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Virulence of *Beauveria bassiana* and *Metarhizium anisopliae* on different stages of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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Abstract

Background: The pink bollworm, *Pectinophora gossypiella*, is the most destructive pests of the cotton plant in Egypt. Due to the several problems of insecticides, the present study was conducted to evaluate the toxicity effect of the entomopathogenic fungi (EPF), *Beauveria bassiana* and *Metarhizium anisopliae*, against the different stages of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

Results: The fungal isolates exhibited a toxic effect against the treated stages, egg, larva, and pupa. According to the obtained data of LC_{50}, *B. bassiana* was more potent in inducing toxicity than *M. anisopliae*. However, eggs of *P. gossypiella* were less susceptible to the EPF than the other stages. Based on total mortality, LC_{50} was 4.97×10^{11}, 6.03×10^{12} spores/ml for egg; 8.25×10^{8}, 6.03×10^{9} spores/ml for neonate; 2.52×10^{8}, 1.29×10^{10} spores/ml for early 4th instar larvae; and 6.79×10^{8}, 8.36×10^{9} spores/ml for pupae after treatment with *B. bassiana* and *M. anisopliae*, respectively.

Conclusions: Entomopathogenic fungi exhibited an activity in inducing mortality against different stages of *P. gossypiella*.

Keywords: Entomopathogenic fungi, Pink bollworm, *Pectinophora gossypiella*, Mortality

Background

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is one of the most injurious cotton pests worldwide (Parmar and Patel 2016). It decreases quantities and qualities of the cotton yield (Moustafa et al. 2019).

Because of the adverse effects of chemical insecticides to human and environment generally, there is a serious need for insecticidal alternatives. Microbial insecticides such as fungus, bacterium, virus, or protozoan are one of such alternatives characterized by not having chemical residues and/or insect resistance (Dhakal and Singh 2019).

More than 700 species of EPF were widely used as biopesticides (e.g., *B. bassiana*, *M. anisopliae*, *Verticillium lecanii*, *Purpureocillium lilacinum*, and *Isaria fumosorosea*) against many agricultural pests (Rizwan et al. 2019). EPF are present within natural insect populations and are often considered as an effective microbial control agent in integrated pest management (Vega et al. 2009). The mode of action of the EPF is through invading the insect cuticle and production of toxic enzymes that overcome the insect immune system (Niu et al. 2019).

EPF proved its effect against eggs, immature stages, and adult stage of many insect species (Hanem 2012) in both natural and artificial ecosystem (Cuthbertson and Audsley 2016). There is a growing interest to use them as a biocontrol agent in integrated pest management programs (Ali et al. 2017). The present study aimed to

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evaluate the virulence of EPF, *B. bassiana* and *M. anisopliae*, against the *P. gossypiella* different stages under laboratory conditions.

Methods

Rearing of insect

A culture of *P. gossypiella* was established under constant laboratory conditions of (27±1 °C and 65% R.H) in a rearing room at the Bio Insecticides Production Unit, Plant Protection Research Institute, Giza, Egypt. The neonate larvae were reared on an artificial diet described by Rashad et al. (1993). The pupae were placed in clean glass vials plugged with cotton until moth emergence.

Entomopathogenic fungi

Entomopathogenic fungi isolates, used in the present study, were *B. bassiana* and *M. anisopliae*. *B. bassiana* was isolated from the red palm weevil, *Rhynchophorus ferrugineus* in Ismailia governorate, while *M. anisopliae* was isolated from the white fly, *Bemisia tabaci* (Genn.) in Sharkia governorate (Ibrahim 2006).

Mass production of entomopathogenic fungi

The obtained conidia from fungal culture of *B. bassiana* and *M. anisopliae* were grown at 25 °C, in dark, on Sabouraud dextrose agar (SDA), consisting of peptone 10 g/l, glucose 20g/l, and agar-agar 20 g/l, (constant volume of 15 ml) in standard Petri-dishes (9-cm diameter). Conidia were harvested from 15-day-old plates by scraping into sterile Triton-X. The suspension was vortexed for 2 min and agitated for 1.5 h on a flask shaker at room temperature before filtering through 4 layers of sterile muslin. The conidial concentrations were estimated stock suspension were estimated using an improved Neubauer bright line hemocytometer (Reichert) under a Leitz Dialux × 40 EB microscope (400× magnification). A series of dilutions were made to give range concentrations of 10^8, 10^9, 10^{10}, 10^{11}, and 10^{12} spores/ml.

Bioassay

To study the virulence of *M. anisopliae* and *B. bassiana* against the different stages of pink bollworm *P. gossypiella*, 5 concentrations of each fungus (10^8, 10^9, 10^{10}, 10^{11}, and 10^{12} (spores/ml)) were prepared. All studies were carried out and incubated at 27±1°C and 65% R.H.

Treatment of eggs

Egg cards of *P. gossypiella* were sprayed using air brush directly with 5 concentrations of each EPF through laboratory bioassays then placed into plastic container measuring 7×5 cm and observed daily for the number of hatched larvae in each treatment. Four replicates (50 eggs/replicate). Control eggs were treated with water only.

Treatment of newly hatched larvae (neonate)

Concentrations of fungi were homogeneously mixed with artificial diet (without the antimicrobial agents). Four replicates (each replicate contained 10 newly hatched larvae *P. gossypiella*). A control experiment was done, but diet was mixed with distilled water. Newly hatched larvae at all treatments were allowed to feed on the treated diet for 48 h; then, alive larvae were transferred individually to glass tubes (2×7 cm) containing untreated artificial diet. Tubes were plugged with cotton wool and incubated at the above concentrations.

Treatment of early 4th instar larvae

Early 4th instar *P. gossypiella* larvae were immersed in 5 concentrations of fungi for 30–60 s, and then transferred to a sterile filter paper to dry. Four replicates (each replicate contained 10 individuals of 4th instar larvae). A control experiment was done, but larvae were immersed in distilled water. The 4th instar larvae were transferred by sterile forceps to glass tubes (2×7 cm) containing untreated artificial diet. Tubes were plugged with cotton wool and incubated at the above conditions. Mortality rate was recorded daily until pupation and adult emergence.

Treatment of pupae

One-day-old pupae *P. gossypiella* were immersed in different concentrations of fungi for 30–60 s, and then transferred to a sterile filter paper to dry. Four replicates at each concentration treated contained 10 pupae. Pupae were incubated at the above conditions. The mortality rate was recorded daily until adult emergence.

Studied criteria

- Mortality rate was recorded daily and calculated as percentages.
- Pupation rate was expressed as percent of the successfully developed pupae.
- Emergence rate was expressed as percent of the successfully emerged adults.
- Total mortality percentages were corrected against those of the control by Abbott's formula (Abbott 1925).
- LC_{50} calculation: corrected percentages of total mortality (between 20 and 84% mortality) were plotted versus the corresponding concentrations on logarithmic probability paper to obtain the corresponding log-concentration probit lines. The median lethal concentration (LC_{50}) of treated insects

was determined from the established regression lines (Finney 1971).

Results

Virulence of EPF against eggs

Table 1 displays the virulence of *M. anisopliae* spore suspensions against the egg of *P. gossypiella*. Egg mortalities were recorded at all concentration levels (41, 33, 22, 17, and 9% at 10^{12}, 10^{11}, 10^{10}, 10^{9}, and 10^{8} spores/ml, respectively, compared to 1.50% of control eggs). Also, egg hatchability was gradually decreased with the increase of concentration levels. The used concentration levels decreased the egg hatchability to 59, 67, 78, 83, and 91 than 98.50% in the control eggs.

B. bassiana spore suspensions exhibited also a mortality effect against the egg of *P. gossypiella* (Table 1). The highest egg mortality was recorded by 53% at concentration level 10^{12} spores/ml than 2.5% in the control egg. The same concentration level decreased the egg hatchability to 47% compared to 97.5% of control egg.

No latent toxicity effect was recorded for the 2 EPF. According to the LC_{50} values, *B. bassiana* exhibited the lowest 4.97×10^{11} spores/ml, whereas 6.03×10^{12} spores/ml for *M. anisopliae* (Table 5).

Virulence of EPF against newly hatched larvae

M. anisopliae exhibited a mortality effect against the newly hatched larvae (neonate) of *P. gossypiella* that increased with the increased of concentration levels (Table 2). Obtained data indicated that the highest mortality percent (92.5%) was recorded by the highest concentration (10^{12} spores/ml) while the lowest mortality percent (15.0%) was caused by the lowest concentration (10^{8} spores/ml) compared to 7.5% of control larvae. The mortality effect of *M. anisopliae* extended from the larval treatment to the resulted pupae and adults. Pupal mortality appeared at the highest 3 concentrations: 66.7, 20.0, and 4.5% at 10^{12}, 10^{11}, and 10^{10} spores/ml, respectively than 0.0% in the control pupae. However, *M. anisopliae* induced adult mortality to 37.5, 14.3, and 6.90% at 10^{11}, 10^{10}, and 10^{9}, respectively compared to 0.0% of control adults. Total mortality was increased gradually by increasing the concentration (97.50, 87.5, 55.00, 32.50, and 15.00 at 10^{12}, 10^{11}, 10^{10}, 10^{9}, and 10^{8} spores/ml, respectively compared to 07.50% of control insects). Pupation and adult emergence percentages were concentration dependent. Application of the highest concentration level (10^{12} spores/ml) decreased the pupation percent to 07.50% vs. 92.50 of the control pupae and adult emergence percent to 02.50% vs. 100.00% of control adults.

B. bassiana showed the same effect of *M. anisopliae* on treatment of the newly hatched larvae (neonate) of *P. gossypiella* (Table 2). Complete mortality (100%) was recorded at the highest concentration level (10^{12} spores/ml) compared to control larvae (10.0%) whereas other tested concentrations caused mortality of 82.5, 70.0, 47.5, and 32.5% at 10^{11}, 10^{10}, 10^{9}, and 10^{8} spores/ml, respectively. Mortality effect of *B. bassiana* was extended in the resulted pupae and adults. In pupal stage, only 2 concentration levels (10^{11} and 10^{10} spores/ml) revealed a mortality rate of 28.60 and 8.0% compared to control (0.0%). In the adult stage, mortality rates were revealed at all concentration levels with the highest mortality 60.0% at 10^{11} spores/ml. Total mortality percent was recorded in a concentration-dependent manner (100.00, 95.50, 77.50, 52.50, and 37.50% at 10^{12}, 10^{11}, 10^{10}, 10^{9}, and 10^{8} spores/ml, respectively vs. 10.00% of control insects). Pupation and adult emergence percent decreased with increasing the concentration levels (Table 2).

According to the LC_{50} values of total mortality, *B. bassiana* exhibited the lowest LC_{50}, 8.25×10^{8} spores/ml, whereas it was 6.03×10^{9} spores/ml at *M. anisopliae* (Table 5).

Table 1 Effect of the fungal isolates against the egg stage of *Pectinophora gossypiella*

Entomopathogenic fungi	Concentration spores/ml	Eggs mortality (%)	Corrected mortality (%)	Egg hatchability (%)
Metarhizium anisopliae	**10^{12}**	41.00	40.0	59.00
	10^{11}	33.00	31.98	67.00
	10^{10}	22.00	20.81	78.00
	10^{9}	17.00	15.73	83.00
	10^{8}	9.00	7.61	91.00
	Control	1.50	---	98.50
Beauveria bassiana	**10^{12}**	53.00	51.81	47.00
	10^{11}	44.00	42.51	56.00
	10^{10}	32.0	30.21	68.00
	10^{9}	24.00	22.1	76.00
	10^{8}	12.0	9.7	88.0
	Control	2.5	---	97.5

Table 2 Effect of the fungal isolates against the newly hatched larvae (neonate) of *Pectinophora gossypiella*

Entomopathogenic fungi	Concentration spores/ml	Larval mortality (%)	Pupation (%)	Pupal mortality (%)	Adult emergency (%)	Adult mortality (%)	Total mortality (%)	Corrected mortality (%)
Metarhizium anisopliae	10^{12}	92.50	07.50	66.7	02.50	0.00	97.50	97.30
	10^{11}	75.00	25.00	20.0	20.00	37.5	87.5	78.41
	10^{10}	45.00	55.00	4.5	52.50	14.3	55.00	43.24
	10^{9}	27.50	72.50	00.00	100.00	6.90	32.50	21.62
	10^{8}	15.00	85.00	00.00	100.00	0.00	15.00	8.11
	Control	07.50	92.50	00.00	100.00	0.00	07.50	---
Beauveria bassiana	10^{12}	100.00	------	----	------	--------	100.00	100.00
	10^{11}	82.50	17.50	28.60	12.50	60.0	95.50	86.11
	10^{10}	70.00	30.00	8.0	27.50	18.1	77.50	69.44
	10^{9}	47.50	52.50	00.00	100.00	9.25	52.50	41.67
	10^{8}	32.50	67.50	00.00	100.00	7.41	37.50	25.00
	Control	10.00	90.00	00.00	100.00	0.00	10.00	---

Virulence of EPF against early 4th instar larvae

Obtained data exhibited that the highest mortality percent in the treated larvae (57.5%) was caused by the highest concentration level of 10^{12} spores/ml whereas the concentrations of 10^{11}, 10^{10}, 10^{9}, and 10^{8} spores/ml induced mortality of 45.0, 32.5, 22.5, and 15.0%, respectively, compared to 0.00% in control larvae (Table 3). The mortality effect of *M. anisopliae* extended to the pupae and adults that resulted from the treatment. Pupal and adult mortalities were recorded at all concentration levels except the lowest concentration. Pupal mortality rates were 52.9, 31.8, 18.5, and 6.5% at 10^{12}, 10^{11}, 10^{10}, and 10^{9} spores/ml, respectively, vs. 0.00% mortality in control. Also, 25.0% of adult mortality that formed the highest percent was recorded at the highest concentration level (10^{12} spores/ml) compared to 2.5% in control. Total mortality increased with the increased concentration levels to 85.0, 67.5, 50.0, 30.0, and 15.0%, respectively, against 2.5% in control. The highest concentration (10^{12} spores/ml) decreased pupation and adult emergence percent with the highest percent to 42.5 and 20.0%, respectively vs. 100% of control insects.

The fungal isolate *B. bassiana* showed the same trend with *M. anisopliae* but with somewhat high effect (Table 3). All spore suspensions exhibited mortal effects against the treated larvae (65, 55, 47.5, 35, and 22.5% at 10^{12}, 10^{11}, 10^{10}, and 10^{9} spores/ml, respectively) vs. 2.5% mortality in control larvae. The virulence activity of the fungus *B. bassiana* was continued in the resulted pupae and adults from larval treatment. Pupal mortality was in a concentration dependent manner. The highest pupal mortality was recorded at the 2 highest spore

Table 3 Effect of the fungal isolates against the early 4th instar *Pectinophora gossypiella* larvae

Entomopathogenic fungi	Concentration spores/ml	Larval mortality (%)	Pupation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)	Total mortality (%)	Corrected mortality (%)
Metarhizium anisopliae	10^{12}	57.5	42.5	52.9	20.00	25.0	85.0	84.6
	10^{11}	45.0	55.0	31.8	37.50	13.3	67.5	66.7
	10^{10}	32.5	67.5	18.5	55.00	9.1	50.00	48.7
	10^{9}	22.5	77.5	6.5	72.50	3.5	30.00	28.2
	10^{8}	15.0	85.0	0.00	100.00	0.00	15.0	12.8
	Control	0.00	100.0	0.00	100.00	2.50	2.50	---
Beauveria bassiana	10^{12}	65.0	35.0	78.6	7.5	66.7	97.5	97.4
	10^{11}	55.0	45.0	55.6	20.0	25.0	85.00	84.6
	10^{10}	47.5	52.5	38.1	32.5	7.70	70.00	69.2
	10^{9}	35.0	65.0	26.9	47.5	0.00	52.5	51.3
	10^{8}	22.5	77.5	16.1	65.0	0.00	35.0	33.3
	Control	2.5	97.5	0.00	100.0	0.00	2.5	---

suspensions, 78.6 and 55.6% at 10^{12} and 10^{11} spores/ml than 0.0% mortality in control pupae. The highest 3 spore suspensions induced adult mortality; the highest mortality was 66.7% at 10^{12} spores/ml vs. 0.0% mortality in control adults. The total mortality was gradually increased by increasing spore suspensions as 97.5, 84.6, 69.2, 51.3, and 33.3% vs. 2.2% mortality in control insects. All concentrations decreased pupation and adult emergence percent in a concentration-dependent manner.

According to LC_{50} values of total mortality, *B. bassiana* was more effective than *M. anisopliae* against the early 4th instar larvae of *P. gossypiella*. The entomopathogenic fungus *B. bassiana* exhibited the lowest LC_{50}, 2.52×10^{8} spores/ml, whereas the LC_{50} of *M. anisopliae* recorded 1.29×10^{10} spores/ml (Table 5).

Virulence of EPF against the pupae

The highest spore suspension of *M. anisopliae*, 10^{12} spores/ml, caused a high percent of pupal mortality, 72.5% and 45.6% of adult mortality after treatment of *P. gossypiella* pupae, compared to 5.0 and 2.5%, respectively, for control (Table 4), whereas the other concentrations of 10^{11}, 10^{10}, 10^{9}, and 10^{8} spores/ml caused 65.0, 47.5, 32.5, and 17.5% pupal mortality rates and 24.6, 14.3, 11.1, and 3.0% adult mortalities. Total mortality was increased with the increase of concentration levels as 85.0, 75.0, 55.0, 40.0, and 20.0% at 10^{12}, 10^{11}, 10^{10}, 10^{9}, and 10^{8} spores/ml, respectively, compared to 7.5% of control insects. The percent of adult emergence decreased gradually to 82.5, 67.5, 52.5, 35.0, and 27.5% at the same ascending fungal concentrations than 95.0% of control adults.

The effect of *B. bassiana* treatment against *P. gossypiella* pupae and its extended to the emerged adults is showed in Table 4. *B. bassiana* showed pupal mortality rates of 85.0, 77.5, 70.0, 47.5, and 35.0% at concentrations of 10^{12}, 10^{11}, 10^{10}, 10^{9}, and 10^{8} spores/ml, respectively, compared to 2.5% for control pupae. Also the effect was spread in the emerged adults, where the same spore suspensions exhibited a slight activity by 5.0, 7.5, 7.5, 5.0, and 2.5% vs. 2.5% for control adults. Total mortality was in a concentration-dependent manner as 90.0, 85.0, 77.5, 52.5, 37.5% at 10^{12}, 10^{11}, 10^{10}, 10^{9}, and 10^{8} spores/ml, respectively, compared to 5.0% for control insects. Adult emergence was inhibited by the highest percent to 15.0, 22.5, 30, 52.5, and 65.0% at the used fungal concentrations vs. 97.5% of control adults.

Based on LC_{50} values for total mortality, *B. bassiana* was more potent than *M. anisopliae* against *P. gossypiella* after treatment of the early pupal stage. Obtained data exhibited that the *B. bassiana* recorded the lowest LC_{50}, 6.79×10^{8} spores/ml, whereas the LC_{50} of *M. anisopliae* was 8.36×10^{9} spores/ml (Table 5). Depending on the previous data of LC_{50} values for EPF, the lowest affected immature stages were eggs.

Discussion

Entomopathogenic fungi currently present as an alternative tool for pest control. Several strains of *B. bassiana*, *M. anisopliae*, and other fungal isolates have been evaluated against *P. gossypiella* (El-Akad et al. 2016; Moustafa et al. 2019) and several other insect pests.

In the present study, the 2 fungal isolates, *B. bassiana* and *M. anisopliae*, proved their virulence against *P. gossypiella* irrespective of the treated stage. The newly hatched larvae were more susceptible than the 4th instar larvae after treatment with *B. bassiana* than *M. anisopliae*. Accordingly, the LC_{50}, *B. bassiana* isolate was more potent than *M. anisopliae* isolate in inducing mortality. Farooq et al. (2020) found that *B. bassiana*, *V. lecanii*, and *M. anisopliae* concentrations and *A. indica*

Table 4 Effect of the fungal isolates against pupal stage of *Pectinophora gossypiella*

Entomopathogenic fungi	Concentration spores/ml	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)	Total mortality (%)	Corrected mortality (%)
Metarhizium anisopliae	**10^{12}**	72.50	27.5	45.6	85.0	83.75
	10^{11}	65.00	35.00	28.6	75.0	72.9
	10^{10}	47.50	52.50	14.3	55.0	51.35
	10^{9}	32.5	67.50	11.1	40.0	35.13
	10^{8}	17.5	82.5	3.0	20.0	13.5
	Control	5.00	95.0	2.5	7.5	---
Beauveria bassiana	**10^{12}**	85.0	15.00	5.00	90.0	89.47
	10^{11}	77.5	22.50	7.50	85.00	84.21
	10^{10}	70.0	30.00	7.5	77.5	76.32
	10^{9}	47.5	52.50	5.0	52.5	50.0
	10^{8}	35.0	65.00	2.5	37.5	34.2
	Control	2.50	97.50	2.5	5.0	---

Table 5 LC_{50} values of the fungal isolates on different stages of *Pectinophora gossypiella*

Entomopathogenic fungi	LC_{50}* spores/ml	Lower limit spores/ml	Upper limit spores/ml
Egg stage			
Metarhizium anisopliae	6.03×10^{12}	1.74×10^{12}	3.94×10^{13}
Beauveria bassiana	4.97×10^{11}	2.25×10^{11}	1.43×10^{12}
Newly hatched larvae			
Metarhizium anisopliae	6.03×10^{9}	3.23×10^{9}	1.11×10^{10}
Beauveria bassiana	8.25×10^{8}	3.25×10^{8}	1.74×10^{9}
Early 4th instar larvae			
Metarhizium anisopliae	1.29×10^{10}	5.48×10^{9}	3.11×10^{10}
Beauveria bassiana	2.52×10^{8}	2.03×10^{9}	8.35×10^{8}
Pupal stage			
Metarhizium anisopliae	8.36×10^{9}	3.4×10^{9}	2.02×10^{10}
Beauveria bassiana	6.79×10^{8}	1.47×10^{8}	1.93×10^{9}

*LC_{50} was calculated for total mortality (between 20 and 84% mortality)

extract (alone and in combination) at different exposure intervals caused high mortality rates among the treated 2nd instar *P. gossypiella* larvae. Also, LC_{50} values for *P. gossypiella* larvae treated with different concentrations of *B. bassiana* and *M. anisopliae* were 1.42 and 0.98 g/l, respectively (El-Akad et al. 2016). Moustafa et al. (2019) showed that the virulence of *Paecilomyces lilicanus* was high on *P. gossypiella* whereas the toxicity of *M. anisopliae* was high in case of *Earias insulana* after treatment of the newly hatched larvae. *Trichoderma harzianum* spores induced mortalities against the larvae of *E. insulana* and *P. gossypiella* (El-Massry et al. 2016).

The present study revealed that the treated eggs of *P. gossypiella* were less susceptible to the EPF than the other treated immature stages including the pupal stage depending on the LC_{50}. It is broadly accepted that insect eggs are more difficult to be infected than the larval stage (Skinner et al. 2014) and that the pupal stage is typically very resistant to succumb to injury (Vestergaard et al. 1995). However, the tested EPF, *B. bassiana* and *M. anisopliae*, decreased the egg hatchability of *P. gossypiella.* This result agrees with the study of Ullah et al. (2019) who reported that the EPF, *I. fumosorosea* and *B. bassiana*, significantly affected the egg hatchability of *Spodoptera litura*. Obtained data corroborated the findings of other studies that recorded pupal mortalities that extended after treatment of the larvae of some insects by some fungal isolates as in *E. insulana* (Abd-ElAzeem et al. 2019). In addition, the toxic effect of the 2 fungal isolates was reflected on the percent of pupation and adult emergence that decreased. These data are in accordance with other studies on certain insects by fungal isolates as in *P. gossypiella* (El-Akad et al. 2016) and *E. insulana* (Abd-ElAzeem et al. 2019).

Enzyme activity is considered one of the main mechanisms of fungal infection to insect host and inducing mortality by desiccation through the imperfect cuticle (Hamadah et al. 2018). Several studies revealed the presence of hydrolytic enzyme activity or cuticle-degrading enzymes, as chitinase, protease, and lipase, that play the main mechanism of fungal infection to insect host and inducing mortality. Also, some studies revealed that the virulence of EPF is associated with cuticle-degrading enzymes (Sargin et al. 2013; Cristina and Gheorghe 2017; Hamadah et al. 2018). These proteases are believed to help the fungal hyphae in penetrating the host tissue by breaking down the protein linkages in the insect cuticle and/or the utilization of the host proteins for fungal nutrition (Pozo et al. 2004). On the other hand, another suggestion that may be acceptable for the toxic effect of the fungal isolates is the complete destruction of the fat bodies; thus, the fat bodies lose their ability to synthesize and capacity to store nutrients (Mitchell and Cali 1994).

Conclusions

The study proved the activity of EPF, *B. bassiana* and *M. anisopliae*, against different stages of *P. gossypiella* under laboratory conditions. So, there is a possibility to use EPF as an alternative control tool to synthetic insecticides. Further study is needed to show their activity under field conditions.

Abbreviations

P. gossypiella: *Pectinophora gossypiella*; *B. bassiana*: *Beauveria bassiana*; *M. anisopliae*: *Metarhizium anisopliae*; *I. fumosorosea*: *Isaria fumosorosea*; *E. insulana*: *Earias insulana*; *V. lecanii*: *Verticillium lecanii*; EPF: Entomopathogenic fungi

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Authors' contributions

AI prepared fungal isolates and concentrations; GO conducted experiments and prepared statics; KH wrote the paper and interpreted the data. The authors read and approved the final manuscript.

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Declarations

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Competing interests
The authors declare that they have no competing interests.

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