Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to Entomopathogenic Fungi and their Extracts

Maria-Angeles Castillo[a], Pilar Moya[b], Enrique Hernández[a], and Eduardo Primo-Yúfera[ab]*

Departamento de Biotecnología and Instituto de Tecnología Química UPV-CSIC, Universidad Politécnica de Valencia, Valencia, Spain

* Address correspondence to this author at Instituto de Tecnología Química UPV-CSIC, Universidad Politécnica de Valencia, Avenida de los Naranjos s/n, Apartado 22012, 42022 Valencia, Spain (fax 34-6-3877809; telephone 34-6-3877805; e-mail eprimo@itq.upv.es).

[a] Departamento de Biotecnología

[b] Instituto de Tecnología Química.

Running head: Pathogenicity of entomopathogenic fungi against *Ceratitis capitata*.

Eduardo Primo-Yúfera. Address: Instituto de Tecnología Química UPV-CSIC, Universidad Politécnica de Valencia, Avenida de los Naranjos s/n, Apartado 22012, 42022 Valencia, Spain. Telephone 34-6-3877805; fax 34-6-3877809; e-mail eprimo@itq.upv.es

ABSTRACT

The effectiveness of seven strains of entomopathogenic fungi against *Ceratitis capitata* adults was evaluated in the laboratory. Adults were susceptible to five out of seven aqueous suspensions of conidia. *Metarhizium anisopliae* and strain CG-260 of *Paecilomyces fumosoroseus* were the most pathogenic fungi with 10-day LD_{50} values of 5.1 x 10^3 conidia/fly and 6.1 x 10^3 conidia/fly, respectively, when applied topically. Sublethal effects on fecundity and fertility of the fungal exposed females were also studied. The most effective fungus in reducing fecundity was *P. fumosoroseus* CECT 2705, with reductions in the order of 65% at 1 x 10^6 conidia/fly. *M. anisopliae* and *Aspergillus ochraceus* also showed significant reductions of fecundity (40-50% for most of the assayed concentrations). Fertility was moderately affected by the fungi. *M. anisopliae* at 1 x 10^6 conidia/fly was the most effective fungus, showing egg eclosion reduction of over 50%, compared with the control. In addition, culture broth dichloromethane extracts from the entomopathogenic fungi were tested for insecticide activity against *C. capitata*, including effects on fecundity and fertility. The extract from *M. anisopliae* was the most toxic, resulting in about 90% mortality at the concentration of 25 mg/g of diet; under these conditions, fecundity and fertility of treated females were reduced by 94% and 53%, respectively, compared with untreated controls.

KEY WORDS: entomopathogenic fungi; *Metarhizium anisopliae*; *Paecilomyces fumosoroseus*; *Aspergillus ochraceus*; Mediterranean fruit fly; *Ceratitis capitata*; fecundity; fertility; organic extracts.

INTRODUCTION

Development of an effective control method against the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, is needed because it does serious damage to many important agricultural crops. Its high reproductive potential and adaptability, combined with the scarcity of natural enemies and its wide host range, cause great concern to growers.

As an alternative to chemical control or as part of IPM programs, there is a resurgence of interest in the use of microbial insecticides for biological control of insect pests. Fungal agents are among the most promising groups of biological control agents against pest insects. Particularly, the Deuteromycete fungi have long been known to cause epizootics in fly populations under laboratory and field conditions (Barson *et al*., 1994; Watson *et al*., 1996; Reithinger *et al*., 1997). *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin, and *Paecilomyces fumosoroseus* (Wize) Brown & Smith have been recognized as some of the most important entomopathogens of dipteran insects (Steinkraus *et al*., 1990; Kuramoto and Shimaku, 1992; Samson *et al*., 1994; Watson *et al*., 1995).

Published research on fungal pathogens of *C. capitata* is limited to the pathogenicity of *M. anisopliae* against adults of Mediterranean fruit fly in the laboratory (Garcia *et al.*, 1980, 1984), but the potential of other fungi as biological control agents for *C. capitata* has not been explored. Hence, experiments presented herein were conducted to further evaluate the susceptibility of *C. capitata* to different entomopathogenic fungi.

Moreover, production of insecticidal metabolites has been directly implicated in the mode of action of entomopathogenic fungi, although reports are uncommon (Gillespie and Claydon, 1989). However, *in vitro* production of bioactive compounds by fungal entomopathogens against different orders of insects, has been widely reported (Claydon and Grove, 1982; Krasnoff *et al.*, 1991; Balcells *et al.*, 1995). In order to establish a

possible relationship between the virulence of a given strain and the toxicity of its metabolites, we also evaluated organic extracts from culture broth of the tested fungi against *C. capitata.*

MATERIALS AND METHODS

Insects

Adults of *C. capitata* were used as hosts. Flies were reared at 27 ± 2°C, 50 - 60 % relative humidity and 16 h light - 8 h dark photoperiod. Adult flies were provided with water and a diet consisting of protein yeast autolysate (Sigma, Madrid, Spain) and sucrose in a 1:4 ratio.

Fungi

Table 1 shows the tested fungi, their origin and their percentage of conidial germination. All fungal isolates were cultured on potato dextrose agar (PDA) (Difco, Detroit, MI, USA).

Germination studies

Conidial viability of each isolate was determined 48-72 h before the bioassay was performed. Conidia from seven-day-old PDA cultures were suspended in sterile distilled water containing 0.05% Tween 80 and removed from the plates with 5 ml pipete. The final conidia concentration, estimated using a hemocytometer (improved Neubauer chamber), was in the range 1 - 5 x 10^6 conidia/ml. A 0.1 ml aliquot of each conidia suspension was spread onto Sabouraud dextrose agar plates and the plates were incubated for 18 h, at 28°C, in darkness. Three agar discs (5 mm diameter) were aseptically removed from each plate using a cork borer, and a total of 50 single conidia

per disc were microscopically (40x) examined. Conidia were considered to have germinated when the germ tube was longer than the diameter of the conidium. All assays were carried out with at least three Petri plates, simultaneously.

Pathogenicity of fungi to C. capitata *adults*

Conidial suspensions of each fungal isolate were prepared as described in the above section. The concentration of conidia in each suspension was adjusted to 1 x 10^9 conidia/ml. Ten-fold serial dilutions were made to 1 x 10^5 conidia/ml.

One µl of each conidial suspension (ranging from 1 x 10^6 to 1 x 10^2 conidia/fly) was applied, using a micropipet, on the ventral surface of the abdomen of two day-old adults flies (5 males and 5 females per dose), which were previously anaesthetized with ice. Controls were similarly grouped, and each fly was treated with 1.0 µl of 0.05% Tween 80. The described bioassay was done on 5 occasions. After treatment, the flies were placed into a metacrilate box (10 x 10 x 10 cm) which contained a circular hole (6 cm diameter) covered with a net cloth, and diet and water were provided *ad libitum*. Test flies were held under the same temperature, humidity and photoperiod as the colony. Mortality was assessed at intervals of 24 h for 10 days. After surface sterilization using 3% sodium hypochlorite solution, cadavers were incubated on PDA at 28°C for 10 days to confirm mycosis, which was assumed when sporulated mycelia mass of the corresponding fungus was superficially observed (Selman *et al.*, 1997).

Effects of treatment on fecundity and fertility

Pathogenicity assays were also used to evaluate the effects on fecunditiy and fertility. To study fecundity, eggs laid for 24 h were collected and counted every 2 days, from the start to the end of treatment. At the same time, female mortality was recorded to obtain

the number of eggs per female/day; these data, accumulated over the 10-day period, were used to compare fecundity among treatments.

To study fertility, at the 6^{th} and 10^{th} day after treatment, 30 eggs per dose and day were randomly chosen among all the eggs laid in each dose of each fungus and placed on a Petri plate coated with 0.03% agar. Plates were incubated in a rearing chamber (27 ± 2°C, 50-60% RH, 16 h light-8 h dark photoperiod) and emerged larvae were counted 5 days after collection of the eggs.

Toxicity of extracts from entomopathogenic fungi against C. capitata

Culture conditions and extraction

Seven-day-old PDA cultures of each test fungus were used to obtain conidial suspensions containing ~ 10^6 conidia/ml, as described in germination studies section. Each suspension was then added, in a 1:9 ratio (v:v), to 10 liters of antibiotic test broth [Yeast extract (Difco, Detroit, Mi), 2.0 g; Bacto Peptone (Difco), 3.0 g; dextrose, 2.0 g; sucrose, 30.0 g; corn steep, 5.0 g; Na NO_3, 2.0 g; $K_2HPO_4 \cdot 3\ H_2O$, 1.0 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; KCl, 0.2 g; $FeSO_4 \cdot 7\ H_2O$, 0.01 g; distilled water, 1000 ml; pH = 5.5] and incubated in static conditions for 14 days at 28°C. After incubation, the culture broth was obtained by filtration through filter paper and extracted 3 times with dichloromethane (1:1; v/v).

Oral bioassay

The toxicity of the different extracts was tested on 2-day-old flies. Each of 5 replicates, of a single bioassay, consisted of 10 flies (5 males and 5 females). Treated food was supplied as a mixture of sucrose and protein yeast autolysate (4:1) which contained the extract at a concentration of 50 mg/g of diet. To appraise a potential antifeeding effect, a non-toxic xanthene dye, neutral red (Aldrich, Madrid, Spain), was

added to the diet at a concentration of 0.3 mg/g of diet; when flies showed red abdomens and colored feces, no antifeeding effect was assumed. Mortality was monitored daily for 12 days. Treated food was not replaced during the assay. Controls, provided with the diet and the xanthene dye, were carried out in parallel. Studies on fecundity and fertility were also carried out according to the method described above.

Statistical analysis

Mortality, fecundity and fertility data were all analyzed with one-way analysis of variance (ANOVA) and the least significant difference (LSD) test was used to compare means (STATGRAPHICS PLUS 4.0). Probit analysis (Finney, 1971) was used to determine the LD_{50} values (SPSS 7.0). LT_{50} values were calculated by mortality versus time regressions. All linear regressions were also performed using SPSS 7.0 program.

In Table 6 percentage mortality values have been corrected for mortality in the control using Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

Pathogenicity of fungi to C. capitata adults

Adults of *C. capitata* were susceptible to infection by five of the seven fungal isolates tested. Figures 1 and 2 show percentages of mortality 6 and 10 days after exposure to different concentrations of conidia, respectively. *P. fumosoroseus* CECT 2705 and *Verticilium lecanii* (Zimmermen) Viegas are not included in the figures because mortalities of less than 10% were found at the end of the treatment.

Six days after treatment, *P. fumosoroseus* CG-260 and *M. anisopliae* exhibited similar levels of activity, being significantly more pathogenic than the other fungi (F = 35.24, df = 4, 20, $P \leq 0.001$ for 1 x 10^6 conidia/fly; F = 36.91, df = 4, 20, $P \leq 0.001$ for 1 x 10^5 conidia/fly). Thus, at the two highest concentrations, no significant differences were found, while at 1 x 10^4 and 1 x 10^3 conidia/fly, *M. anisopliae* was slightly more virulent than *P. fumosoroseus* CG-260 (F = 6.55, df = 1, 8, $P \leq 0.05$ for 1 x 10^4 conidia/fly; F = 9.80, df = 1, 8, $P \leq 0.05$ for 1 x 10^3 conidia/fly). The concentration-response tests for *M. anisopliae* and *P. fumosoroseus* CG-260 showed linear correlations, with regression coefficients of r = 0.864 and r = 0.927, respectively. The calculated LD_{50} values (Table 2) were 2.6 x 10^5 conidia/fly for *M. anisopliae* and 2.2 x 10^5 conidia/fly for *P. fumosoroseus* CG-260; results of χ^2 analysis for goodness of fit for the regression equation revealed the existence of considerable homogeneity in data. Thus, judged by the overlap among confidence limits of the LD_{50} values, *C. capitata* was equally susceptible to *M. anisopliae* and *P. fumosoroseus* CG-260.

Ten days after treatment, the mortality caused by the fungi had increased, mainly at the three highest concentrations. *M. anisopliae* and *P. fumosoroseus* CG-260 were the most pathogenic fungi (F = 74.44, df = 4, 20, $P \leq 0.001$ for 1 x 10^6 conidia/fly; F = 71.10, df = 4, 20, $P \leq 0.001$ for 1 x 10^5 conidia/fly) compared with the other isolates. Mortality data confirmed the linear relation between mortality and concentration already observed at day 6 (regression coefficients: r = 0.971 and r = 0.970, for *M. anisopliae* and *P. fumosoroseus* CG-260, respectively). The 10-day LD_{50} values were 5.1 x 10^3 conidia/fly (2.5 x 10^3 – 9.8 x 10^3) and 6.1 x 10^3 conidia/fly (3.2 x 10^3 – 1.1 x 10^4) for *M. anisopliae* and *P. fumosoroseus* CG-260, respectively (Table 2). As in the previous case, the χ^2 values showed homogeneity in data.

Decreasing LD_{50} values with time is an indication that mortalities at the higher concentrations (over 10^4 conidia/fly) rise faster than at the lower concentrations. Following this approach, time-mortality responses could be useful to determine specific

clues of fungal-host interactions and, thus, LT_{50} values of *M. anisopliae* and *P. fumosoroseus* were calculated (Table 3). In general, speed of mortality of the host is correlated positively with conidial concentration (Fargues and Rodriguez-Rueda, 1980). In our case, differences in *M. anisopliae* LT_{50} values between 1 x 10^4 and 1 x 10^6 conidial doses were found, but this relation was not maintained among the consecutive doses (confidence limits overlapped), likely owing to that only one order of magnitude exists among doses. Regarding *P. fumosoroseus* CG-260, positive correlation was found between 1 x 10^4 conidia/fly and each of the other analyzed doses (Table 3), although no relation was found between 1 x 10^5 and 1 x 10^6 conidia/fly.

Taking into account these data, time-susceptibility of *C. capitata* to *M. anisopliae* and *P. fumosoroseus* was similar at higher doses. However, at 1 x 10^4 conidia/fly the former was faster causing mycosis and, consequently killing flies (8.8 against 11.0 days required by *P. fumosoroseus*).

In summary, it seems that *M. anisopliae* and *P. fumosoroseus* CG-260 show a similar level of pathogenicity against *C. capitata*, as can be concluded from both LC_{50} and LT_{50} values.

All the other isolates were less pathogenic, giving about 30% mortality at 1 x 10^6 conidia/fly. There was no evidence of mycosis in any control cadavers, whereas mycosis was confirmed on all dead treated adults.

As mentioned above, *V. lecanii* and *P. fumosoroseus* showed low mortalities (<10%) against *C. capitata*. According to these data, a lack of pathogenicity could be assumed under our experimental conditions. Several facts support this assumption. Although, in a first sight, the low viability of *V. lecanii* conidia (49.0%) (Table 1) could explain the low mortalities achieved against *C. capitata*, studies testing *V. lecanii* against *Musca domestica* L., have shown that high mortalities can be obtained with low viability of conidia (Barson *et al.*, 1994). On the other hand, germination studies were extended to a longer period and increased germination was observed with the time (89% at 36 h); this

result seems to indicate that exists slow germination rather than low viability. This fact could affect the fungal pathogenicity because conidia are exposed more time to adverse environmental conditions decreasing the probability of infection. In addition, *V. lecanii* is not a characteristic dipteran pathogen, being more frequently recovered, in natural conditions, from aphids and scale insects, but rarely from Diptera (Hall, 1981).

Although *P. fumosoroseus* is reported from >40 insect species in 8 orders, including dipterans (Samson, 1974; Smith, 1993; Lacey *et al.*, 1996), our results suggest that *C. capitata* is not a susceptible host for *P. fumosoroseus* CECT 2705 isolate.

Our results have confirmed the susceptibility of adults of *C. capitata* to infection by several isolates of entomopathogenic fungi. Only the pathogenicity of *M. anisopliae* var. *anisopliae* against *C. capitata* has been previously reported by Garcia *et al*. (1984), who found a 11-day LD_{50} of 8 x 10^6 conidia/ml, under experimental conditions similar to ours. Although Garcia´s LD_{50} value is enclosed in the 95% CL of our LD_{50} [5.1 x 10^6 (2.5 x 10^6 - 9.8 x 10^6) in conidia/ml], it is very close to the upper limit. In addition, the former value was obtained after 11 days of treatment as contrasted with our 10-day value. *M. anisopliae* CECT 2952 thus appears more efficacious against *C. capitata* than the isolate tested by Garcia *et al.* (1984).

P. fumosoroseus CG-260, which was isolated from the tephritid *Anastrepha* sp., was highly virulent to Mediterranean fruit fly in our laboratory study, stressing that isolation of fungi from naturally-infected insects is a good practice in the search of isolates with potential as biological control agents (Watson *et al.*, 1995; Vanderberg, 1996).

Effects on fecundity and fertility

Female exposure to fungal treatment usually resulted in reduced fecundity (Table 4). The highest fecundity reduction was obtained following treatment with *P. fumosoroseus* CECT 2705; the effect was concentration-dependent ranging from 8.0% for 1 x 10^2 to 66.1% for 1 x 10^6 conidia/fly (regression coefficient, r = 0.956). However, this was not

the rule for the other fungi, which showed concentration-independent effects. In these cases, *M. anisopliae* and *Aspergillus ochraceus* Wilhelm were the most active fungi that reduced fecundity between 40 and 50% for nearly all concentrations. In contrast, *P. fumosoroseus* CG-260 showed a moderate activity on fecundity, with significant reduction observed only at 1 x 10^6 conidia/fly.

To further study fungal-host interactions, time-fecundity relationships were obtained for the most active fungus reducing fecundity at the two highest doses (Figure 3). As shown, the effect on fecundity followed the same trend in nearly all cases; reductions were already observed on day 4 and, usually, a similar level of reduction was maintained during the experiment. The unique exception was found for *M. anisopliae* at 1 x 10^5 conidia/fly, where an obvious decreasing trend with time was observed.

The effects of treatments on fertility were moderate although significant reductions in the percentages of hatching were observed for almost all the fungi, mainly at 1 x 10^6 conidia/fly (F = 94.49, df = 6, 28, $P \leq 0.001$) (Table 5). *M. anisopliae* was the most effective fungus showing >50% reduction at 1 x 10^6 conidia/fly; in addition, this reduction of fertility was concentration-dependent (regression coefficient, r = 0.953). On the other hand, *Penicillium chrysogenum* Thom and *V. lecanii* exhibited moderate effects (34 and 25% of reduction, respectively) at the highest concentration tested, but they were practically ineffective at lower concentrations.

As in the case of fecundity, studies on time-fertility relationship were carried out; however, not a clear relation was established.

We have shown that fungal treatment affected negatively both fecundity and fertility. Similar effects have been reported in *M. domestica*, where the fecundity and fertility of females treated with conidia of *Entomophthora muscae* (Cohn) Fresenius and *M. anisopliae* were considerably reduced (Mullens, 1990; Bywater *et al.*, 1994). These effects are important when considering entomopathogenic fungi as fruit fly biocontrol

agents, because a decrease in these reproductive parameters after infection should further contribute to the reduction of fly populations.

Although we did not investigate the physiological effects of these fungi on *C. capitata* females, the reduction of fecundity and fertility was likely due to the invasive action of mycelia or the toxic action of fungal metabolites, or a combination of both (Hajek and St. Leger, 1994).

Activity of the fungal organic extracts

Table 6 shows the percentages of mortality in *C. capitata* adults at 6 and 10 days after treatment with the different fungal extracts. The most active extract produced by *M. anisopliae*, achieved 88.0% mortality at day 6 and almost 100% mortality at the end of the experiment. A lower level of activity was obtained with the extract of *P. chrysogenum* which gave 44.0% mortality 6 days after treatment, a value which remained unchanged until the end of the experiment.

Since the *M. anisopliae* extract was highly toxic, it was assayed at lower concentrations. Mortalities of 52 and 90% were observed at the concentration of 25 mg/g, 6 and 10 days after treatment, respectively. The assay at 12.5 mg/g resulted in 22.0% mortality at day 10, but no activity was detected at day 6 after treatment. The high activity obtained with the extract of *M. anisopliae* might be due to the presence of destruxins. *M. anisopliae* is a main producer of these mycotoxins, and some of them are highly toxic to insects, including dipterans such as *M. domestica*, *Drosophila melanogaster* Meigen and the mosquito *Culex pipiens* L. (Vey *et al*., 1987). Work is in progress to isolate the active products from the extract.

The effects of the extracts on fecundity and fertility were variable. The fecundity of females was reduced by all the extracts, except in the case of *B. bassiana* (Table 6). The most significant reductions were observed for *P. fumosoroseus* CG-260 (53.6%) and *P.*

chrysogenum (66.1%). The fact that reduced fecundity was found in almost all the extracts might be due to a lack of fitness in the treated females instead of a specific effect on reproduction; thus, it is possible that the extracts contained toxic products having sublethal toxicity that provoked a general weakness in the fly, secondarily affecting fecundity. With regard to fertility (Table 6), three extracts (*P. chrysogenum*, *V. lecanii* and *P. fumosoroseus* CECT 2705) showed percentages of reduction of hatching significantly different from the control ($F = 58.69$, $df = 5, 24$, $P \leq 0.001$).

The high mortality of females treated with the *M. anisopliae* extract prevented us from assessing these parameters at 50 mg/g; however, when assayed at 25 mg/g, fecundity and fertility of treated females were reduced to 94% and 53%, respectively. At 12.5 mg/g, no negative effects on these parameters were found.

Little information is available on sublethal effects of fungal metabolites on reproduction in dipterans. Only a few data about aflatoxins are reported. Matsumura and Knight (1967) found that *Aedes aegypti* L. was not killed when treated with mixed aflatoxins, but it exhibited reduced fecundity and fertility. Additionally, they noted that *M. domestica* and *D. melanogaster* underwent high mortality as well as the effects noted above.

Many entomopathogenic fungi produce toxins, but although some of them are fully described chemically, the paucity of toxicological studies leaves their role in pathogenesis unclear (Gillespie and Claydon, 1989; Roberts *et al*., 1992). In our approach for establishing a pathogenesis-toxicosis relationship, no conclusions could be drawn. However, results concerning *M. anisopliae*, either in entomotoxicity or in the studied sublethal effects, seem to indicate such a relationship, although further studies are necessary. This would be in agreement with data reported by Samuels *et al*. (1988a, b) about the destruxins metabolized by different isolates of *M. anisopliae*. In this case, the amount of destruxins from each isolate allowed the correlation of toxicosis and differential virulence against some insects. Moreover, this fungus has been reported as

pathogenic to several insect species which have also been shown to be susceptible to injected destruxins (Van der Laan, 1967). Thus, the destruxins must play an important role in the pathogenesis of this entomopathogenic fungus.

No relationship seems to exist between the toxicosis and pathogenesis shown by *P. chrysogenum* against *C. capitata*. The percentage of mortality produced by the extract and its effectiveness in reducing fecundity and fertility warrant further work that focuses on the isolation and identification of the active compound(s). In a previous study we found that the dichloromethane extract of the culture broth of this same isolate exhibited high toxicity against the hemipteran *Oncopeltus fasciatus* Dallas (Castillo *et al.*, 1999); this observation further supports the isolation goal because, a priori, the active product (products) could be a broad spectrum insecticide affecting different orders of insects.

Our study has shown the susceptibility of *C. capitata* to different species and isolates of entomopathogenic fungi, some of them with considerable potential for control of the pest. Future work will be directed towards selection of strains with higher virulence through assaying new isolates, mainly of *M. anisopliae* and *P. fumosoroseus*, laboratory evaluation of their tolerance to abiotic factors, and development of an effective field trial. Complementary, studies of the secondary metabolites from the different isolates will be carried out.

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REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18,** 265-267.

Balcells, M., Canela, R., Coll, J., Sanchis, V., and Torres, M. 1995. Effect of fungal metabolites and some derivatives against *Tribolium castaneum* (Herbst) and *Nezara viridula* (L.). *Pestic. Sci.* **45,** 319-323.

Barson, G., Renn, N., and Bywater, A. F. 1994. Laboratory evaluation of six species of entomopathogenic fungi for the control of the house fly (*Musca domestica* L.), a pest of intensive animal units. *J. Invertebr. Pathol.* **64,** 107-113.

Bywater, A. F., Barson, G., and Renn, N. 1994. The potential of oil-based suspensions of *Metarhizium anisopliae* conidia for the control of the house fly (*Musca domestica)*, a pest of intensive animal units. *Brighton Crop Protection Conference, Pest and Diseases*, 1097-1102.

Castillo, M. A., Moya, P., Cantín, A., Miranda, M. A., Primo, J., Hernández, E., and Primo-Yúfera, E. 1999. Insecticidal, anti-juvenile hormone, and fungicidal activities of organic extracts from different *Penicillium* species and their isolated active compounds. *J. Agric. Food Chem.* **47,** 2120-2124

Claydon, N., and Grove, J. F. 1982. Insecticidal secondary metabolic products from the entomogenous fungus *Verticillium lecanii*. *J. Invertebr. Pathol.* **40,** 413-418.

Fargues, J. and Rodriguez-Rueda, D. 1980. Sensibilitè des larves de *Spodoptera littoralis* [Lep. Noctuidae] aux hyphomycètes entomopathogènes *Nomuraea rileyi* et Paecilomyces fumosoroseus. *Entomophaga* **25,** 43-54.

Finney, D. J. 1971. *Probit Analysis*; Cambridge University Press: Cambridge.

Garcia, A. S., Baracho, I. R., and de Souza, H. M. L. 1980. Patogenicidades de linhagens de *Metarhizium anisopliae* a *Ceratitis capitata*. *Cienc. e Cult.* **32,** 671.

Garcia, A. S., Messias, C. L., de Souza, H. M. L., and Piedrabuena, A. E. 1984. Patogenicidade de *Metarhizium anisopliae* var. *anisopliae* a *Ceratitis capitata* (Wied.) (Diptera, Tephritidae). *Rvta. Bras. Entomol.* **28,** 421-424.

Gillespie, A. T. and Claydon, N. 1989. The use of entomogenous fungi for pest control and the role of toxins in pathogenesis. *Pestic. Sci.* **27,** 203-215.

Hall, R. A. 1981. The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. *In* "Microbial Control of Pests and Plant Diseases" (H. D. Burges, Ed.), pp. 483-498. Academic Press, London.

Hajek, A. E. and St. Leger, R. J. 1994. Interactions between fungal pathogens and insect hosts. *Annu. Rev. Entomol.* **39,** 293-322.

Krasnoff, S. B., Gupta, S., St. Leger, R. J., and Renwick, J. A. A. 1991. Antifungal and insecticidal properties of the efrapeptins: metabolites of the fungus *Tolyplocladium niveum*. *J. Invertebr. Pathol.* **58,** 180-188.

Kuramoto, H. and Shimaku, M. 1992. Pathogenicity of some entomogenous fungi of the adult house fly. *J. Jpn. Appl. Entomol. Zool.* **36,** 202-203.

Lacey, L. A., Fransen, J. J., and Carruthers, R. 1996. Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. *In* "Bemisia 1995: Taxonomy, Biology, Damage, and Management" (D. Gerling and R. Mayer, Eds.), pp. 401-433. Intercept, Andover, UK.

Matsumura, F. and Knight, S. G. 1967. Cited in: Wright, V. F., Vesonder, R. F., Ceigler, A. 1982. Mycotoxins and other fungal metabolites as insecticides. *In* "Microbial and Viral Pesticides" (E. Kurstak, Ed.), pp. 559-583. Marcel Dekker Inc., New York.

Mullens, B. A. 1990. *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) as a pathogen of filth flies. *In* "Biocontrol of Arthropods Affecting Livestock and

Poultry" (D. A. Rutz and R. S. Patterson, Eds.), pp. 231-245. Westview, Boulder, CO.

Reithinger, R., Davies, C. R., Cadena, H., and Alexander, B. 1997. Evaluation of the fungus *Beauveria bassiana* as a potential biological control agent against phlebotomine sand flies in Colombian coffee plantations. *J. Invertebr. Pathol.* **70,** 131-135.

Roberts, D. W., Gupta, S., and St. Leger, R. J. 1992. Metabolite production of entomopathogenic fungi. *Pesqui. Agropecu. Bras.* **27**, 325-347.

Samson, R. A. 1974. *Paecilomyces* and some allied Hyphomycetes. Studies in Mycology, No 6. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

Samson, P. R., Milner, R. J., and McLennan, P. D. 1994. Field trials of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) against *Inopus rubriceps* (Diptera: Stratiomyidae) in sugarcane. *Environ. Entomol.* **23,** 749-754.

Samuels, R. I., Charnley, A. K., and Reynolds, S. E. 1988a. The role of destruxins in the pathogenicity of three strains of *Metarhizium anisopliae* for the tobacco hornworm *Manduca sexta*. *Mycopathologia* **104**, 51-58.

Samuels, R. I., Reynolds, S. E., and Charnley, A. K. 1988b. Calcium channel activation of insect muscle by destruxins, insecticidal compounds produced by the entomopathogenic fungus, *Metarhizium anisopliae. Comp. Biochem. Physiol.* **90C**, 403-412.

Selman, B. J., Saaid Dayer, M., and Hasan, M. 1997. Pathogenicity of *Beauveria bassiana* (Baals.) Vuill. to the larvae of diamondback moth, *Plutella xylostella* L. (Lep., Yponomeutidae). *J. Appl. Entomol.* **121,** 47-49.

Smith, P. 1993. Control of *Bemisia tabaci* and the potential of *Paecilomyces fumosoroseus* as a biopesticide. *Biocontr. News Inf.* **14,** 71N-78N.

Steinkraus, D. C., Geden, C. J., Rutz, D. A., and Kramer, J. P. 1990. First report of the natural occurrence of *Beauveria bassiana* (Moniliales: Moniliaceae) in *Musca domestica* (Diptera: Muscidae). *J. Med. Entomol.* **27,** 309-312.

Vanderberg, J. D. 1996. Standardized bioassay and screening of *Beauveria basssiana* and *Paecilomyces fumosoroseus* against the Russian Wheat aphid (Homoptera: Aphididae).

Van der Laan, P. A. (Ed.). 1967. Insect Pathology and Microbial Control. North–Holland, Amsterdam.

Vey, A., Quiot, J. M., and Vago, C. 1987. Mode d'action insecticide d'une mycotoxine, la dextrusine E, sur les diptères vecteurs et disséminateurs de germes. *C. R. Acad. Sci. Paris* **304,** 229-234.

Watson, D. W., Geden, C. J., Long, S. J., and Rutz, D. A. 1995. Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae). *Biol. Contr*. **5,** 405-411.

Watson, D. W., Rutz, D. A., and Long, S. J. 1996. *Beauveria bassiana* and sawdust bedding for the management of the house fly, *Musca domestica* (Diptera: Muscidae) in calf hutches. *Biol. Contr*. **7,** 221-227.

TABLE 1

Isolates of Fungi Tested against Adults of *Ceratitis capitata*, and Percentage of Conidial Germination

Fungi	Code	Host	% Germination[d]
Metarhizium anisopliae	CECT 2952[a]	soil	98.3±1.2
Paecilomyces fumosoroseus	CG-260[b]	*Anastrepha* sp.	94.5±0.6
P. fumosoroseus	CECT 2705[a]	unknown	86.3±1.6
Aspergillus ochraceus	AV1[c]	*Ceratitis capitata*	77.3±1.5[e]
Beauveria bassiana	CECT 2704[a]	unknown	94.8±1.1
Verticillium lecanii	CECT 2953[a]	*Thaumetopea pitycampa*	49.0±1.9[e]
Penicillium chrysogenum	P87[c]	cereal	91.5±1.2

[a] Fungi provided by Colección Española de Cultivos Tipo (CECT).

[b] Isolated from *Anastrepha* sp. and provided by the Collection of Entomopathogenic Fungi, CENARGEN/EMBRAPA (Brazil).

[c] Isolated in our laboratory and mantained at the Colección de la Cátedra de Microbiología of the Universidad Politécnica de Valencia.

[d] Percentage of conidial germination measured at 18 h. Values are means ± standard error of 3 replicates of 50 conidia each.

[e] Percentage of conidial germination after 36 h was 90.3±1.3 for *A. ochraceus*, and 89.2±0.8 for *V. lecanii*.

TABLE 2

Dose-Mortality Responses of Adult *Ceratitis capitata* Exposed to *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* CG-260 Isolates

Fungi	day	slope ± ES	LD_{50} (95% CL)[a]	χ^2	*df*	*P*
M. anisopliae	6	0.30±0.06	2.6×10^5 (6.6×10^4-3.3×10^6)	8.02	23	0.998
	10	0.62±0.07	5.1×10^3 (2.5×10^3- 9.8×10^3)	7.71	23	0.999
P. fumosoroseus	6	0.49±0.07	2.2×10^5 (8.7×10^4-7.8×10^5)	8.47	23	0.997
	10	0.69±0.08	6.1×10^3 (3.2×10^3-1.1×10^4)	9.35	23	0.995

[a] Values in conidia/fly.

TABLE 3

Time-Mortality Responses of Adult *Ceratitis capitata* Exposed to *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* CG-260 Isolates

Fungi	conidia /fly	slope ± ES	r	LT_{50} (95% CL)[a]	*F*	*df*	*P*
M. anisopliae	10^4	6.84±0.28	0.963	8.8 (7.7 - 10.2)	605.0	1, 48	0.000
	10^5	9.09±0.38	0.960	6.5 (5.5 - 7.7)	568.8	1, 48	0.000
	10^6	10.92±0.41	0.969	5.9 (5.1 - 6.9)	728.5	1, 48	0.000
P. fumosoroseus	10^4	5.50±0.36	0.913	11.0 (9.0 - 13.6)	239.8	1, 48	0.000
	10^5	9.84±0.39	0.964	6.7 (5.8 - 7.8)	631.0	1, 48	0.000
	10^6	11.64±0.38	0.975	5.6 (4.8 - 6.4)	920.2	1, 48	0.000

[a] Values in days.

TABLE 4

Effects on Fecundity of Female *Ceratitis capitata* 10 days after treatment with Suspensions of Conidia

Conidia/fly	Fecundity									
	1 x 10^2		1 x 10^3		1 x 10^4		1 x 10^5		1 x 10^6	
	Eggs/female	% reduction	Eggs/female	% reduction	Eggs/female	% reduction	Eggs/female	% reduction	Eggs/female	% reduction
M. anisopliae	220.5±14.3^{AC}	29.7±4.5	182.4±13.8^{A}	41.8±4.4	141.2±7.6^{A}	54.9±2.5	171.5±12.8^{A}	45.3±4.1	165.8±13.8^{A}	47.1±4.4
P. fumosoroseus CG-260	311.2±6.1^{B}	2.0±1.6	304.1±6.9^{B}	4.0±1.8	285.9±7.8^{B}	2.6±2.1	285.9±9.7^{B}	12.7±5.6	199.7±8.1^{B}	35.9±2.7
P. fumosoroseus CECT 2705	289.6±12.8^{B}	8.1±3.8	238.7±6.4^{CD}	23.9±2.0	163.7±8.2^{A}	47.8±2.6	123.1±4.4^{C}	60.7±3.1	106.5±3.0^{C}	66.1±0.9
A. ochraceus	206.5±3.0^{C}	34.1±1.0	201.8±35.7^{A}	35.7±3.0	165.7±17.1^{A}	47.2±5.5	204.3±11.1^{D}	34.9±3.5	187.5±5.4^{AB}	40.2±1.7
B. bassiana	225.0±8.3^{AC}	28.2±2.6	214.3±10.8^{AD}	31.7±3.5	259.4±7.5^{C}	17.3±2.4	192.0±4.6^{AD}	38.9±1.5	307.1±12.1^{DF}	4.6±3.2
V. lecanii	249.6±12.1^{A}	20.4±3.9	259.0±12.8^{C}	17.4±4.0	239.1±12.9^{C}	23.8±4.1	236.7±10.0^{E}	24.5±3.2	233.7±6.7^{E}	25.5±2.2
P. chrysogenum	241.4±12.0^{A}	19.9±5.1	246.1±15.7^{CD}	21.5±5.0	264.1±11.0^{C}	15.8±3.5	183.1±17.8^{AD}	41.6±5.7	269.3±13.1^{D}	14.2±4.2
Control	313.6±10.5^{B}		313.6±10.5^{B}		313.6±10.5^{B}		313.6±10.5^{B}		313.6±10.5^{F}	

[a] Values are means ± standard error of cumulative number of eggs laid per female over a 10-day period in 5 experiments made overtime (5 males and 5 females per experiment). Within each column mean values with the same superscripts are not significantly different ($P > 0.05$).

TABLE 5

Effects on Fertility of Female *Ceratitis capitata* 10 days after treatment with Suspensions of Conidia

Conidia/fly	Fertility									
	1×10^2		1×10^3		1×10^4		1×10^5		1×10^6	
	% eclosion[a]	% reduction	% eclosion	% reduction	% eclosion	% reduction	% eclosion	% reduction	% eclosion	% reduction
M. anisopliae	83.8 ± 2.2^{A}	9.0±2.4	68.7 ± 1.7^{A}	25.3±1.9	63.3 ± 0.7^{A}	31.1±1.6	53.8 ± 2.3^{A}	41.5±2.5	43.3 ± 1.8^{A}	52.9±2.0
P. fumosoroseus CG-260	94.2 ± 2.5^{B}	0.9±0.7	88.6 ± 2.5^{B}	4.6±3.2	90.2 ± 1.0^{BE}	2.7±1.3	90.8 ± 1.8^{BD}	3.4±1.3	80.1 ± 1.3^{B}	13.5±1.4
P. fumosoroseus CECT 2705	92.3 ± 1.9^{B}	1.6±1.5	83.7 ± 1.4^{C}	11.0±0.7	89.1 ± 2.6^{BE}	4.5±1.7	79.3 ± 1.5^{C}	13.8±1.7	74.9 ± 1.7^{C}	18.5±1.9
A. ochraceus	90.8 ± 1.7^{BC}	2.3±1.4	89.9 ± 2.1^{B}	3.3±1.6	85.4 ± 2.7^{BC}	7.2±3.0	88.6 ± 3.0^{BD}	4.7±2.6	90.2 ± 2.1^{DG}	3.4±1.1
B. bassiana	85.3 ± 1.6^{A}	7.3±1.7	77.0 ± 2.0^{D}	16.3±2.2	79.4 ± 1.9^{CD}	13.7±2.1	81.2 ± 2.0^{C}	11.7±2.1	86.1 ± 1.6^{D}	6.4±1.7
V. lecanii	85.7 ± 1.8^{AC}	6.9±2.0	81.9 ± 0.6^{CD}	11.0±0.7	77.3 ± 1.9^{D}	16.0±2.1	85.0 ± 2.0^{BC}	7.5±2.2	68.9 ± 2.2^{E}	25.1±2.4
P. chrysogenum	86.3 ± 1.2^{AC}	6.2±1.3	81.6 ± 1.1^{CD}	11.3±1.1	78.5 ± 4.0^{D}	14.7±4.3	81.5 ± 1.1^{C}	10.3±1.9	60.3 ± 1.5^{F}	34.4±1.6
Control	92.6 ± 1.5^{B}		92.6 ± 1.5^{B}		92.6 ± 1.5^{E}		92.6 ± 1.5^{D}		92.6 ± 1.5^{G}	

[a] Values are means ± standard error of 5 experiments made overtime of percentages of hatching on day 6 and 10 after treatment. Within each column mean values with the same superscripts are not significantly different ($P > 0.05$).

TABLE 6

Insecticidal Activity of Organic Fungal Extracts against Adult *Ceratitis capitata* and Sublethal Effects on Fecundity and Fertility

Organic extracts	% Mortality[a]		Fecundity		Fertility	
	6 days	10 days	Eggs/female[b]	% reduction	% eclosion[c]	% reduction
M. anisopliae	88.0±3.7^{A}	95.6±2.7^{A}	*		*	
P. fumosoroseus CG-260	8.0±2.0^{B}	15.2±2.2^{B}	108.7±3.7^{A}	53.6±1.6	85.9±0.4^{A}	8.8±0.4
P. fumosoroseus CECT 2705	8.0±3.7^{B}	12.0±2.2^{B}	162.5±11.7^{B}	30.8±5.0	71.3±3.1^{B}	24.4±3.2
A. ochraceus	0	1.8±0.4^{C}	151.9±9.9^{B}	35.3±4.2	88.1±2.7^{AC}	6.6±2.8
B. bassiana	0	2.2±0.0^{C}	285.5±4.3^{C}	-[d]	90.0±3.0^{AC}	4.8±3.2
V. lecanii	0	28.3±4.4^{D}	193.4±11.9^{D}	17.6±5.1	51.0±3.6^{D}	45.9±3.8
P. chrysogenum	44.0±6.0^{C}	44.0±6.0^{E}	79.6±9.6^{E}	66.1±3.8	37.6±3.3^{E}	60.1±3.5
Control	4.2±0.8^{B}	4.2±0.8^{F}	234.9±7.7^{F}	0	94.3±1.5^{C}	0

Extract concentration: 50 mg/g.

[a] Values are means ± standard error of percentage of mortality of 1 bioassay of 5 replicates of 10 flies each. Data corrected using Abbott's formula.

[b] Means ± standard error of 5 replicates of cumulative number of eggs laid per female.

[c] Values are means ± standard error of 5 replicates of averaged eclosion percentages on day 6 and 10 after initiating the assay. Within each column, values with the same superscripts are not significantly different ($P > 0.05$).

[d] No reduction

* Parameter not estimated because high mortality of females.