Pathogenicity of three entomopathogenic fungi against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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Abstract

The use of pesticides against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) as a tool to control the pest has become an obstacle to the fresh agricultural products export to many countries that restrict pesticides residues. The effectiveness of three local strains of entomopathogenic fungi: *Metarhizium anisopliae*, *Beauveria bassiana*, and *Paecilomyces lilacinus* against the adult and immature stages of *C. capitata* was evaluated under laboratory conditions. Obtained results showed that *M. anisopliae* and *B. bassiana* were superior in its pathogenicity and potential to kill the pest than *P. lilacinus*. These results may be important to be used for the control of the pest in IPM program.

Keywords: *Ceratitis capitata*, Entomopathogenic fungi, Pathogenicity, Fruit fly

Background

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the economic horticultural pests in Egypt and Mediterranean basin due to its ability to infest wide range of fruits. *Ceratitis capitata* attacks more than 300 different hosts and leave negatively economic impact (Papadopoulos et al. 2001). Flies control depends mainly on chemical sprays (organophosphates, especially malathion, pyrethroids, and spinosad) mixed with protein baits (Martinez-Ferrer et al. 2012) in the orchards as well as larvae and pupae found in soil (Stark and Vargas 2009). However, synthetic pesticides for crop protection in aggravating use cause a number of undesirable effects on human health and the environment (Perry et al. 1998).

Entomopathogenic fungi (EPF) are soil-borne microorganisms, where optimal temperature in agricultural soil for their growth, sporulation, and infection often ranges from 20 to 30 °C, but variation in temperature tolerance within a strain can be significant. There are several factors negatively affecting on EPF presence in the soil such as copper content and fungicides (Uzman et al. 2019), nevertheless, *Metarhizium* spp. can be the dominant EPF genus in agricultural soil (Thaczuk et al. 2014) than *Beauveria bassiana* (Balsamo) Vuillemin. Also, organic matter (for *M. anisopliae* (Metschnikoff) Sorokin) and pH clay content (for *B. bassiana*) did not affect the occurrence of EPF (Quesada-Moraga et al. 2007). Consequently, EPF form an important component of an integrated pest management strategy for fruit flies via soil treatment. Also, EPF had effective role in fly regulation in the field (Sookar 2014). Entomopathogenic fungi, compared to other soil micro-organisms, have the advantage that they can infect their hosts via contact, invade via the epicuticle of integument, and do not need to be ingested by the insect to cause infection (Lacey and Shapiro-Ilan 2008).

Many investigations revealed that EPF have a significant role as biological control agents against different
The objective of this investigation was to evaluate the virulence of locally isolated strains of the EPF; *M. anisopliae*, *B. bassiana*, and *Paecilomyces lilacinus* against *C. capitata* stages via different concentrations under laboratory conditions.

**Material and methods**

**Elaboration of rearing**

The Mediterranean fruit fly *C. capitata* was obtained from the rearing laboratory of Horticultural Pest Department, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt. Flies fed on sugar and protein hydrolysate in a ratio 3:1, respectively (El-Sayed 1979), in addition to a source of water. Produced eggs were collected daily and reared on the artificial rearing medium described by Tanaka et al. (1969). After larvae complete the third larval instar, larvae were received in water then collected in fine sand obtained from Al-Arish City (Saini peninsula) for pupation. A day before the emergence of flies, pupae were sieved and located in screen cage (30 cm × 30 cm × 30 cm) allowing the emergence of flies, pupae were sieved and located in screen cage (30 cm × 30 cm × 30 cm) allowing the emerged flies to feed, mate, and produce eggs.

**Fungal strains and conidiospores production**

Local strains of *B. bassiana* 5133, *M. anisopliae* 5130, and *P. lilacinus* 1062 were used in the current study. These isolates were obtained from the Center for Fungi, Faculty of Science, Assiut University, Assiut, Egypt. Conidial viability test was achieved on these isolates. Conidia obtained from the 3 fungal cultures were grown on potato dextrose agar (PDA), consisting of 10 g/l potato, 20 g/l glucose, and 20 g/l agar-agar in Petri dishes (9 cm diameter and 1.5 cm in height). After 15 days of incubation at 25 °C and 60% RH, the conidia were harvested from the plates by grazing with sterile spatula into 1% Tween-80. The suspension was vortexed for 30 min using agitator at room temperature. The conidial concentration of the resulting stock suspension was estimated using hemocytometer under light microscope for each fungus. A series of dilutions were made to give concentrations range of $10^6$, $10^7$, $10^8$, $10^9$, and $10^{10}$ conidia/ml suspensions were held on ice at 4 °C to prevent conidial germination until using in bioassay as described by Gabarty et al. (2014).

**Bioassay tests**

Adults, third larval instar, and pupae were used in this bioassay. Ten third instar larvae were dipped in 2 ml of the above-mentioned fungal concentrations (conidia/ml) for 30 s, then transferred to a Petri dish containing 10 mg standard steam-autoclaved-sterilized fine sand, covered by the lid, sealed with plastic parafilm to avoid external contamination, and kept at 25 °C in a complete darkness. A plastic cage, 0.5 L capacity, was sprayed by 2 ml of conidial suspension and kept to dry, and then 10 newly emerged flies (unequal in sex) were entered. Control cages were treated by 2 ml of 1% tween 80. Artificial diet was supplied. Flies mortality were recorded 6 days after treatment. Dead flies were incubated in 25 °C and 65% RH to observe mycosis. All bioassays were recorded and analyzed based on four independent replicates.

**Statistical analysis**

Mortalities were corrected using Abbott formula (Abbott 1925). Probit analysis was used to determine lethal and sublethal levels (Finney 1971). The difference among treatments in rate of mortality was determined by analysis of variance (ANOVA) and Tukey’s method was used to compare mean significant differences among treatments ($p < 0.05$) using SPSS 26 statistical software.

**Results and discussion**

This study addressed the pathogenicity of three EPF: *M. anisopliae*, *B. bassiana*, and *P. lilacinus*, against different stages of *C. capitata*, with different levels (Tables 1, 2, 3, and 4). To address the pathogenicity, it was needed to analyze the effect of different fungal concentrations on different immature stages of *C. capitata* as well as adult stage.

**Susceptibility of *C. capitata* larvae**

Mean percentage of larval mortality of *C. capitata* treated with different concentrations of fungi showed significant increase in mortality ($p$ value $< 0.05$) in a concentration-dependent manner (Table 1). The fungus *M. anisopliae* caused the highest percentages of larval mortality in late third larval instar that ranged 15–60% at the $1 \times 10^6$ and $1 \times 10^{10}$ conidia/ml, respectively. The mortality rate appeared to increase by increasing concentration. Similarly, *B. bassiana* ranked second recording (15–57.50 %) at the same concentrations like *M. anisopliae*. On the contrary, *P. lilacinus* caused the lowest percentage of larval mortality ranging 10–17.5%. Interestingly, concentration increase in *P. lilacinus* treated larvae did not affect the mortality rate (Table 1).

To further select which of the tested fungi is high in pathogenicity, the differences in mortality among EPF at the same concentration were statistically analyzed (Fig. 1). The obtained results revealed no differences in larval sensitivity among the three EPF, at the lowest concentration, $1 \times 10^6$ (Fig. 1a). However, at high concentrations,
|                        | Conidia/ml | Conidia/ml | Conidia/ml | Conidia/ml | Conidia/ml | Control |
|------------------------|------------|------------|------------|------------|------------|---------|
| Mean mortality % of 1-day-old pupae |            |            |            |            |            |         |
| **Metarhizium anisopliae** |            |            |            |            |            |         |
| 1.0 × 10^6            | 1.0 × 10^7 | 1.0 × 10^8 | 1.0 × 10^9 | 1.0 × 10^10 | Control |
| Conidia/ml            | 25.0 ± 5.00 | 35.0 ± 5.77 | 45.0 ± 5.77 | 60.0 ± 8.16 | 62.5 ± 5.00 | 77.5 ± 5.00 | 5.00 ± 5.77 |
| F = 49.533            | p value = .000 | df = 5, 18 | p value = .000 | df = 5, 18 | df = 5, 18 |
| **Beauveria bassiana** |            |            |            |            |            |         |
| 15.0 ± 5.77           | 22.5 ± 5.00 | 32.5 ± 5.00 | 45.0 ± 5.77 | 57.5 ± 5.00 | 5.00 ± 5.77 |
| F = 50.684            | p value = .000 | df = 5, 18 | df = 5, 18 | df = 5, 18 | df = 5, 18 |
| **Paecilomyces lilacinus** |            |            |            |            |            |         |
| 10.0 ± 0.00           | 10.0 ± 0.00 | 15.0 ± 5.77 | 15.0 ± 5.77 | 17.5 ± 5.00 | 5.00 ± 5.77 |
| F = 4.563             | p value = .051 | df = 5, 18 | df = 5, 18 | df = 5, 18 | df = 5, 18 |
| Mean mortality % of 5-day-old pupae |            |            |            |            |            |         |
| **Metarhizium anisopliae** |            |            |            |            |            |         |
| 1.0 × 10^6            | 1.0 × 10^7 | 1.0 × 10^8 | 1.0 × 10^9 | 1.0 × 10^10 | Control |
| Conidia/ml            | 25.0 ± 5.00 | 35.0 ± 5.77 | 45.0 ± 5.77 | 60.0 ± 8.16 | 62.5 ± 5.00 | 77.5 ± 5.00 | 5.00 ± 5.77 |
| F = 73.174            | p value = .000 | df = 5, 18 | p value = .000 | df = 5, 18 | df = 5, 18 |
| **Beauveria bassiana** |            |            |            |            |            |         |
| 15.0 ± 5.77           | 22.5 ± 5.00 | 32.5 ± 5.00 | 45.0 ± 5.77 | 57.5 ± 5.00 | 5.00 ± 5.77 |
| F = 58.267            | p value = .000 | df = 5, 18 | df = 5, 18 | df = 5, 18 | df = 5, 18 |
| **Paecilomyces lilacinus** |            |            |            |            |            |         |
| 12.5 ± 5.00           | 12.5 ± 5.00 | 15.0 ± 5.77 | 20.0 ± 5.00 | 22.5 ± 5.00 | 5.00 ± 5.77 |
| F = 5.463             | p value = .003 | df = 5, 18 | df = 5, 18 | df = 5, 18 | df = 5, 18 |
| Mean mortality % of 8-day-old pupae |            |            |            |            |            |         |
| **Metarhizium anisopliae** |            |            |            |            |            |         |
| 1.0 × 10^6            | 1.0 × 10^7 | 1.0 × 10^8 | 1.0 × 10^9 | 1.0 × 10^10 | Control |
| Conidia/ml            | 25.0 ± 5.00 | 35.0 ± 5.77 | 45.0 ± 5.77 | 60.0 ± 8.16 | 62.5 ± 5.00 | 77.5 ± 5.00 | 5.00 ± 5.77 |
| F = 29.286            | p value = .000 | df = 5, 18 | p value = .000 | df = 5, 18 | df = 5, 18 |
| **Beauveria bassiana** |            |            |            |            |            |         |
| 22.5 ± 5.00           | 25.0 ± 5.00 | 32.5 ± 5.00 | 32.5 ± 5.00 | 47.5 ± 5.00 | 5.00 ± 5.77 |
| F = 26.314            | p value = .000 | df = 5, 18 | df = 5, 18 | df = 5, 18 | df = 5, 18 |
| **Paecilomyces lilacinus** |            |            |            |            |            |         |
| 12.5 ± 5.00           | 12.5 ± 5.00 | 15.0 ± 5.77 | 15.0 ± 5.77 | 17.5 ± 5.00 | 5.00 ± 5.77 |
| F = 4.560             | p value = .007 | df = 5, 18 | df = 5, 18 | df = 5, 18 | df = 5, 18 |

Means followed by the same letter are not significantly different
Table 2 Toxicity of Metarhizium anisopliae, Beauveria bassiana and Paecilomyces lilacinus to different stages of Ceratitis capitata

| Stages                        | LC_{10} Conidia/ml | LC_{30} Conidia/ml | LC_{10} Conidia/ml | LC_{30} Conidia/ml | Slope ± SE | \( \chi^2 \) | \( p \) |
|-------------------------------|--------------------|--------------------|--------------------|--------------------|------------|-------------|-------|
| **Metarhizium anisopliae**    |                    |                    |                    |                    |            |             |       |
| Third larval instar           | 9.81 \times 10^4   | 2.53 \times 10^7   | 1.18 \times 10^5   | 1.42 \times 10^5   | 0.314 \pm 0.068 | 3.284       | 1.000 |
| 1-day-old pupae               | 4.09 \times 10^10  | 7.08 \times 10^4   | 6.10 \times 10^7   | 9.10 \times 10^7   | 0.179 \pm 0.064 | 2.871       | 1.000 |
| 5-day-old pupae               | 1.62 \times 10^4   | 4.83 \times 10^6   | 2.49 \times 10^8   | 3.83 \times 10^8   | 0.306 \pm 0.067 | 6.181       | 0.995 |
| 8-day-old pupae               | 6.66 \times 10^4   | 1.54 \times 10^8   | 3.27 \times 10^{10} | 1.61 \times 10^{10} | 0.225 \pm 0.069 | 2.375       | 1.000 |
| **Beauveria bassiana**        |                    |                    |                    |                    |            |             |       |
| Third larval instar           | 1.86 \times 10^5   | 5.22 \times 10^7   | 2.59 \times 10^9   | 3.62 \times 10^9   | 0.309 \pm 0.069 | 2.296       | 1.000 |
| 1-day-old pupae               | 1.06 \times 10^3   | 1.55 \times 10^5   | 1.18 \times 10^7   | 1.32 \times 10^7   | 0.182 \pm 0.064 | 1.672       | 1.000 |
| 5-day-old pupae               | 2.54 \times 10^5   | 4.62 \times 10^5   | 4.11 \times 10^8   | 6.65 \times 10^8   | 0.178 \pm 0.065 | 7.813       | 0.981 |
| 8-day-old pupae               | 2.61 \times 10^3   | 9.88 \times 10^7   | 1.46 \times 10^{11} | 8.19 \times 10^{11} | 0.165 \pm 0.067 | 3.463       | 1.000 |
| **Paecilomyces lilacinus**    |                    |                    |                    |                    |            |             |       |
| Third larval instar           | 1.54 \times 10^6   | 2.07 \times 10^{14} | 8.84 \times 10^9   | 5.08 \times 10^9   | 0.093 \pm 0.080 | 2.338       | 1.000 |
| 1-day-old pupae               | 8.46 \times 10^5   | 1.97 \times 10^{18} | 7.23 \times 10^9   | 6.17 \times 10^9   | 0.061 \pm 0.081 | 2.689       | 1.000 |
| 5-day-old pupae               | 3.89 \times 10^3   | 2.35 \times 10^{13} | 1.40 \times 10^9   | 5.05 \times 10^9   | 0.077 \pm 0.074 | 3.000       | 1.000 |
| 8-day-old pupae               | 3.47 \times 10^5   | 2.77 \times 10^{13} | 8.22 \times 10^{18} | 1.95 \times 10^{18} | 0.096 \pm 0.077 | 3.850       | 1.000 |

Table 3 Mean percentage of mortality (±SD) of Ceratitis capitata adults at different treatment times (h) using different concentrations of Metarhizium anisopliae, Beauveria bassiana, and Paecilomyces lilacinus

| H    | Control | Metarhizium anisopliae | Beauveria bassiana | Paecilomyces lilacinus |
|------|---------|------------------------|--------------------|------------------------|
|      | 1 \times 10^6 Conidia/ml | 1 \times 10^7 Conidia/ml | 1 \times 10^8 Conidia/ml | 1 \times 10^9 Conidia/ml | 1 \times 10^{10} Conidia/ml | F  | p value |
| 24   | 0.00 ± 0.00a  | 10.00 ± 0.00b         | 2.50 ± 5.00c       | 15.00 ± 5.77d  | 12.50 ± 5.00e         | 20.00 ± 0.00f   | 16.560 | 0.000 |
| 48   | 5.00 ± 5.77a  | 7.50 ± 5.00a          | 20.00 ± 0.00c      | 25.00 ± 5.77e  | 15.00 ± 5.77f         | 32.50 ± 5.00g   | 17.600 | 0.000 |
| 72   | 0.00 ± 0.00a  | 30.00 ± 0.00a         | 27.50 ± 5.00f      | 30.00 ± 0.00a  | 20.00 ± 0.00b         | 30.00 ± 0.00a   | 135.400 | 0.000 |
| 96   | 10.00 ± 0.00a | 32.50 ± 5.00a        | 35.00 ± 5.77d      | 20.00 ± 0.00b  | 20.00 ± 0.00b         | 17.50 ± 5.00e   | 25.920 | 0.000 |
| 120  | 15.00 ± 5.77a | 15.00 ± 5.77a        | 15.00 ± 5.77a      | 7.50 ± 5.00b   | 20.00 ± 0.00b         | 0.00 ± 0.00b    | 9.800  | 0.000 |
| 144  | 0.00 ± 0.00a  | 35.00 ± 0.00a        | 30.00 ± 8.16c      | 17.50 ± 5.00d  | 0.00 ± 0.00d          | 2.50 ± 5.00d    | 25.000 | 0.000 |
| 24   | 0.00 ± 0.00a  | 2.50 ± 5.00a         | 5.00 ± 5.77a       | 10.00 ± 0.00a  | 20.00 ± 0.00b         | 17.50 ± 5.00d   | 19.200 | 0.000 |
| 48   | 5.00 ± 5.77a  | 10.00 ± 0.00b        | 15.00 ± 5.77b      | 15.00 ± 5.77b  | 17.50 ± 5.00d         | 20.00 ± 0.00e   | 8.127  | 0.000 |
| 72   | 0.00 ± 0.00a  | 20.00 ± 0.00a        | 20.00 ± 0.00a      | 30.00 ± 0.00   | 30.00 ± 0.00          | 30.00 ± 0.00    | –     | –     |
| 96   | 10.00 ± 0.00a | 20.00 ± 0.00a        | 20.00 ± 0.00a      | 30.00 ± 0.00   | 30.00 ± 0.00          | 30.00 ± 0.00    | –     | –     |
| 120  | 15.00 ± 5.77a | 12.50 ± 5.00b        | 15.00 ± 5.77b      | 25.00 ± 5.77e  | 20.00 ± 0.00a         | 2.50 ± 5.00d    | 9.200  | 0.000 |
| 144  | 0.00 ± 0.00a  | 0.00 ± 0.00a         | 0.00 ± 0.00a       | 0.00 ± 0.00    | 0.00 ± 0.00           | 0.00 ± 0.00     | –     | –     |
the performances of *M. anisopliae* and *B. bassiana* induced higher mortality than *P. lilacinus*.

Sub-lethal concentrations; LC_{10}, LC_{30}, and lethal concentrations; LC_{50} and LC_{90} of the 3 EPF showed that *M. anisopliae* ranked first, causing mortality to late third-instar larvae followed by *B. bassiana*. *P. lilacinus*, which showed the weakest pathogenic activity against the larval stage (Table 2).

Generally, it was recorded that EPF vary in their pathogenicity on insects and other arthropods. For instance, the pathogenicity of four species of EPF: *B. bassiana*, *M. anisopliae*, *M. flavoviride*, and *Paecilomyces fumosoroseus*, to various developmental stages of *Rhipicephalus sanguineus* (Acari: Ixodidae) ticks was compared under laboratory conditions. The most virulent isolate, *M. anisopliae*-108, caused 92–96% mortality (Samish et al. 2001). Similarly, the pathogenicity of 13 isolates of *M. anisopliae* and 2 isolates of *B. bassiana* to *C. capitata* and *C. var. rosa fasicentrinis* showed fluctuation in the mortality rates among larvae (Ekesi et al. 2002).

**Susceptibility of *C. capitata* pupae**
The EPF *M. anisopliae* appeared superior in causing pathogenicity recording the highest mean percentages of mortality in 1-day-old pupae. Using the concentrations ranging between 1 × 10^6 and 1 × 10^{10} conidia/ml, the mortality rate ranged 35–62.5%, while *B. bassiana* caused 30–57% pupal mortality. Both fungi increased the mortality in pupae by increasing concentration (Table 1). However, *P. lilacinus*, showed the least percentages of pupal mortality which ranged (10–15%) without any effect of fungal concentration increase on pupal mortality (Table 1).

The 5-day-old pupae were susceptible to all tested fungi. *Metarhizium anisopliae* achieved (25 and 77.50%) pupal mortality at the concentrations (1 × 10^6 and 1 × 10^{10} conidia/ml), respectively. While *B. bassiana* treated pupae recorded 30–65% mortality. Unlike to other developmental stages (third larval instar and 1-day-old pupa), *P. lilacinus* showed slightly more activity at 5-day-old pupae and resulted (15 and 22.50 %) pupal mortality. In 5-day-old pupae, all fungi increased mortality as the concentration increases (Table 1).

Similar pattern to those 5-day-old pupae was obtained while using 8-day-old pupae, showed significant differences (p value < .05) in the mean percentages of mortality among different concentrations. The fungus *M. anisopliae* showed pupal mortality between 15 and 45% at the concentrations (1 × 10^6 and 1 × 10^{10} conidia/ml) while *B. bassiana* and *P. lilacinus* caused 22.5–47.5% and 12.5–22.5% pupal mortality, respectively.

Comparing the effect of equal concentrations of EPF on different ages of pupae, the mortality rates were compared at the same concentration (Fig. 1b–d). On 1- and 5-day-old pupae (Fig. 1b, c), the

### Table 4 Lethal time LT (h) for *Ceratitis capitata* treated with different concentrations of *Metarhizium anisopliae, Beauveria bassiana,* and *Paecilomyces lilacinus*

| Concentration | LT_{25} | LT_{50} | LT_{75} | LT_{90} | Slope ± SE | χ² | p   |
|---------------|---------|---------|---------|---------|-----------|-----|-----|
| *Metarhizium anisopliae* |         |         |         |         |           |     |     |
| 1 × 10^6      | 45.083  | 64.390  | 91.967  | 126.755 | 4.357 ± 0.470 | 17.398 | .741 |
| 1 × 10^7      | 49.929  | 64.182  | 82.502  | 103.424 | 6.185 ± 0.698 | 28.008 | .147 |
| 1 × 10^8      | 33.704  | 49.332  | 72.208  | 101.741 | 4.077 ± 0.447 | 12.463 | .947 |
| 1 × 10^9      | 41.718  | 63.467  | 96.555  | 140.859 | 3.701 ± 0.424 | 13.909 | .905 |
| 1 × 10^10     | 29.356  | 40.922  | 57.046  | 76.926  | 4.675 ± 0.530 | 7.440  | .998 |
| *Beauveria bassiana* |         |         |         |         |           |     |     |
| 1 × 10^6      | 75.240  | 100.199 | 133.439 | 172.688 | 5.421 ± 0.697 | 25.201 | .288 |
| 1 × 10^7      | 50.084  | 79.337  | 125.678 | 190.137 | 3.376 ± 0.429 | 37.434 | .021 |
| 1 × 10^8      | 56.669  | 83.214  | 122.193 | 172.670 | 4.043 ± 0.501 | 24.617 | .316 |
| 1 × 10^9      | 31.858  | 53.344  | 89.319  | 142.045 | 3.013 ± 0.379 | 21.962 | .462 |
| 1 × 10^10     | 23.271  | 41.498  | 74.001  | 124.547 | 2.685 ± 0.367 | 4.944  | 1.00 |
| *Paecilomyces lilacinus* |         |         |         |         |           |     |     |
| 1 × 10^6      | 61.611  | 98.800  | 158.438 | 242.359 | 3.289 ± 0.470 | 7.033  | .999 |
| 1 × 10^7      | 55.873  | 91.294  | 149.170 | 232.063 | 3.163 ± 0.436 | 8.145  | .997 |
| 1 × 10^8      | 42.199  | 61.589  | 89.889  | 126.328 | 4.108 ± 0.475 | 10.356 | .961 |
| 1 × 10^9      | 31.626  | 49.303  | 76.861  | 114.620 | 3.498 ± 0.483 | 6.632  | .980 |
| 1 × 10^10     | 33.788  | 47.665  | 67.242  | 91.651  | 4.513 ± 0.488 | 16.125 | .810 |
performances of *M. anisopliae*, *B. bassiana*, and *P. lilacinus*, showed similar pattern but the difference between *M. anisopliae* and *B. bassiana* and *P. lilacinus* was clear at all concentrations giving the highest pathogenicity to *M. anisopliae* followed by *B. bassiana* and finally *P. lilacinus* (Fig. 1b, c).

For 8-day-old pupae (Fig. 1d), the pattern of pathogenicity was very similar to that of larvae (Fig. 1a) where...
there was no differences between all treatments only at the lowest concentration, $1 \times 10^6$ (Fig. 1d). However, at the highest concentrations, the performances of *M. anisopliae* and *B. bassiana* caused higher mortality than *P. lilacinus*. Similar results were obtained when analyzing sub-lethal concentrations; LC$_{10}$, LC$_{30}$, and lethal concentrations; LC$_{50}$ and LC$_{90}$ of the 3 EPF which also revealed that *M. anisopliae* ranked first in its pathogenicity to 1-, 5-, and 8-day-old pupae, followed by *B. bassiana* and *P. lilacinus*, which showed the weakest pathogenic activity against the mentioned stages (Table 2).

Oreste et al. (2015) studied the interaction between age of puparia (at 2, 4, 6 days old) and fungal strains. They addressed both the emergence of *C. capitata* adults and the mortality of pupae. They found that the fungal treatments of different *B. bassiana* strains was higher on 2-day-old puparia (49.16 and 51.33% of mycosed puparia for ATCC 74040 and AL1 strain, respectively), while the rate of mycoses was low and ranged between 39 and 27.16%, when fungal treatments were performed on 4- and 6-day-old puparia. Lozano-Tovar et al. (2013) found significant differences among strains of *Beauveria* spp. and *Metarhizium* spp. in the total percentage of non-viable puparia of *C. capitata* and non-viable puparia showing fungal outgrowth, with percentages ranged from 5.0 to 45.0%. Quesada-Moraga et al. (2006) tested the pathogenicity of 10 strains of *B. bassiana* and 5 of *M. anisopliae* against puparia and adults of *C. capitata*, finding that only 2 strains of *B. bassiana* and one of *M. anisopliae* caused mortality higher than 50%, when puparia were immersed in the conidial suspensions. Imoulan et al. (2011) tested several *B. bassiana* strains against medfly pupae, finding that when insects were exposed to $10^8$ conidia/ml, the adult emergence ranged from 0 to 23.33% after 10 days post-treatment.

Susceptibility of *C. capitata* flies to entomopathogenic fungi

The three fungal species were pathogenic to the flies; however, it was difficult to identify a clear pattern of mortality either with time during conidial suspensions by concentrations of the used fungi (Table 3). Mortality of *C. capitata* adults by all of the used fungi showed fluctuated increases and decreases with time when compared to control (Table 3). Similar limitation was achieved while calculating the lethal time where the lethal time to kill 25% (LT$_{25}$), 50% (LT$_{50}$), 75% (LT$_{75}$), and 90% (LT$_{90}$) varied among the three fungal isolates (Table 4).

The fluctuation in mortality lethal time and concentration among adults might be linked to the method of treatment where adults were kept in 0.5 l plastic cage in which only 2 ml of conidial suspension was sprayed. Based on the fact that they can fly away from the contaminated area, adult might avoid infection with the fungi. The red palm weevil adults treated with *B. bassiana* and *M. anisopliae* showed different lethal time by changing the spray method between dry powder and aqueous suspension (Gindin et al. 2006). Beris et al. (2013) verified the pathogenicity of several strains of *B. bassiana*, *Isaria fumosorosea* Wize—formerly *Paecilomyces fumosoroseus* (Wize) Brown et Smith and *M. anisopliae* under laboratory conditions against pupal and adult stages of Mediterranean fruit fly via different exposure routes. Castillo and Moya (2000) had confirmed the susceptibility of adults of *C. capitata* to infection by several isolates of EPF, especially, *M. anisopliae* and *P. fumosoroseus* CG-260, which 6 days after treatments, exhibited similar levels of activity, being significantly more pathogenic than the other fungi. Thus, at the two highest concentrations, non-significant differences were found, while at the lowest concentrations, *M. anisopliae* was slightly more virulent than *P. fumosoroseus* CG-260. Ten days after treatment, the mortality caused by the fungi increased, mainly at the three highest concentrations. *M. anisopliae* and *P. fumosoroseus* CG260 were the most pathogenic fungi. In general, the speed of mortality of the host is correlated positively with conidial concentration (Fargues and Rodriguez-Rueda 1980). In this work, time—mortality responses could be useful to determine specific clues of fungal–host interactions. The application of conidial preparations to natural soil reduced insect emergence and the adult life span and represents a promising strategy for fruit fly integrated management.

The present findings are in agreement with many works who confirmed the susceptibility of other tephritids to infection with *M. anisopliae* and *B.bassiana* (Dimbi et al. 2003; Quesada-Moraga et al. 2006; Ladurner et al. 2008). Current research in controlling fruit fly and other harmful pests is highly focusing on searching for safe biopesticides and other safe alternative methods of control (Ali and Ibrahim 2018; Ibrahim and Ali 2018). Such safe biopesticides as well EPF are believed to serve as promising tools in pest management rather than chemical insecticides.

Conclusion

Entomopathogenic fungi, as soil-borne micro-organisms, are one of the most important agent in biological control programs against *C. capitata*. Three species of fungi (local strains) were tested against the adults and different immature stages (larvae and pupae) of *C. capitata* to evaluate their pathogenicity. Our results suggest *M. anisopliae* and *B. bassiana* have a great value in its virulence to *C. capitata*. 
Abbreviations
IPM: Integrated pest management; EPF: Entomopathogenic fungi;
PDA: Potato dextrose agar; RH: Relative humidity; ANOVA: Analysis of variance; LC: Lethal concentration; LT: Lethal time; SD: Standard deviation

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NAS reared C. capitata and put strategy to achieve this work. SMA achieved this investigation. NAS and SMA are the contributors in writing the manuscript. AMAI and AMAI revised the manuscript. All authors read and approved the final manuscript.

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