on *G. mellonella* larvae presenting 83.33% mortality, $LC_{50} = 6.31\times10^7$ conidia.ml^{-1} and LT_{50}= 5.18 days. Bb79MI strain presented 78% mortality in *T. molitor*, $LC_{50} = 1.03\times10^7$ conidia.ml^{-1} and $LT_{50} = 5.57$ days. Ma58MI *M. anisopliae* strain presented 82% mortality in *T. molitor,* $LC_{50} = 1.00\times10^6$ conidia.ml^{-1} and $LT_{50} = 4.05$ days.

The LT_{90} values to *B. bassiana* strains on *G. mellonella* larvae ranged from 8.03 to 10.23 days, and for *M. anisopliae* strains on *G. mellonella* larvae ranged from 8.29 to 10.47 days. In *T. molitor* larvae, the LT_{90} *B. bassiana* strains ranged from 9.24 to 10.04 days, in *M. anisopliae* strains the LT_{90} ranged from 7.18 to 11.26 days. The LC_{90} also varied depending upon the insect species and strain. The *B. bassiana* strain presenting the

Table 1. Mortality *G. mellonella* larvae at different conidia concentrations of *B. bassiana* and *M. anisopliae*.

Concentration Conidia.ml^{-1}	Bb11MI	Bb27MI	Bb53MI	Bb79MI	Ma10MI	Ma11MI	Ma12MI	Ma58MI
1×10^{9}	100.0^{Aa}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}
1×10^{8}	93.3^{bB}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}	76.7^{bC}	93.3^{bB}	100.0^{aA}
1×10^{7}	83.3^{cB}	100.0^{aA}	96.6^{aA}	100.0^{aA}	100.0^{aA}	63.3^{cD}	70.0^{cC}	100.0^{aA}
1×10^{6}	70.0^{dC}	73.3^{bC}	80.0^{bB}	83.3^{bB}	86.7^{bB}	56.7^{dD}	73.3^{cC}	96.7^{aA}
Control	20.0^{eA}	20.0^{cA}	20.0^{cA}	20.0^{cA}	20.0^{cA}	20.0^{eA}	20.0^{dA}	20.0^{bA}

Averages followed by different letters, small letters on columns and capital letters on lines differ by Scott Knott test at 5%. Mortality average at 8 days.

Table 2. Mortality *T. molitor* larvae at different conidia concentrations of *B. bassiana* and *M. anisopliae*.

Concentration Conidia.ml^{-1}	Bb11MI	Bb27MI	Bb53MI	Bb79MI	Ma10MI	Ma11MI	Ma12MI	Ma58MI
1×10^{9}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}
1×10^{8}	90.0^{bB}	100.0^{aA}	100.0^{aA}	100.0^{aA}	100.0^{aA}	90.0^{bB}	100.0^{aA}	100.0^{aA}
1×10^{7}	86.7^{bC}	100.0^{aA}	93.3^{bB}	100.0^{aA}	100.0^{aA}	70.0^{cD}	83.3^{bC}	100.0^{aA}
1×10^{6}	63.3^{c}E	70.0^{bD}	80.0^{cC}	80.0^{bC}	100.0^{aA}	66.7^{cD}	86.7^{bB}	100.0^{aA}
Control	10.0^{dA}	10.0^{cA}	10.0^{dA}	10.0^{cA}	10.0^{bA}	10.0^{dA}	10.0^{cA}	10.0^{bA}

Averages followed by different letters, small letters on columns and capital letters on lines differ by Scott Knott test at 5%. Mortality average at 8 days.

Table 3. LC_{50} and LT_{50} values of *B. bassiana* and *M. anisopliae* strains against *G. mellonella* and *T. molitor*.

Strain/Insect	LC_{50}	Confidence interval 95%	LT_{50} (days)	Confidence interval 95%
Bb11MI				
G. mellonella	9.12×10^{8}	4.97×10^{6}-2.28×10^{9}	6.99	5.69-7.02
T. molitor	7.94×10^{7}	4.76×10^{6}-2.23×10^{8}	5.42	5.31-5.90
Bb53MI				
G. mellonella	3.98×10^{7}	1.34×10^{7}-2.29×10^{8}	5.46	5.36-6.09
T. molitor	5.87×10^{7}	1.68×10^{7}-2.05×10^{8}	5.73	5.43-6.12
Bb79MI				
G. mellonella	1.04×10^{7}	1.45×10^{6}-5.75×10^{8}	5.27	5.11-5.74
T. molitor	1.03×10^{7}	1.32×10^{6}-4.76×10^{8}	5.57	5.39-6.03
Bb27MI				
G. mellonella	5.53×10^{7}	7.74×10^{6}-2.05×10^{8}	5.56	5.37-5.99
T. molitor	3.98×10^{7}	7.74×10^{6}-2.05×10^{8}	5.83	5.63-6.18
Ma58MI				
G. mellonella	6.31×10^{7}	1.67×10^{7}-2.38×10^{9}	5.18	4.79-5.36
T. molitor	1.00×10^{6}	2.88×10^{5}-3.48×10^{9}	4.06	3.89-4.68
Ma10MI				
G. mellonella	1.10×10^{8}	1.96×10^{7}-1.28×10^{8}	6.37	5.79-6.89
T. molitor	1.00×10^{7}	2.88×10^{6}-3.48×10^{10}	5.22	4.97-5.66
Ma12MI				
G. mellonella	1.59×108	3.93×107-1.21×109	6.17	6.00-6.45
T. molitor	1.13×108	4.60×106-2.77×107	6.59	5.15-5.87
Ma11MI				
G. mellonella	2.12×109	1.28×108-3.51×1010	7.69	6.87-7.91
T. molitor	6.21×108	3.15×106-1.22×109	6.27	6.11-6.46

Table 4. CL_{90} and LT_{90} values of *B. bassiana* and *M. anisopliae* strains against *G. mellonella* and *T. molitor*.

Strain/Insect	LC_{90}	Confidence interval 95%	LT_{90} (days)	Confidence interval 95%
Bb11MI				
G. mellonella	8.31×10^{11}	1.44×10^{10}-4.79×10^{12}	10.23	9.42-10.52
T. molitor	8.46×10^{11}	1.49×10^{11}-4.82×10^{12}	10.04	9.98-10.14
Bb53MI				
G. mellonella	2.15×10^{10}	2.15×10^{10}-6.84×10^{11}	9.38	9.10-9.67
T. molitor	3.16×10^{10}	3.29×10^{9}-2.11×10^{11}	9.48	9.23-9.68
Bb79MI				
G. mellonella	6.31×10^{9}	1.67×10^{8}-2.39×10^{10}	8.03	7.85-8.16
T. molitor	1.00×10^{10}	1.45×10^{9}-6.88×10^{10}	9.42	9.23-9.57
Bb27MI				
G. mellonella	1.00×10^{10}	6.95×10^{9}-1.44×10^{11}	9.19	9.00-9.31
T. molitor	8.34×10^{10}	6.95×10^{10}-1.44×10^{11}	9.68	9.46-9.78
Ma58MI				
G. mellonella	1.00×10^{9}	6.60×10^{8}-1.51×10^{10}	8.29	8.06-8.47
T. molitor	9.90×10^{8}	8.48×10^{8}-1.18×10^{9}	7.18	7.02-7.38
Ma10MI				
G. mellonella	9.98×10^{9}	2.88×10^{9}-3.48×10^{10}	8.51	8.29-8.88
T. molitor	9.98×10^{8}	8.48×10^{8}-1.18×10^{9}	7.80	7.32-8.12
Ma12MI				
G. mellonella	4.38×10^{10}	3.84×10^{9}-1.21×10^{11}	9.20	9.11-9.37
T. molitor	3.56×10^{9}	1.47×10^{9}-1.08×10^{10}	8.41	8.26-8.69
Ma11MI				
G. mellonella	2.88×10^{11}	1.82×10^{10}-4.55×10^{11}	10.47	10.19-10.59
T. molitor	1.26×10^{12}	1.06×10^{12}-1.20×10^{13}	11.26	11.09-11.46

lowest LC_{90} on *G. mellonella* larvae was Bb79MI (6.31×10^{9} conidia.ml^{-1}) and the most LC_{90} was presented by Bb11MI strain (8.31×10^{11} conidia.ml^{-1}). Ma11MI *M. anisopliae* showed the highest LC_{90} (2.88×10^{11} conidia.ml^{-1}) and the lowest LC_{90} was presented by Ma58MI strain (1.00×10^{9} conidia.ml^{-1}) on *G. mellonella* larvae. Finally, for *T. molitor* larvae the LC_{90} varied with the *B. bassiana* strains from 1.00×10^{10} to 8.46×10^{11} conidia.ml^{-1} concentration; in *M. anisopliae* strains the LC_{90} ranged from 9.90×10^{8} to 1.26×10^{12} conidia.ml^{-1} (Table 4).

DISCUSSION

The development of PCR amplification from different rDNA regions has greatly facilitated the fungi taxonomic studies. Alignments and phylogenetic analyses confirmed the *B. bassiana* and *M. anisopliae* strains taxonomic identity. Analysis of ITS-rDNA sequences have been applied to determine the genetic diversity of *M. anisopliae* and *B. bassiana* (Wall et al., 2005; Entz et al., 2005; Becerra et al., 2007; Carneiro et al., 2008; Freed et al., 2011). Thus, Bautista-Galvez et al. (2012), made the genetic characterization of *M. anisopliae* strains obtaining fragments of 600 to 800 bp by PCR amplification from the ITS1-ITS4 rDNA regions. The size of the DNA fragments from *M. anisopliae* strains collected in sugarcane crops were similar to those ones reported for the *M. anisopliae* (L0909) reference strain. Comparison of ITS rRNA obtained from fungal isolates in regarding to the GenBank database (NCBI) indicated that the MM0801, AD0702, AD0803, CD0804, FC0805, FC0706, AS0807, GB0808, L0909, BC0710, SF0811, M370 and M374 strains corresponding to *M. anisopliae* and the JC0816 and AR0814 corresponding to *B. bassiana*. Mergulhão et al. (2014) used the ITS rDNA region, as well as assessed the genetic variability and phylogeny from five *B. bassiana* strains (API 145, API 148, API 223, API 225 and API 226). The ITS region was amplified by the ITS1

and ITS4 primers, wherein amplicon was observed with an average size of 800 bp which was sequenced. The results showed that all strains, except for IPA145, presented themselves as monophyletic group. The IPA145 showed a genetic identity with *B. bassiana* and *Cordyceps bassiana* 99%. The IPA148 was the most genetically distant in regarding to the other ones, at less than 40% similarity.

G. mellonella and *T. molitor* are known to be susceptible to *B. bassiana* and *M. anisopliae*, for this reason they have been used as baits for isolation of entomopathogenic fungi from the soil (Zimmerman, 1986; Bidochka et al., 2002; Montesinos et al., 2011) or, as a probe to pathogenicity of other insect species (Bharadwaj et al., 2011). Mortality and concentrations were considered parameters in the study of the best strains behavior once indicating the pathogen colonization capacity, surpassing all competitor agents present in the insect (Neves, 1998). It was observed that it requires less inoculums amount by Bb79MI *B. bassiana* and Ma58MI *M. anisopliae* treatments to kill the *G. mellonella* and *T. molitor* larvae. Similar results by Garcia et al. (2011) were obtained, evaluating the insecticidal activity from 8 *B. bassiana* strains and from 4 *M. anisopliae* on *Spodoptera frugiperda* and *Epilachna varivestis* larvae at six concentrations (10^4 to 10^9); the BB18 *B. bassiana* strain was more virulent for *E. varivestis* larvae with a 93.3% mortality, LC_{50}= 1.20×10^6 conidia.ml^{-1} and LT_{50}= 5.1 days. Bb42 *B. bassiana* strain presented the highest mortality on *S. frugiperda* larvae (96.6%, LC_{50}= 5.92×10^3 conidia.ml^{-1} and LT_{50}= 3.6 days). Differences among lethal times is a tool widely used in selecting strains, because it is interesting that the fungus quickly eliminate its host, as well (Lohmeyer and Miller, 2006).

A significant interaction was also observed between the conidial concentration and the time. The highest conidia concentrations require less time causing 100% insects mortality. The amount of conidia used should to attain a certain concentration and thus, achieving an efficacious penetration of the fungus on the insect cuticle and causing host death (Zhioua et al., 1997). All strains were able to cause infection and mortality against *G. mellonella* and *T. molitor* by contact. In the *T. molitor* larvae the shortest time to cause the maximum mortality was 6 days at 1×10^9 conidia.ml^{-1} concentration with the Ma12MI, Ma10MI and Ma58MI *M. anisopliae* strains, while for the Bb79MI *B. bassiana* strain the time was 7 days. On *G. mellonella* the shortest time to cause 100% mortality was 6 days with the Bb79MI *B. bassiana* and Ma58MI *M. anisopliae* strains. At the 1×10^9 conidia.ml^{-1} concentration, 100% mortality for the *T. molitor* larvae with the Bb11MI, Bb53MI, Bb72MI *B. bassiana* and Ma11MI of *M. anisopliae* strains was not obtained, as well as for the *G. mellonella* larvae with Bb11MI and Bb27MI *B. bassiana* and Ma12MI and Ma11MI *M. anisopliae* strains. These results are similar to the ones obtained by Khalid et al. (2012), evaluating the virulence of 90 *B. bassiana* and 15 *M. anisopliae* strains on *G. mellonella* larvae using 10^2, 10^3, 10^4, 10^5 and 10^6 conidia.ml^{-1} concentration. The BbaAUMC3076, BbaAUMC3263 and ManAUMC3085 strains caused 100% mortality at concentrations of 5.5×10^6, 5.86×10^5 and 4.8×10^6 conidia.ml^{-1}. The LC_{50} values were 1.43×10^3, 1.04×10^5 and 5.06×10^4 conidia.ml^{-1} Bba3263AUMC, Bba3076AUMC and Man3085AUMC, respectively.

Khosravi et al. (2015), evaluated pathogenicity from *B. bassiana* strains against *Arge rosae* larvae. The bioassay was performed by the immersion method at 2×10^4, 2×10^5, 2×10^6, 2×10^7 and 2×10^8 conidia.ml^{-1} concentrations; IRAN403C strain presented the highest mortality being 70% at 2×10^7 conidia.ml^{-1} concentration, LC_{50} and LT_{50} obtained was 5.54×10^5 conidia.ml^{-1} and 3.92 days at 2×10^8 conidia.ml^{-1} concentration, respectively. However, the findings of this study demonstrated direct proportional relationship between the amount of conidia applied on the insects and mortality from the same ones, so the more diluted suspensions the virulence and pathogenicity were less pronounced, resulting in lower mortality for a longer time than the observed one at higher concentrations. This fact was also observed by other authors studying *M. anisopliae* and *B. bassiana* strains on different insect species (Filho et al., 2002; Silva et al., 2003; Cunha et al., 2008). According to Neves and Alves (2000), as more conidia penetrating, more toxins or enzymes are released, increasing the insect mortality. However, the fungus action speed depends, besides the concentration, of the host species involved (Sosa-Gomez and Moscardi, 1992). According Paccola-Meirelles and Azevedo (1990), St. Leger (1991) and Kleespies and Zimmermann (1998), variation in virulence of entomopathogenic strains is a result of differences in the enzymes and toxins production in conidia germination speed, mechanical activity in the cuticle penetration, colonization capacity and cuticle chemical composition.

It might be concluded that *B. bassiana* and *M. anisopliae* fungi present different capacity cause mortality of the insects, with the Bb53MI and Bb79MI *B. bassiana* strains as the most pathogenic for *G. mellonella*, as well as the Bb79MI *B. bassiana* strain was the most pathogenic for *T. molitor*. Ma58MI and Ma10MI *M. anisopliae* strains presented the highest virulence for *G. mellonella* and *T. molitor*, and also presenting the lowest LC_{50} and LT_{50} values.

Conflict of Interests

The authors have not declared any conflict of interests.

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