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*Full Length Research Paper*

# Pathogenicity of native isolates of *Isaria fumosorosea* (Deuteromycotina: Hyphomycetes) on immature whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and their genetic variability

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**The use of chemical insecticides to manage *Bemisia tabaci* has caused serious damage to the environment and has also selected resistant populations. The use of biological control agents, such as entomopathogenic fungi is an excellent component of integrated pest management programs. In the present study, the virulence of four native isolates (Pf-Tim, Pf-Tiz, Pf-Hal and Pf-Tic), as well as a commercial strain (Pae-sin) of *Isaria fumosorosea* was evaluated on eggs and second instar nymphs of *B. tabaci*. In addition, by the random amplified polymorphic DNA (RAPD) analysis, the genetic variability of the *I. fumosorosea* isolates was determined. All fungal isolates were more virulent on nymphs than on eggs. The most virulent native isolate for eggs was Pf-Tim, which showed the same effect than that of the commercial strain Pae-sin. As for the effects on nymphs, the native isolate Pf-Tim ($5.5 \times 10^4$ conidia $mL^{-1}$) showed the lowest median lethal concentration. This value was not significantly different than that observed for the commercial strain Pae-sin ($2.6 \times 10^4$ conidia $mL^{-1}$). As for the RAPD analysis, there was low genetic variability among *I. fumosorosea* isolates Pf-Tim, Pf-Tic and Pf-Tiz.**

**Key words:** Biological control, pest management, entomopathogenic fungi.

## INTRODUCTION

The whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), is considered one of the most serious pest of field and greenhouse crops worldwide (Musa and Shun 2005, Pineda et al., 2007). *B. tabaci* causes direct damage to plants by phloem-sap feeding, which can result in reduction of plant growth and yield. In addition, *B. tabaci* feeding induces plant physiological disorders due to the transmission of begomoviruses in a wide range of crops (Leshkowitz et al., 2006). The management of *B. tabaci* has been typically carried out with synthetic insecticides, such as organophosphates, insect growth regulators and neonicotinoids, which have produced high levels of environmental contamination and insecticide resistance (Gorman et al., 2001; Nauen and Stumpf 2002; Gutiérrez et al., 2007; Erdogan et al., 2008). To reduce the use of synthetic insecticides, the implementation of integrated pest management (IPM) has been utilized as an alternative to efficiently suppress *B.*

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**Table 1.** Name and origin of *Isaria fumosorosea* isolates.

| Isolate | Host resource | Location |
|---|---|---|
| Pf-Tim | *Bemisia tabaci* | Timucuy, Yucatán, Mex. |
| Pf-Tiz | *Bemisia tabaci* | Tizimín, Yucatán, Mex. |
| Pf-Hal | *Bemisia tabaci* | Halachó, Yucatán, Mex. |
| Pf-Tic | *Bemisia tabaci* | Ticul,Yucatán, Mex. |
| Pae-sin® | Unknown | Commercial strain |

*tabaci* populations (Oliveira et al., 2001). For IPM, the use of entomopathogenic fungi is one of the most important strategies (Faria and Wraight, 2001).

The entomopathogenic fungus *Isaria fumosorosea* Wize (Deuteromycotina: Hyphomycetes) (formerly *Paecilomyces fumosoroseus*) is a geographically widespread fungus infecting various orders of insects at all developmental stages (Wraight et al., 2000; Luangsa et al., 2005). The virulence of this fungus on *B. tabaci* and its potential use as a biological control agent have been reported in various regions where this pest causes severe damage to valuable crops (Osborne and Landa, 1992; Wraight et al., 2000). Several dozen isolates of *I. fumosorosea* have been evaluated on eggs, nymphs and adults *B. tabaci* under laboratory and greenhouse conditions (Osborne and Landa, 1992; Vidal et al., 1997; Negasi et al., 1998; Herrera et al., 1999; Wraigth et al., 2000; Saito and Sugiyama, 2005). Various *I. fumosorosea* isolates are currently produced at the commercial levels as biopesticides for *B. tabaci* control in countries like USA (PFR-97®, ECO-tec), Belgium (PreFeRal®, Biobest N.V.), Venezuela (Bemesin®) and Mexico (Pae-sin®, Agrobiológicos del Noreste S.A. de C.V.) (Faria and Wraight, 2001; Chan-Cupul et al., 2010). The search for new isolates to detect highly virulent strains that are environmentally adapted for a particular region of interest is of particular importance since the most successful markets for entomopathogenic fungi has been local (Sahayaraj and Namasivayam, 2008).

To develop an entomopathogenic-based biopesticide, the isolation and bioassays of the strains are the first step in this program. In addition, molecular characterization (finger prints) of the strains should be carried out for regulatory purposes prior to the release of commercial products (Tigano-Milani, 1995a). For molecular characterization, the random amplified polymorphic DNA (RAPD) analysis is a powerful tool for the estimation of genetic variability among species or strains (Williams et al., 1990; Oborník et al., 2000; Souza et al., 2000; Bieliková, 2002).

In the present work, we investigated the virulence of *I. fumosorosea* isolates from the Yucatán Peninsula, Mexico against eggs and second instar nymphs of *B. tabaci*, and in addition random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was employed to determine the genetic variability and fingerprint of such isolates.

## MATERIALS AND METHODS

### Insects

Adult whiteflies were collected from field-establish habanero pepper (*Capsicum chinense* Jacq) in Conkal, Yucatan, Mexico. The colony was maintained in entomological cages made of anti-aphid mesh with aluminum frame, and kept in a greenhouse at 28±6°C with a natural photoperiod (L:D) of approximately 12:12 h. Insects in the cages were fed on 40 to 70 day-old *C. chinense.* These plants were grown in 1 L plastic pots containing peat moss (Canadian Sphagnum Peat Moss, Canada) and fertilized daily with triple 19, 1 g $L^{-1}$.

### Fungal isolates

All *I. fumosorosea* strains were isolated from *B. tabaci* collected on vegetable crop fields, where microbial pesticides have never been used (Table 1). Fungal identities were confirmed using morphologic characteristics according to Humber (1998) and Barnett and Hunter (2003). Fungi were isolated and cultured on Sabouraud dextrose agar (Difco, USA), in 90 x 15 mm plastic Petri dishes. Fungal cultures were carried out in total darkness at 25±2°C.

### Bioassays

For bioassays, eggs and second instar *B. tabaci* nymphs were used. Immature *B. tabaci* were obtained as described by Muñiz and Nombela (2001). Briefly, groups of 10 *B. tabaci* adults were aspirated from the rearing stock colony and transferred to clip cages set for 24 h in upper leaves of 30-day-old *C. chinense* plants. To form experimental units, groups of 30 eggs or nymphs were selected and the rest removed from leaves under a stereoscopic microscope. Fungal inoculation on eggs was carried out immediately after obtaining eggs from adult whiteflies. Fungal inoculation on nymphs was carried out eight days after obtaining eggs, when second-instar-nymphs were observed in the experimental units.

Conidial suspensions for the bioassays were obtained from 15-day fungal colonies. Conidia were harvested using sterilized distilled water (0.05% Tween 80) and filtered through several layers of cheesecloth to remove mycelia mats and strand displacement amplification (SDA) fragments (Chan-Cupul et al., 2010). For bioassays, conidial concentrations were adjusted to $10^4$, $10^5$, $10^6$ and $10^7$ conidia $mL^{-1}$ with the use of a standard Neubauer chamber.

Fungal inoculation was carried out as described by Gindin et al. (2000) and Saito and Sugiyama (2005). Briefly, individual *C. chinense* leaves (experimental units) bearing the selected 30 eggs or nymphs were immersed in the respective conidial suspension for 10 s. Control leaves were immersed in water with 0.05% Tween 80 for the same period of time. All fungal strains or conidial concentrations were assayed with six replicates. After fungal inoculation, plants were maintained under laboratory conditions at 25±3°C and 75% humidity. Percentages of egg mortality were obtained from the proportion of non-hatched/necropsied eggs and the total number of eggs in the experimental units. Nymphs were scored as dead if the body of the individual was discolored, necropsied and/or with hyphae growing on the surface. Observations were made daily for eight days in egg bioassays, and for 15 days in nymph bioassays. Natural mortality in the control group was lower than 10%; therefore the respective values were not subtracted from those obtained by the fungal effects.

**Table 2.** Primer sequences employed for RAPD-PCR.

| Primer | Sequence 5´to 3´ |
|---|---|
| OPB 02 | 5'-TGATCCCTGG-3' |
| OPB 06 | 5'-TGCTCTGCCC-3' |
| OPB 07 | 5'-GGTGACGCAG-3' |
| OPB 09 | 5'-TGGGGGACTC-3' |
| OPB 10 | 5'-CTGCTGGGAC-3' |

**Table 3.** Percent mortality (mean ± standard error of the mean) of *Bemisia tabaci* eggs after six days post inoculation ($1x10^7$ spores $mL^{-1}$) of *Isaria fumosorosea*.

| Isolate | Mean |
|---|---|
| Pf-Tim | $55.5±7.4^{a}$ |
| Pf-Tiz | $33.3±1.6^{bc}$ |
| Pf-Hal | $21.3±1.9^{cd}$ |
| Pf-Tic | $48.8±5.1^{ab}$ |
| Pae-sin | $61.3±3.8^{a}$ |
| Control | $9.8±6.3^{d}$ |

Means (±standard error of the mean) followed by the same letter(s) are not significantly different (Tukey test, $P > 0.05$).

#### Data analysis

Analysis of variance and mean comparison (Tukey, $P<0.05$) of percentage of egg mortality were performed using GraphPad InStat (GraphPad Software Inc., 2000). Median lethal concentrations ($LC_{50}$) and median lethal times ($LT_{50}$) of fungal strains on *B. tabaci* nymphs were calculated by Probit procedure in SAS (SAS Institute, 1998). $LT_{50}$ for second instar nymphs were calculated using a fungal concentration of $1x10^7$ conidia $mL^{-1}$.

#### DNA extraction for molecular characterization

Fungal isolates were cultured in Sabouraud dextrose broth (Difco Microbiology Media, Kansas, USA) pH 5.5 and cloramphenicol 100 µg $L^{-1}$. Fungal culture was carried out for five days in an orbital shaker at 150 rpm and 22°C. The mycelium was harvested by filtration through filter paper (Whatman No.1) and washed twice with wash buffer (100 Mm Tris-HCl pH 8.5, 150 Mm CaCl and 5 Mm ethylenediaminetetraacetic acid (EDTA)). Mycelium was stored at -80°C until usage. Genomic DNA was extracted as described by Raeder and Broda (1985). Briefly, 200 mg of ice-cold mycelium was macerated in liquid nitrogen, then suspended in 500 µL lysis buffer (200 Mm Tris-HCl pH 8.5, 250 mM NaCl, 25 mM EDTA and 0.5% sodium dodecyl sulfate (SDS) and shaken gently for 10 min. Subsequently, 500 µL phenol-chloroform was added to the mix. The supernatant was extracted by adding chloroform and the nucleic acids were precipitated by adding ice-cold isopropanol. The precipitate was centrifuged at 12,000 rpm for 10 min and treated with 5 µL of RNase (10 mg/mL) for 30 min at 37°C. Nucleic acids were precipitated with isopropanol. The pellet was washed with 70% ethanol, dried, and resuspended in 100 µL of sterilized ultrapure water. The extracted DNA was quantified by spectrophotometer and verified by electrophoresis on 1% Tris/acetic acid/EDTA (TAE) agarose gel stained with ethidium bromide.

#### RAPD analyses

Primers (Kit OPB) used were purchased from Operon Technologies (Alameda CA) (Table 2). Taq DNA polymerase and DNA polymerization mix (dNTPs) were supplied by Invitrogen, Brasil. The RAPD reactions were performed in 25 µL reaction mix containing 15.5 µL ultra-pure sterilized water, 2.5 µL buffer PCR 10x, 1.5 µL dNTPs (50 mM each), 2.0 µL primer (20 pmol $µL^{-1}$), 0.5 µL Taq DNA polymerase (2.5 units), 2.0 µL $MgCl_2$ (50 mM) and 1.0 µL DNA (80 ng $µL^{-1}$). The amplification was carried out in a Thermocycler Techgene FTgene2D (Techgene, USA), under the following conditions: 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 36°C for 1 min , 72°C for 2 min and 72°C for 7 min. Control reactions were run with *Beauveria bassiana* HBb5 and *B. bassiana* HBb22.

RAPD products were electrophoresed in 1% agarose gels run with Tris-borate-EDTA (TBE). Gels were stained with ethidium bromide and photographed under ultraviolet (UV) light. For the analyses of data, each strain was scored for the presence or absence of amplification products. The data were input into a binary matrix and pairwise similarity matrix was constructed using the Jaccard coefficient. Clustering was done using the unweighted pair group arithmetic method (UPGMA) in FreeTree 0.9.1 program and dendrogram visualized through TreeView 4.1 program (Pavlicek et al., 1999; p. 1996).

## RESULTS

### Virulence of *I. fumosorosea* on *B. tabaci* eggs

All *I. fumosorosea* isolates caused mortality on *B. tabaci* eggs under the tested conditions. The commercial strain Pae-sin and the native isolate Pf-Tim caused 61.3 and 55.5% mortality, respectively. These percent mortalities were significantly higher ($F$=17.28; n=5; $P<0.05$) than those caused by Pf-Tic, Pf-Tiz and Pf-Hal (Table 3).

### Virulence of *I. fumosorosea* on *B. tabaci* nymphs

All *I. fumosorosea* isolates caused mortality on second instar *B. tabaci* nymph. The commercial strain Pae-sin and the native isolate Pf-Tim were the most virulent, which showed the lowest values for $CL_{50}$, $2.6x10^4$ and $5.5x10^4$ conidia $mL^{-1}$, respectively (Table 4). The $LC_{50}$ values calculated for the isolates Pf-Tiz and Pf-Hal were $3.5x10^5$ and $2.3x10^6$ conidia $mL^{-1}$. These isolates showed the lowest virulence. The median lethal time ($LT_{50}$) for nymph mortality when a conidial suspension of $1x10^7$ conidia $mL^{-1}$ was used, was significantly different among isolates (Table 5). The commercial strain Pae-sin showed

**Table 4.** $LC_{50}$ values for *Isaria fumosorosea* isolates against second instar nymphs *Bemisia tabaci*.

| Isolate | $LC_{50}$ (conidia $mL^{-1}$) | CI (conidia $mL^{-1}$) | Slope ± S.E.M. | Pr>f |
|---|---|---|---|---|
| Pae-sin | $2.6 \times 10^{4a}$ | $1.2x10^4$ - $4.7x10^4$ | 0.31 ± 0.029 | <0.0001 |
| Pf-Tim | $5.5 \times 10^{4a}$ | $3.4x10^4$ - $8.2x10^4$ | 0.40 ± 0.029 | <0.0001 |
| Pf-Tic | $1.5 \times 10^{5b}$ | $1.1x10^5$ - $2.0x10^5$ | 0.53 ± 0.032 | <0.0001 |
| Pf-Tiz | $3.5 \times 10^{5c}$ | $2.0x10^5$ - $5.9x10^5$ | 0.28 ± 0.028 | <0.0001 |
| Pf-Hal | $2.3 \times 10^{6d}$ | $1.6x10^6$ - $3.3x10^6$ | 0.53 ± 0.024 | <0.0001 |

$LC_{50}$, Median lethal concentration; CI, confidence interval; S.E.M., standard error of the mean; Pr>f, adjustment of the probit analysis model for $LC_{50}$ calculation. $LC_{50}$ values followed by the same letter are not significantly different based on non-overlapping confidence intervals.

**Table 5.** Median lethal time ($LT_{50}$) values for the isolates of *Isaria fumosorosea* against second instar nymphs *Bemisia tabaci*.

| Strain | $LT_{50}$ (Days) | CI (Days) | Slope ± ES | Pr>f |
|---|---|---|---|---|
| Pae-sin | $3.72^a$ | 3.41 - 4.04 | 3.47 ± 0.308 | <0.0001 |
| Pf-Tic | $4.35^b$ | 4.11 - 4.59 | 5.86 ± 0.484 | <0.0001 |
| Pf-Tiz | $4.66^b$ | 4.22 - 5.22 | 2.93 ± 0.308 | <0.0001 |
| Pf-Tim | $5.04^b$ | 4.70 - 5.45 | 3.70 ± 0.323 | <0.0001 |
| Pf-Hal | $6.36^c$ | 5.76 - 7.22 | 2.53 ± 0.242 | <0.0001 |

$LT_{50}$, Median lethal time; CI, confidence interval; S.E.M., standard error of the mean; Pr>f, adjustment of the probit analysis model for $LT_{50}$ calculation. $LT_{50}$ values followed by the same letter are not significantly different based on non-overlapping confidence intervals.

significantly lower $LT_{50}$ value (3.7 days) than the rest of the isolates. The $TL_{50}$ values for the native isolates ranged from 4.3 to 6.3 days.

### Genetic variability of *Isaria fumosorosea* isolates

The RAPD analysis of *I. fumosorosea* isolates showed that the five primers selected for this study produced 72 well resolved consistent bands, where 34 were polymorphic. The number of bands generated by each primer was variable, ranging from seven for primer OPB 02 to 14 for primer OPB 07 (Figure 1). The primer OPB 06 and OPB 07 revealed more polymorphic bands (8 in each one) among *I. fumosorosea* isolates (Figure 1).

Cluster analysis arranged *I. fumosorosea* isolates into three groups (Figure 2). The first group was formed by the isolate Pf-Hal. The second group was formed by the commercial isolate Pae-sin, and the third group was formed by the isolates Pf-Tim, Pf-Tic and Pf-Tiz. As for the genetic similarity coefficients, Pf-Tic and Pf-Tiz showed the highest value with 100%. In contrast, the lowest value for genetic similarity coefficient was observed in Pf-Hal and Pae-sin (Figure 3).

## DISCUSSION

Various species of entomopathogenic fungi have been found worldwide to infest *B. tabaci*. There are currently fungal biopesticides commercially available to manage *B. tabaci*. Field work using these biological control agents, however, has suggested that this type of microbial biopesticides show higher effectiveness when the strains used are adapted to the environmental conditions of the agroecosystems where these products are employed. In addition, some native strains are also easily produced under unsophisticated conditions using low-cost agricultural byproducts (Sahayaraj and Namasivayam, 2008). For instance, the search for native entomopathogenic fungi for local markets contributes to the use of microbial biopesticides for plant pest management.

In a previous work from our group, a field search of *I. fumosorosea* in the Yucatan Peninsula yielded four entomopathogenic isolates (Chan-Cupul et al., 2010). In the present work, we extended the study and evaluated the virulence of such isolates on eggs and second instar nymphs of *B. tabaci*. In addition, the genetic variability of the isolates was also determined.

All native *I. fumosorosea* isolates were moderately virulent to *B. tabaci* eggs and nymphs under laboratory conditions. The most virulent isolates, Pf-Tim and Pf-Tic, caused 48 to 55% mortality of eggs. Of particular significance, the native isolate Pf-Tim in our study showed similar effectiveness on *B. tabaci* eggs than the commercial strain Pae-sin.

The effects of the fungal isolates on *B. tabaci* nymphs were also evaluated in the present work. The median lethal concentration of all isolates ranged from $2.6x10^4$ to $2.3x10^6$ conidia $mL^{-1}$. Other studies have also reported *I. fumosorosea* strains with similar virulence with those of the present work. For example, Saito and Sugiyama (2005) recorded a $LC_{50}$ of $1.1x10^4$ conidia $mL^{-1}$ for a native *I. fumosorosea* isolate on *B. tabaci*. In contrast, less virulent isolates were found by Vidal et al. (1997), who reported higher $LC_{50}$ values ($1.7x10^8$ to $9.9x10^8$ conidia $mL^{-1}$) than those reported in the present work. Other studies, such as those from Cabanillas and Jones (2009), Lacey et al. (1999) and Wraigth et al. (2000) are difficult to compare with the present study as they reported the $LC_{50}$ values in spores/$mm^2$. Our native isolate Pf-Tim showed similar virulence on nymphs than

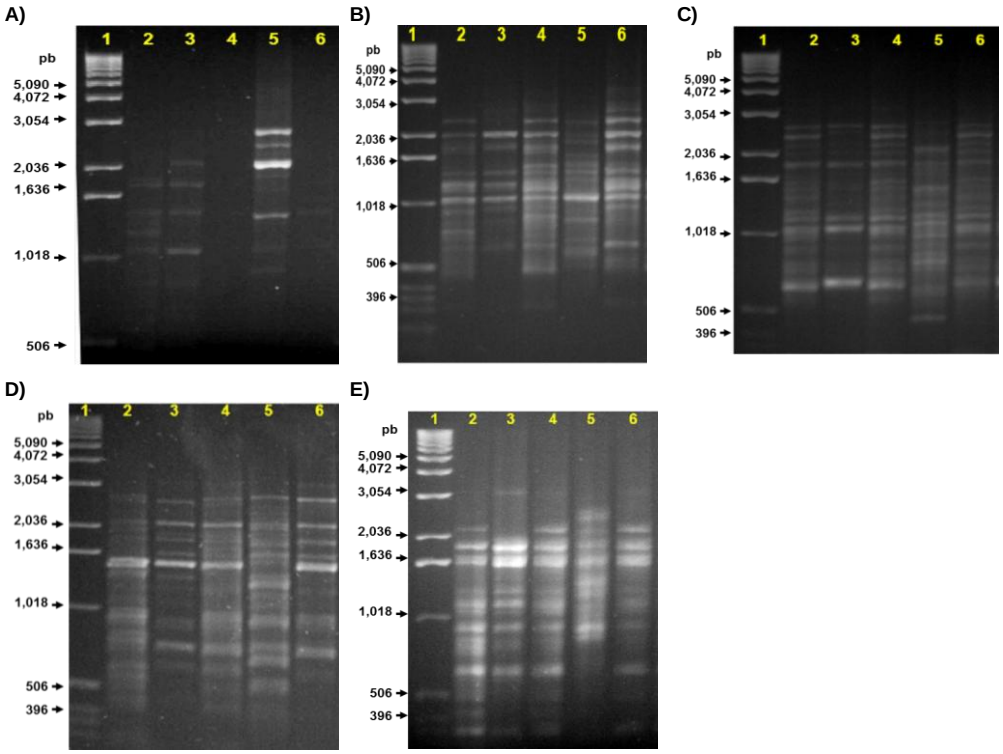


**Figure 1.** RAPD-PCR profiles of the five isolates of *Isaria fumosorosea* generated by the primers. **A)** OPB 02, **B)** OPB 06, **C)** OPB 07, **D)** OPB 09 and **E)** OPB 10. Line 1, molecular weight marker; lines 2 to 6, *Isaria fumosorosea* isolates: Pf-Tiz, Pf-Tim, Pf-Tic, Pf-Hal, Pae-sin, respectively.

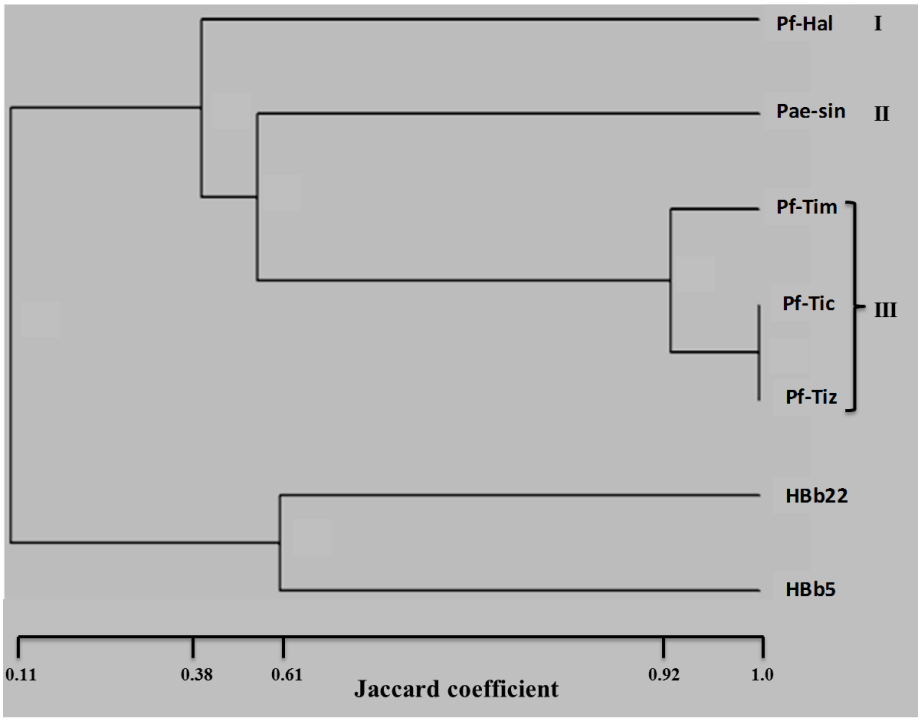


**Figure 2.** Dendrogram constructed from analysis of DNA fragments of five *Isaria fumosorosea* isolates and two *Beauveria bassiana* isolates (HBb22 and HBb) amplified by RAPD. The matrix was created with the Jaccard similarity coefficient. Clustering was performed with UPGMA.

| | | | | | |
|---|---|---|---|---|---|
| Pf-Tiz | --- | | | | |
| Pf-Tim | 92.31 | --- | | | |
| Pf-Tic | 100.00 | 92.31 | --- | | |
| Pf-Hal | 57.14 | 53.33 | 57.14 | --- | |
| Pae-sin | 53.85 | 61.54 | 53.85 | 38.46 | --- |
| | Pf-Tiz | Pf-Tim | Pf-Tic | Pf-Hal | Pae-sin |

**Figure 3.** Genetic similarity coefficient matrix of five isolates of *Isaria fumosorosea*. Matrix was calculated from 72 bands yield by five primers. Simple matching coefficients given in percentage similarity.

the commercial strain Pae-sin, consistent with its effects on eggs (see above).

In our study, the median lethal time ($LT_{50}$) for all fungal isolates were also determined. We observed that the commercial strain Pae-sin showed the lowest value for $LT_{50}$ (3.7 day). The native isolate Pf-Tim showed a $LT_{50}$ value of 5.0 days. The $LT_{50}$ values for *I. fumosorosea* on *B. tabaci* reported by other authors are lower to those found in our most virulent isolate. For example, Gindin et al. (2000), Saito and Sugiyama (2005) and Cabanillas and Jones (2009) found a $LT_{50}$ value of three to four days under similar experimental conditions. Even though our most virulent native isolate showed a $LT_{50}$ higher than those of the aforementioned studies, we suggest that *I. fumosorosea* Pf-Tim might be a good candidate to develop into a microbial control agent for *B. tabaci* at the regional level.

The development and utilization of a microbial control agent should be preceded by a clear identification and characterization of the microorganism. The characterization provides a mechanism for tracking the progress and fate of the microorganism in the environment. The characterization may also be used to validate the purity of a formulated product and provide a useful reference to register or protect an individual isolate (Jenkins and Grzywacz, 2000). The RAPD-PCR technique has been widely used to detect genetic variability in entomopathogenic fungi, even with small number of isolates (Tigano-Milani, 1995a; Castrillo and Brooks, 1998; Souza et al., 2000; Suey et al., 2002). In the present work, we used the primers sequences OPB from Operon Technologies (Alameda, CA), which have been previously used for characterization of the genus *Isaria* (Tigano-Milani, 1995a; Tigano-Milani, 1995b; Bielikova et al., 2002; Fargues and Bon, 2004). Particularly for *I. fumosorosea* strains, the primers OPB 02 and OPB 10 have been used for genetic characterization of this species. In such study, OPB 02 and OPB 10 yielded 13 and 15 representative bands, respectively (Bieliková et al., 2002). In our study, we only detected 7 and 13 representative bands for OPB 02 and OPB 10, respectively. Although the observed RAPD-PCR profiles were different, all primers used in the analysis revealed the same basic grouping pattern. In the present work, the primers OPB 06 and OPB 07 revealed more polymorphic bands (8 in each one) among *I. fumosorosea* isolates, which allowed differentiation of all tested isolates.

The Jaccard similarity coefficient for pairwise comparisons of the *I. fumosorosea* isolates varied from 38.4 to 100%, with a mean value of 65.9%. This mean value is similar to that obtained by Tigano-Milani et al. (1995b) for the entomopathogenic fungi *Paecilomyces lilacinus* (other). Although most of the studies have been carried out on the genetic variability of entomopathogenic fungi collected from differ insect host, here we detected high variability in two *I. fumosorosea* isolates obtained from the same insect host. We also observed very low genetic variability between the two isolates, Pf-Tic and Pf-Tiz (Jaccard similarity coefficient = 100%). In this regard, Tigano-Milani et al. (1995b) and Souza et al. (2000) mentioned that this low genetic variability is expected when isolates are collected from the same host.

In summary, this study provides evidence of native *I. fumosorosea* isolates that are pathogenic to eggs and nymphs of *B. tabaci*. The isolates Pf-Tic, Pf-Tiz and Pf-Tim had low genetic variability as determined by RAPD analysis. Based on the bioassay, the native isolate Pf-Tim might be a good candidate to develop into a microbial biopesticide for the management *B. tabaci* in the Yucatan Peninsula. Future studies will be carried out to explore the mass production and formulation of a product derived from our most virulent strain.

## Conclusions

This study shows that *I. fumosorosea* isolates from the Yucatan Peninsula are virulent to eggs and nymphs of *B. tabaci*. *I. fumosorosea* Pf-Tim may be considered a good candidate to develop into a fungal biopesticide for whitefly management as its effect on nymphs was similar to that observed by the commercial strain Pae-sin. The RAPD analysis showed that there is low genetic variability

among *I. fumosorosea* isolates of Pf-Tim, Pf-Tic and Pf-Tiz.

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