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Control of *Bemisia tabaci* by entomopathogenic fungi isolated from arid soils in Argentina.

Entomopathogenic Hypocreales were isolated from arid soils in Argentina using *Tenebrio molitor* as bait and tested for their biological performance at 30 °C and 45-65% RH. Conidial germination was tested in three vegetable oils (Sunflower, Olive and Maize) at two concentrations (1 and 10%) to evaluate their compatibility for further liquid formulations. According to radial growth and germination results, we selected 4 isolates to test their pathogenicity against second instar *B. tabaci* nymphs with the selected oil formulations at 30 °C. CEP381 and CEP401 showed the highest radial growth. Isolates CEP381, CEP401, CEP413 and CEP409 (*Metarhizium* spp.) had similar germination percentages as compared with water control when germinated on either Sunflower, Olive and or Maize oils at 10% v/v. The highest mortality of *B. tabaci* were observed for the isolates CEP381 in Sunflower oil and CEP401 in Olive oil. Molecular identification of isolates was performed using ITS4-5 primers. All isolates belong to the *Metarhizium* core group. Tested isolates could grow and infect *B. tabaci* nymphs at 30 °C in some of the vegetable oils as carriers, providing new possibilities for IPM of *Bemisia tabaci.*

Keywords: Entomopathogenic fungi; *Metarhizium*; low humidity; whiteflies; oil conidia germination; pathogenicity.

Subject classification codes: include these here if the journal requires them

1. Introduction

The sweetpotato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) currently represents a major pest to several crops globally (Nomikou, Janssen, Schraag, & Sabelis, 2001; Ofori et al., 2014; Segura, Vera & Cladera, 2004). This threat is due mainly to its worldwide distribution (Holguín-Peña, Hernández-Montiel, & Latisnere-Barragán, 2010), large number of hosts species (Li et al., 2011), high capacity to generate resistance to several pesticides (Wegore, 2005; Yaqoob & Arora, 2005) and economic damage from local to global scale (Rebolledo, 1994; Novoa, Rubio, & Hodges, 2010). While the control of *B. tabaci* has been almost exclusively based on chemical methods, there has been much research aiming to address the problem from a different approach, that being, biological control (Cuthbertson et al., 2012; Cuthbertson, 2013).

Entomopathogenic fungi (EF) constitute important tools in biological control of insect pest populations (Tangtrakulwanich et al., 2014) in several agricultural ecosystems (Pell, Eilenberg, Hajek, & Steinkraus, 2001). However, effective inoculation and infection in arid and semiarid regions could be difficult due to high temperatures and low humidity; an optimal inoculation system must be developed to ensure the conidial germination and the further infection of the insect host. Vegetable oils seem to be a potential answer to this problem, protecting the spores from the UV agents and desiccation (Alves, Bateman, Gunn, Prior, & Leather, 2002; Kaaya & Hedimbi, 2012; Luz & Batagin, 2005). Also, oil formulations unlike water, can provide an improvement in the attachment to the insect surface (Malsam, Kilian, Oerke, & Dehne, 2002). Developing appropriate formulations, with correct application methods for host infection, are considered crucial issues for successful utilization of EF on a large scale (Faria & Wraight, 2007).

The aim of this study was to select native EF isolates from arid soils and to estimate their growth and germination. Since there are no previous reports for EF in arid soils from Argentina, molecular analysis was used to understand phylogenetic and functional relationships between these isolations and closely related EF. Moreover, the aim was also to find isolates that could be functional and pathogenic against *B. tabaci* at 30 °C, providing new possibilities for IPM.

2. Materials and methods

2.1. Soil sampling

Samples were collected in San Juan (Argentina). Poblete and Minetti (1989) classified the region within the "Dry desert climate" (according to the Koeppen classification) with less than 250 mm of annual precipitation and maximum and minimum temperatures that range between 42 and -4 °C. Soil samples were collected from August to October 2012 from 4 agricultural fields; two fields under organic production systems and two fields under conventional production systems (*Vitis vinifera* L.). Soil samples were collected with a sterilized garden spade from a 5–15 cm depth. According to Ali-Shtayeh et al. (2003) the soil sample (1.5-2 Kg) was formed by five (300-400 g) sub-samples taken at 20 cm distances from each other. Between samples the spade was sterilized first with 96% ethanol and then washed with sterile distilled water twice. The sub-samples were placed in sterile polypropylene bags (32 × 16 cm) and sealed with a rubber band. In the laboratory, the five sub-samples were homogenized, mixed thoroughly, filtered through a 4 mm sieve and stored at 4 °C until use (no longer than 30 days) (Asensio, Carbonell, López-Jiménez, & Lopez-Llorca, 2003). A total of 50 samples were obtained from the fields; 25 from organic (OP) and 25 from conventional production (CP) systems.

2.2. Fungal isolation

Entomopathogenic fungi were isolated from soil samples using *Tenebrio molitor* L. larvae as bait (modified from Meyling, 2007). Homogenized soil samples were placed in 900 g plastic cups (400-420 g each) with two replicates. Twenty *T. molitor* larvae (2-3.5 cm) were added to each cup (n= 2000). Larvae were not fed during the bait process and the cups were incubated at 28 °C and 65% relative humidity with a photoperiod of 14:10 h L:D. Cups were

turned upside-down every two days to improve the contact of the larvae with the soil spores (Meyling & Eilenberg, 2006). The inspection of dead larvae was carried out daily from the fifth day of incubation. According to Ali-Shtayeh et al., (2003) dead larvae without external mycosis were removed, surface-sterilized in 1% sodium hypochlorite for 2 min and washed twice with sterile distilled water. External mycosed larvae were rinsed in sterile distilled water and fungi were isolated on selective media (Dobersky & Tribe, 1980) and grown for 15 days at 28 ºC in darkness. Morphological characterization of the colonies was made using light and stereomicroscopes and isolates were identified according to the classification of Humber (2012). The isolates were deposited at the Fungal Culture Collection CEPAVE-EF (La Plata-Argentina).

2.3. Radial Growth

Fungal spores were obtained from the growing border of each isolate using a sterile gauge needle and placed in 90 mm Petri dishes containing 25 mL of specific culture media (dextrose 20 g; peptone 5 g; agar; 7.5 g; gentian violet 0.005 g; cycloheximide 0.125 g; chloranphenicol 0.25 g; distilled water 500 mL) (Dobersky & Tribe, 1980). Isolates were grown for 12 days at 30 ± 1 °C in darkness and the colony diameter was measured in two perpendicular directions (Weitz, Ballard, Campbell, & Killham, 2001) every 48 h. Three replicate plates were made for each isolate. A growth curve was estimated according to Kalm & Kalyoncu (2008).

2.4. Conidial biomass production for bioassays and germination tests in oils

The isolates were grown in 125 mL conical flasks. Previous to spore inoculation, flasks containing 20 g water-soaked rice grains were sterilized after the addition of 10 mL distilled water for 40 min at 120 °C. After cooling, each flask was inoculated with two plugs (10 mm diameter) of sporulated mycelium from each isolate and incubated for 15 days at 30 °C in darkness. Then, 3-5 grains of sporulated rice were placed first in a sterile humid chamber (90 mm Petri dish with moistened filter paper) for 30 min to avoid possible rehydration damage (Moore, Langevald, & Obognon, 1997). Rice grains were then submerged in 50 mL sterile distilled water plus 0.05% Tween® 80 to harvest conidia and agitated in a vortex for 60 s. Three oils were used to evaluate the effect of vegetable oils in the spore germination (Malsam et al., 2002): Legítimo® (Argentina) sunflower, Almazara® (Argentina) olive, and Arcor® (Argentina) maize oils. The influence of germination was tested in two oil concentrations: 1 and 10% v/v. After mixing and vortexing, rice grains were removed and the fungal suspension was adjusted to 3 x 10^4 spores/mL. Then, 100 µL of the adjusted suspension were carefully placed on a concave slide and the slide placed in a 90 mm sterile Petri dish with moistened filter paper for 24 h at 30 ± 1 °C in darkness. The counting was made on at least 300 conidia and the complete experiment was repeated twice. The control treatments were made with sterile distilled water and sterile distilled water plus Tween® 80 (0.05% w/v). The aim of this trial was to find a combination of oils/concentration that could give better or similar germination values compared to controls.

2.5. Molecular analysis

Isolates were grown for 7-10 days on SDY ¼ (Peptone 2.5 g - Dextrose 5 g - Agar 15 g - Yeast extract 2.5 g - Distilled water 1 L) at 27 °C in the dark. Fresh mycelium (80 – 100 mg) was scraped from the plates with a sterile tooth-pick, placed in a sterile 2 mL micro centrifuge tube and submerged for 3 min in liquid nitrogen. Frozen mycelia were initially ground manually using a plastic pestle in 400 µL Buffer AP1 and 4 µL RNase A. After 10 min incubation at 65 °C, samples were processed using the DNeasy Plant Kit (Qiagen) for extraction of Genomic DNA according to the standard protocol provided by the manufacturer. DNA was finally eluted in 100 µL Buffer AE.

Polymerase chain reactions (PCRs) were performed to amplify complete internal transcribed spacer (ITS) sequences together with the 5.8S ribosomal RNA (rRNA) encoding sequence from fungal rRNA operons. PCR reactions were performed using oligonucleotide primers ITS4 and ITS5 (White, Bruns, Lee, & Taylor, 1990) with an initial denaturation at 95 °C (10 min), followed by 35 cycles including 1 min denaturation at 95 °C, annealing at 52 °C

for 30 s, and elongation for 2 min at 72 °C. PCR products were subjected to a final elongation process at 72 °C for 10 min.

Success of amplification reactions was controlled electrophoretically using 1% agarose gels in 1x TAE buffer that were stained with Roti-GelStain (Roth, Germany). 50 µL of PCR product was used for DNA purification using the Qiaquick PCR Purification Kit® (Qiagen) following the manufacturer's standard protocol. Purified PCR products were sequenced at StarSeq GmbH, Mainz, Germany. DNA sequences were subjected to a Basic Local Alignment Search Tool (BLAST) to identify sequences deposited in GenBank that had a significant similarity. BlastN search results were ordered by decreasing maximum identity values.

2.6. Whiteflies rearing and bioassays

Primula sp. were used as *B. tabaci* biotype MEAM 1 (Middle East-Asia Minor 1) (Alemandri, Martino, Di Feo, & Truol, 2014) host plants in the infection assays, since they are small and easy to maintain with big leaves able to support large whitefly populations. Ornamental Primulas were obtained from a commercial nursery. Plants were not treated with any chemical product before or after the exposure to whiteflies. *Bemisia tabaci* adults were collected from infested tomatoes (*Lycopersicon esculentum* Mill.) and watermelon (*Cucumis melo* L.) commercial fields in San Juan (Argentina) and kept under quarantine conditions on Primulas plants within a glass box (25 x 25 x 45 cm) for 48 h. After eggs were laid, whitefly adults were removed, and the infested leaves labelled; the plants were kept in a growth chamber with controlled temperature (30 °C) and humidity (45-65% RH). No fungal infection was observed in whitefly populations originally sampled from fields. Second instar *B. tabaci* (Cuthbertson, Walters, & Northing, 2005) were used to assess the pathogenicity of the fungal isolates. The fungal suspensions tested had 10^7 spores/mL plus Tween® 80 (0.05% w/v). The oils were used according to the concentration that did not negatively influence the germination process. In the control, nymphs were sprayed only with sterile distilled water and/or the oils at the selected concentrations without conidia. All applications were made on the complete leaf with *B. tabaci* nymphs. Leafs were sprayed to run-of using a commercial sprayer (Johnson Diversey®) and left to drain 15 min. An extra group of three plants (without *B. tabaci*) were treated in the same way and after draining, 3 discs (1 cm^2 each) from three individual leafs on each plant were removed using a cork borer and dipped into an Eppendorf tube with 1 mL of distilled water plus Tween® 80 (0.05% w/v). The tubes were manually agitated for 5 min and the conidia concentration was determined with a Neubauer® Haemocytometer. Treatments resulted in approximately 10^5 spores/cm^2 of leaf surface.

After treatments, the Primula plants with *B. tabaci* were carefully placed on separate glass boxes (previously detailed) and kept in a Fitotron® with controlled temperature and relative humidity (30 °C and 45-65% respectively). The boxes had voile fabric in one side to allow gas exchange and to prevent an excess of humidity. Four plants were placed in each glass box. To confirm temperature and humidity during the assays, a HOBO data logger (Onset®) was placed inside each box. The excess of nymphs were carefully removed according to Ruiz Sánchez, Rosado Calderón, Chan Cupul, Alejo, & Munguía Rosales, (2009) to obtain an experimental unit of 120 nymphs per leaf. Nymphs were checked for mortality daily following 48 h after inoculation until the seventh day. Only the sporulated nymphs were considered as effective infections. This trial was repeated three times.

2.7. Statistics and data analysis

All analyses were performed using Infostat® (2013) statistical software, professional version. A Poisson distribution was used to analyse the number of dead *T. molitor* larvae. The growth curve was analysed with one-way analysis of variance (ANOVA). Conidia germination in oils was analysed by two-way analysis of variance considering oils, isolates and the interaction of both factors. Prior to analysis, data were arcsin square-root transformed (Saito, Takatsuka, & Shimazu, 2012). Tukey HSD test (0.05 significance level) was used for mean comparison. The proportion of infected *B. tabaci* nymphs from trials were analysed by one-way analysis of variance. Analyses of bioassays were contrasted with LSD Fisher test to compare means (0.05 significance level). Before statistical analyses were performed, homogeneity and the normality of error of variances were evaluated for all data groups.

ITS sequences were aligned with homologs from the *Metarhizium* reference strains (Bischoff, Rehner, & Humber, 2009) listed in Table 1 using the ClustalW module incorporated in the MEGA 6® software package (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The TREE-PUZZLE 5.2 software (Schmidt, Strimmer, Vingron, & von Haeseler, 2002) was used to estimate data set specific parameters as transition/transversion ratios and the α parameter for the Γ- distribution based correction of rate heterogeneity among sites. The most appropriate models of DNA sequence evolution for phylogenetic reconstruction were chosen according to the rationale outlined by Posada & Crandall (1998); phylogenies were thus reconstructed with the Maximum Likelihood (ML) method as implemented in the PhyML software tool (Guindon & Gascuel, 2003) using the Hasegawa-Kishino-Yano (HKY) model of nucleotide substitution (Hasegawa, Kishino, & Yano, 1985). A Γ- distribution based model of rate heterogeneity (Yang, 1993) allowing for eight rate categories was assumed. Tree topology confidence limits were explored in non-parametric bootstrap analyses on over 1000 pseudo-replicates.

3. Results

3.1. Fungal isolation

In the soils under organic production system (OP) most of the isolates corresponded to *Metarhizium* sp. (94.7%) followed by *Beauveria* sp. (2.6%) and *Purpureocillium lilacinum* (2.6%). In the soils under conventional production systems (CP), only *Metarhizium* sp. was found. The Poisson distribution detected significant differences between the number and rate of dead larvae in OP vs. CP ($p<0.0001$). However, due to the over dispersion value of the analysis (6.822) a negative binomial distribution was performed to correct this error (data not shown). Subsequently, no significant differences were found in the mortality or rate of *T. molitor* larvae with EF from OP and CP soils ($p=0.193$). Six *Metarhizium* sp. isolates were chosen for radial growth and oil germination trials. The selected isolates were chosen since they showed rapid growth and sporulation (qualitatively) under the culture media used (Saito et al., 2012). Then, according to the growth curves and germination values, four of those six isolates were selected to perform bioassays and molecular analysis.

3.2. Radial growth

One-way ANOVA showed differences among the isolates during the complete growing period ($p<0.0001$). Isolates CEP401 and CEP381 showed the fastest growth with statistical differences ($p<0.001$) among them. Also, they were significantly different from the others isolates (Figure 1). The rest of the isolates showed smaller growth curves with no statistical differences among them.

3.3. Germination test with various oils

Significant interaction among oil treatments and isolates were found ($p<0.0001$) (Table 2). Therefore, none of the used oils could be selected as the best germination stimulator for all isolates. Therefore, a partitioned ANOVA was performed. With this analysis, the best stimulator (oil-concentration) to each isolate was obtained. The highest germination value (97.5 ± 1.8%) was found in the water control without Tween® 80 (CEP388), and the lowest germination value (2.9 ± 1.2%) was found in sunflower oil 1% v/v (CEP401) (Table 2). Control treatments (without Tween® 80) showed germination percentages ranging from 63 to 97%. When Tween® 80 was added to control treatments, the germination percentages only increased slightly in CEP375 (>10.6%), CEP409 (9.2%), and CEP413 (7.1%).

Isolates CEP381 (sunflower oil 10% v/v), CEP401 (olive oil 10% v/v), CEP409 (maize oil 10% v/v) and CEP413 (olive oil 10% v/v) were selected for further assays since they showed similar or higher germination values in those oils without statistical differences compared to controls.

3.4. Bioassays

Four isolates (CEP381, CEP401, CEP409 and CEP413) were chosen to assess the pathogenicity on *B. tabaci* nymphs (Figure 2). Statistical differences ($P<0.0001$) were found between control treatments (without conidia) and the pathogenicity trials. Also between mortality for conidia in water vs oil formulations (Figure 2a, b, c, and d). The highest

cumulative mortality given by the effect of oils was found in maize oil (20.5 ± 5%) (Figure 2 a). Within the fungal treatments, the highest mortality values were found in Olive oil + CEP401 and Sunflower oil + CEP381 formulations (45.8% and 46.9% respectively) (Figure 2b, and c).

When comparing water vs oil formulations, we found statistical differences in formulated conidia for isolates CEP401, CEP409 and CEP413. Isolate CEP381 showed no statistical differences when considering cumulative mortality in water and oil formulations (Figure 2b). Specific mortality by isolates is given by the difference between treatments with and without conidia.

3.5. Molecular identification

PCR products amplified from fungal isolates with primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') comprised between 530 and 620 nucleotides in length. BlastN searches revealed the following highest similarities to ITS sequences assigned to the species *Metarhizium anisopliae:* isolate CEP381 (100%), CEP401 (100%), CEP409 (95%), and CEP413 (92%). Maximum likelihood based phylogenetic reconstruction clearly confirmed that the four isolates belong to the fungal genus *Metarhizium* (Figure 3). However, species level characterization is not unambiguous, as all isolates are located in a 94% bootstrap supported sub-clade comprising reference strains of the *Metarhizium* core species *M. anisopliae*, *M. brunneum*, and *M. robertsii*, but resolution of species delineation is low within this sub-clade. Consequently, CEP isolates lack firm association with a particular species. Isolates CEP381, CEP401 and CEP413 appear most closely related to *M. brunneum* or *M. robertsii*, CEP409 is located in an out-group position relative to all other sequences comprised in the sub-clade. Moreover, isolates CEP409 and CEP413 form long terminal branches indicative of fast sequence evolution.

4. Discussion

The selected *Metarhizium* sp. isolates were able to grow and infect second instar *B. tabaci* nymphs at 30 °C and 45-65% RH. This data shows cumulative mortality obtained in a range from 3-10 °C higher and around 30-50% RH less than some tested isolates for similar biocontrol purposes (Table 3). Humidity has been considered as one of the critical issues related to the outcome of mycelial growth (both laboratory and field-tests), at least in *Beauveria bassiana* (Scholte, Knols, Samson, & Takken, 2004). Nevertheless, Lord, (2005) demonstrates that low ambient moisture can be an advantage rather than an impediment. In his study, when *B. bassiana* conidia were combined with diatomaceous earth, a synergistic effect produced the highest mortality of *Rhyzopertha dominica* at 43% RH. For the same RH, Lord, (2011) again found the highest mortality for *B. bassiana* on *Dermestes maculatus*. It seems that some stress tolerant fungi also may have more opportunities to colonize hosts at even lower ambient moistures. In some insects, desiccation stress may cause changes in their cuticle that may modify the ability of conidia to attach, germinate, and penetrate. It may affect cellular and humoral defences in a manner that favours fungal infection (Lord, 2005). Still, the effect of ambient moisture on the efficacy of entomopathogenic fungi is a complex and controversial matter. If the environmental conditions are not suitable for external mycelia production and further aerial conidia release, it is presumed that inundated conidia releases are to be repeated frequently. However, the aim in those environments could be to infect and kill with contact rather than to establish epizootics (Bateman, Carey, Moore, & Prior, 1992).

In this study, the cumulative mortality suggests that oil formulations can slightly increase virulence. Isolates CEP401, CEP409 and CEP413 showed an increase of mortality when comparing oil formulations against water suspensions. However, we have not found great differences in the cumulative mortality with spores applied in water vs. oil formulations. Perhaps, the thermal protective effect of oils could be more evident at higher temperatures than 30 °C.

It is known that the increased infectivity of entomopathogenic Hypocreales in oil is due to their greater adhesiveness to the lipophilic insect cuticle, whereas water formulations do not adhere and are lost before conidia can germinate and infect (Bateman et al., 1992). A second possible explanation may be the irregular distribution of conidia in water formulations. However we did not see clear differences in mortality among water and oils formulations.

Malsam et al. (2002) found that conidia of *M. anisopliae* can be uniformly distributed in oil formulations, equally covering nymphs of *Trialeurodes vaporariorum* but, without the addition of oil to the spore suspension, the conidia were distributed very irregularly on the whitefly integument. About 30% of the nymphs and eggs were totally covered with masses of conidia and other nymphs were almost free from fungal spores. Also the irregular distribution could be appraised when infested leaf discs were dipped into the spore suspension; some droplets on the discs coalesced to form drops, while other leaf areas remained dry (Malsam et al., 2002).

The ability of EF spores to germinate in oil formulations and to infect and kill specific targets is crucial to implementation of alternative management programmes, especially in places with high temperatures, high UV radiation and low atmospheric humidity (Fernandes, Rangel, Braga & Roberts, 2015). Although there is little research on this particular topic, a similar type of enhanced biocontrol efficacy by *M. acridum* erroneously reported as *M. flavoviride* at that time (Lomer, Bateman, Johnson, Langewald & Thomas, 2001) against *Schistocerca gregaria* at 30 °C and 35% RH was performed by Bateman et al. (1992) obtaining, as in this study, higher efficacy in oil formulations under those environmental conditions. Yousef (2014) found mortality percentages around 50% in *S. gregaria* using *B. bassiana* at 27 ± 2 °C and 70 ± 5% RH. Our study is also in concordance with Malsam et al. (2002) that reports mortality percentages that reach 44% in *B. tabaci* and 57.6% in *T. vaporariorum* using *M. anisopliae* at 25 °C and 80% RH. These species are not frequently associated as natural hosts for *Metarhizium* species, but some strains have shown to be virulent against whiteflies (Islam, Omar, & Shabanimofrad, 2014; Malsam et al., 2002). This study reveals that some species like *M. robertsii* (CEP381 and CEP401) could be virulent enough to infect and kill around 50% on *B. tabaci* nymphs. Fungal growth studies such as radial growth, germination and sporulation have been very helpful in the characterization of EF (Almeida, Albuquerque, & Luna-Alves, 2005), and these parameters seem to be important in defining the virulence of fungal isolates (Islam et al., 2014). Results of our study suggest a relationship between growth rate and pathogenicity in our isolates. Two isolates (CEP381 and CEP401) have shown the highest growth rates and have generated the highest mortality at 30 °C and 45-65% RH. However, the effects on mortality with and without oil formulations at those conditions were similar. Although both isolates have shown promising results, further studies are needed to improve their application for biocontrol purposes.

To assume that the mortality rate can be directly associated with a given isolate becomes relative for being closely related to the conditions under which the isolate was tested, which will not necessarily be the conditions under which the isolate is going to be used. Isolates as those used in this study (CEP381 and CEP401), have generated mortalities close to 50% on *B. tabaci* nymphs. At first 50% mortality did not seem to be highly efficient, but the relatively low mortalities are probably more reflective of a modest challenge than a lack of pathogenicity. However, these isolates can be easily applied with vegetable oils, without altering their viability and can also be functional under temperatures of 30 °C and RH up to 65%. Taxonomically, more studies need to be done on these strains to determine the proper species identification within the *Metarhizium* core group.

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Table 1. List of fungal reference strains used in phylogenetic analysis. ITS GAN (Genbank Accession Number).

Reference Species	Strains	Original Host / Source	Geographic Origin	ITS (GAN)
Metarhizium acridum	ARSEF 324	Orthoptera	Australia	AF137063
Metarhizium acridum	ARSEF 7486	Orthoptera	Niger	HQ331458
Metarhizium anisopliae	ARSEF 7487	Orthoptera	Eritrea	AF135210
Metarhizium brunneum	ARSEF 2107	Coleoptera	U.S.A.	KC178691
Metarhizium brunneum	ARSEF 4152	Soil	Australia	HQ331452
Metarhizium flavoviride	ARSEF 2133	Coleoptera	Czech Rep.	NR_131992
Metarhizium frigidum	ARSEF 4124	Coleoptera	Australia	NR_132012
Metarhizium globosum	ARSEF 2596	Lepidoptera	India	HQ331459
Metarhizium guizhouense	ARSEF 6238	Lepidoptera	China	HQ331447
Metarhizium guizhouense	CBS 258.90	Lepidoptera	China	HQ331448
Metarhizium lepidiotae	ARSEF 7412	Coleoptera	Australia	HQ331455
Metarhizium lepidiotae	ARSEF 7488	Coleoptera	Australia	HQ331456
Metarhizium majus	ARSEF 1015	Lepidoptera	Japan	HQ331444
Metarhizium majus	ARSEF 1914	Coleoptera	Philippines	HQ331445
Metarhizium pingshaense	CBS 257.90	Coleoptera	China	HQ331450
Metarhizium robertsii	ARSEF 727	Orthoptera	Brazil	HQ331453
Metarhizium robertsii	ARSEF 2575	Coleoptera	U.S.A.	NR_132011
Beauveria bassiana	ARSEF 751	Coleoptera	Vietnam	AY532045

Table 2. Conidia germination (% ± SD) of selected isolates in different vegetable oil concentrations. Different letters indicate significant differences among isolates. Tukey HDS test at 5% level of significant.

Oils concentrations (% v/v)	**Isolates**					
	CEP375	**CEP381**	**CEP388**	**CEP401**	**CEP409**	**CEP413**
Control (Water and conidia)	63.43±4^{D}	80.52±1.2^{F}	97.52±1.8^{E}	92.96±4^{E}	69.77±2.2^{C}	79.09±2.7^{CDE}
Control plus Tween 0.05 %	74±1.8^{DE}	67.72±1.1^{E}	63.52±3.2^{B}	65.09±1.9^{C}	78.93±2.6^{D}	86.69±3.2^{E}
Maize 10%	50.67±0.9^{C}	79.47±1.6^{F}	73.08±2^{C}	51.36±0.1^{B}	80.74±2.3^{D}	63.63±2.4^{AB}
Sunflower 10%	15.89±3.8^{A}	77.06±1.3^{F}	93.25±2.8^{E}	69.15±3.1^{C}	31.9±1.6^{B}	67.79±4.5^{ABC}
Olive 10%	77.55±1.7^{E}	22.23±2.1^{B}	91.18±1.4^{DE}	79.46±3^{D}	75.81±1.8^{CD}	81.13±1.4^{DE}
Sunflower 1%	32.47±0.2^{B}	30.87±0.8^{C}	83.36±2.5^{D}	2.92±1.2^{A}	14.83±1.9^{A}	68.98±1.9^{BC}
Maize 1%	23.04±4^{AB}	9.03±2^{A}	5.39±1.7^{A}	3±0.5^{A}	78.55±1.9^{D}	71.24±3.9^{BCD}
Olive 1%	26.43±3.3^{AB}	45.15±0.5^{D}	6.95±1.7^{A}	65.83±2.9^{C}	21.84±1.2^{A}	56.97±2.3^{A}
Sign.	**<0,0001**	**<0,0001**	**<0,0001**	**<0,0001**	**<0,0001**	**0.0003**

Table 3. Comparison between the general conditions used to test pathogenicity of EF on whiteflies. Note the range of effective application (Temperature and RH). Sp/mL (Applied spores per mL); C. Mort (Cumulative Mortality plus SD).

Specimen	**Target specie**	**Temp.**	**R.H.**	**Sp/mL**	**C. Mort**	**Coadjuvant-Oil**	**Source**
M. anisopliae	*B. tabaci*	20±1°C	-	5x10^{6}	100%	Coconut + soybean	Batta, 2003
M. anisopliae	*B. tabaci*	18-30°C	-	6x10^{4}	100%	Coconut + soybean	Batta, 2003
M. anisopliae	*T. vaporarorium*	25°C	80%	5x10^{6}	100%	0.1% sunflower oil	Malsam et al., 2002
M. anisopliae	*T. vaporarorium*	25°C	80%	5x10^{6}	70%	0.1% rape seed oil	Malsam et al., 2002
M. anisopliae	*T. vaporarorium*	25°C	80%	5x10^{6}	50%	0.1% potassium oleate	Malsam et al., 2002
I. fumosorosea	*B. tabaci*	25 ± 2°C	90%	1x10^{7}	99%	-	Murillo et al., 2015
I. javanica	*B. tabaci*	25 ± 1°C	-	1x10^{7}	90%	Barley	Xie et al., 2016
I. javanica	*B. tabaci*	25 ± 1°C	-	1x10^{7}	96%	Barley + 5% moth larva	Xie et al., 2016
B. bassiana	*B. tabaci*	27±3°C	75±8%	1x10^{7}	67.8%	0.05% Tween® 80	Ruiz et al., 2009
M. anisopliae	*B. tabaci*	25±1°C	70±10%	1x10^{8}	73.6%	0.02% Tween® 80	Islam et al., 2014
M. anisopliae	*B. tabaci*	25±1°C	70±10%	1x10^{8}	54.8%	0.02% Tween® 80	Islam et al., 2014
M. anisopliae	*B. tabaci*	25±1°C	70±10%	1x10^{8}	84.3%	0.02% Tween® 80	Islam et al., 2014
CEP381	*B. tabaci*	30±1°C	45-65%	1x10^{7}	45.55±5.67%	0.05% Tween® 80	This study
CEP381	*B. tabaci*	30±1°C	45-65%	1x10^{7}	46.91±3.94%	10% sunflower oil	This study
CEP401	*B. tabaci*	30±1°C	45-65%	1x10^{7}	32.77±4.28%	0.05% Tween® 80	This study
CEP401	*B. tabaci*	30±1°C	45-65%	1x10^{7}	45.83±0.83%	10% olive oil	This study
CEP413	*B. tabaci*	30±1°C	45-65%	1x10^{7}	35±3.33%	0.05% Tween® 80	This study
CEP413	*B. tabaci*	30±1°C	45-65%	1x10^{7}	41.9±4.11%	10% olive oil	This study
CEP409	*B. tabaci*	30±1°C	45-65%	1x10^{7}	30.55±1.27%	0.05% Tween® 80	This study
CEP409	*B. tabaci*	30±1°C	45-65%	1x10^{7}	36.11±2.1%	10% maize oil	This study