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Pathogenicity of Entomopathogenic Fungal Isolates on Immature Stages of Pectinophora gossypiella (Saunders), (Lepidoptera: Gelechiidae)

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ABSTRACT

The pink bollworm, *Pectinophora gossypiella*, (Saunders) (Lepidoptera: Gelechiidae) considers as one of the world's most invasive insects, is a voracious lepidopteran pest of cotton. To control this insect pest and increase crop yield, chemical pesticides was mainly used and that caused resistant population and side effects for human and environment. Therefore, there is a need to increase of alternative methods such as the biological control. During the current study, five fungi isolates, one of *Beauveria bassiana* (AUMC 9896) and four of *Metarhizium anisopliae* (M1, M2, M3 &M4) were evaluated against the eggs, new hatched larvae, and pupa of *P. gossypiella* under laboratory conditions. Bioassay was performed using spores suspension concentrations $(1.0 \times 10^6, 1.0 \times 10^7 \text{ and } 1.0 \times 10^8 \text{ spores /ml})$. Results showed that *B. bassiana* (AUMC 9896) proved to be more toxicity than *M. anisopliae* isolates as depicted by the calculated LC₅₀ values on all treated stages, egg, larva, and pupa. On the other hand, the highest egg and pupal mortality percentages were recorded to *B. bassiana* (76.7 and 80.0%), respectively, at the same concentration $(1 \times 10^8 \text{ spores /ml})$. Whereas the isolate *M. anisopliae* (M4) showed the least one (23.3 and 46.6 %). Also, *M. anisopliae* isolates (M1 & M4) had recorded the highest value of larval mortality (86.7%). Thus, it can be concluded that the isolates *B. bassiana* (AUMC 9896 and M1) were strong pathogenicity while the isolates (M3&M4) were moderate and (M2) was showed low pathogenicity. **Keywords:** Pink bollworm, *Pectinophora gossypiella*, *Beauveria bassiana, Metarhizium anisopliae*, isolates

INTRODUCTION

The pink bollworm is an important cotton pest that drill through cotton bolls to feed on the cotton seeds. Cotton lint is destroyed in this process. As a result of the feeding damage, other insects and fungus entered the boll, inflicting more damage. (Naik *et al.*, 2021). This insect pest has become a commercial problem because its larval stage frequently enters diapause while in seed capsules, which enables the pest to become widespread. (Sarwar, 2017).

Chemical control with synthetic insecticides of these pests results in many problems and their residues are toxic to wildlife (e.g., birds, beneficial insects such as honey bees) (Usta, 2013). These chemical insecticides also cause many damage chemical changes in non-target insects that are harmful to predators, parasitoids, etc. The chemical insecticides are harmful to humans and domesticated animals Entomopathogenic fungi are important natural regulators of insect populations. In terms of insecticidal resistance, insect pests do not easily become resistant to infection by entomopathogenic fungi (Jiang *et al*, 2019). These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins, and growing by utilizing nutrients present in the haemocoel to avoid insect immune responses (Usta, 2013). Microbial control agents include entomopathogenic fungi (EPFs) in particular *Beauveria bassiana* (Ascomycota: Hypocreales) and *Metarhizium anisopliae* (Ascomycota: Hypocreales) are regarded as promising alternatives to traditional synthetic insecticides (Wakil *et al.*, 2017). Entomopathogenic fungi *B. bassiana* and *M. anisopliae* have pathogenic action on various agricultural insect pests (Rodrigues *et al.*, 2016). *M. anisopliae* and *B. bassiana* have shown potential for controlling several insect pests like the cotton leafwarm, *Spodoptera littoralis*, cotton bollworm *Helicoverpa armigera* (Sahar and El- Sabagh, 2015; Hirapara, *et al.*, 2019). Entomopathogenic fungi have been shown to cause mortality at different stages of *P. gossypiella* (Omar *et al.*, 2021).

Therefore, the present study was carried out to assess the efficiency of *B. bassiana* and some isolates of *M. anisopliae* against *P. gossypiella* immature stages under laboratory conditions. The effect of this isolates on some biological aspects of pink bollworm immature stages.

MATERIAL AND METHODS

1. Insects culture:

The culture of *P. gossypiella* was maintained in Bollworms Research Department, Plant Protection Research Institute, Agriculture Research Center (ARC), Sabaheia branch, Alexandria, Egypt. Under laboratory conditions of 27±2°C and 70±5% RH. Newly hatched larvae of the pink bollworm were reared on an artificial diet as described by (Abd El-Hafez *et al.*, 1982). Moths that emerged were sexed and kept under the previously mentioned conditions and provided with sucrose solution (10%). Females were permitted to lay their eggs in a glass cage covered with muslin, it was changed on alternate days and the egg were counted.

2. The entomopathogenic fungi tests:

2.1. Isolation and identification of tested fungi:

Five native fungal isolates were isolated in Bio-insecticide Production Unit, Plant Protection Research Institute, Agriculture Research Center (ARC), Egyp from soil. The first fungus, *Beauveria bassiana* (AUMC 9896) was defined in Mycological of Assiut University (Sahar and Moharram, 2014). Four isolates of *Metarhizium anisopliae* (M1, M2, M3 and M4) were isolated from soil. The dilution plate method was used. Soil samples were collected from the vegetable field in Qalyubia Governorate, Egypt. From each sample, 10g were added to with 100 ml of 0.185 % tetrasodium pyrophosphate was incubated on a shaker at 28 °C for 2 h, then was settled for 15 sec. The suspension (0.1 ml) was spread on semi-selective medium (2 % glucose; 1.8 % agar and 1 % peptone;) then added the antibiotics 0.005% tetracycline, 0.06 % streptomycin, 0.005 % cycloheximide and 0.01% v/v dodine. And incubated at 27 °C for 10-15 days in the dark condition after which the developing colonies were aseptically sub cultures on Czapek-dox agar medium(CZA) in plates and slopes for further research. Detected in Plant Pathology Research Institute, Agricultural Research Center of Egypt.

2.2. Culture conditions:

The isolates were cultured on (CZA) medium with yeast extract (1%) plates in 9 cm in diameter of several Petri dishes and were grown for 15 to 20 days at $27\pm2^{\circ}$ C. The spores were harvested by scraping the surface of 15-20 days old culture gently with a sterilized glass slide. The spores were suspended in distilled water with Tween-80 (0.1%). The mixture was shaken with a magnetic shaker for 10 minutes and was prepared three concentrations of spores suspension for each of isolates (1.0×10^{6} , 1.0×10^{7} and 1.0×10^{8} spores /ml).

3. The bioassay:

3.1. Treated of *P. gossypiella* eggs:

The cards with attached eggs had been divided into three pieces to obtain three replicates of 1-day old eggs (10 eggs/replicate). Then, each replicate was carefully sprayed with serial concentrations of 1.0×10^6 , 1.0×10^7 and 1.0×10^8 spores /ml of *B. bassiana* (AUMC 9896) and *M. anisopliae* (M1, M2, M3 and M4) suspensions. All egg cards were left for 15 min to dryness then placed into glass tubes and every day the cards were checked in each treatment and the numbers of egg hatched were recorded. While the untreated eggs were sprayed with distilled water with Tween-80 using the same technique. After all eggs were hatched in the control, the number of egg hatched and unhatched were recorded in treatments.

3.2. Treated of *P. gossypiella* new hatched larvae:

Pathogenicity tests with *B. bassiana* (AUMC 9896) and *M. anisopliae* (M1, M2, M3 and M4) were carried out at three concentrations $(1.0 \times 10^6, 1.0 \times 10^7 \text{ and } 1.0 \times 10^8 \text{ spores} / \text{ml}$ against newly hatched larvae of *P. gossypiella*. Firstly, three replicates (every replicate was contained twenty newly hatched larvae), those newly hatched larvae in each treatment were carefully sprayed in Petri dishes with the serial concentrations, and then were transferred to a filter paper to dryness and after about 30 min of spraying process newly hatched larvae were transferred to glass tubes (2×7.5 cm) containing untreated diet. Tubes were capped with a clean cotton and incubated at $27\pm2^{\circ}$ C and $70\pm5^{\circ}$ RH; whereas, the untreated larvae were sprayed with water with Tween-80. After three days of treatment, dead and alive larvae were recorded. The same procedure was done with the untreated. All tubes were incubated at the previous concentrations and inspected daily. The counting continues daily until the larvae enter the pupation stage. The larvae mortality, the percentage of pupation, as well as the duration of the larval stage were all counted. The data of larval mortality were corrected using Abbott formula (1925).

3.3. Treated of P. gossypiella pupae:

Three replicates were prepared of one-day-old pupae; each replicate included ten pupae which carefully sprayed directly with the concentrations $(1.0 \times 10^{-6}, 1.0 \times 10^{7} \text{ and } 1.0 \times 10^{8} \text{ spores /ml})$ of different entomopathogenic fungi isolates through laboratory bioassays. Then, at that point, moved to a filter paper to dry then placed into glass jars and incubated till the moth exited then calculated the pupal mortality, adult emergence and malformed adults percentages and pupal duration. Untreated pupae were treated with Water with Tween-80.

6. Statistical Analysis of Data:

LC₅₀ and LC₉₀ and slope values were calculated according to Finny (1971) using Ldp- line software (Bakr, 2005). Biological parameters were analyzed using Costat Statistical Software Program (1990).

RESULTS

A. Competence of isolated fungi against *P. gossypiella* egg stage.

The isolate of *B. bassiana* (AUMC 9896) detected a high significantly mortality percentage of *P. gossypiella* eggs in high concentration compared with the remainder of the isolates and the untreated eggs. As shown in (Table 1), the mortality percentages of *P. gossypiella* eggs were 76.7, 63.3, 53.3, 60.0 and 63.3 % for each isolates of *B. bassiana* (AUMC 9896), *M.anisopliae* M1, M2, M3, and M4, respectively in the highest concentration (1x10⁸ spores/ ml) compared with the untreated egg (10%).

The LC₅₀ and LC₉₀ values were symmetrical to the percentage of hatchability eggs reduced. The lowest LC₅₀value was recorded for the *B. bassiana* (AUMC 9896) and it was 9.82×10^4 spores/ml. While the isolate *M. anisopliae* (M4) was given the highest LC₅₀value 8.24×10^7 spores/ml. The changes in the LC₅₀ values were not effective among the different isolates within the same species of entomopathogenic fungi, *M. anisopliae* Table (1).

Table 1. Egg mortality, LC₅₀ and LC₉₀ values of *P. gossypiella* eggs after treated with three concentrations of five isolates of entomopathogenic fungi.

Isolates		Conc. (spores/ml)	Egg mortality%	LC₅₀ (Spores/ml)	LC∞ (Spores/ml)	Slope ± SE
. bassiana B (AUMC 9896)		1.0 X10 ⁶	50 ^{de}		3.20 × 10 ¹⁰	0.0826 ± 0.157
		1.0 X10 ⁷	56.7 bcd	9.82×10^{4}		
		1.0 X10 ⁸	76.7 °			
M. anisopliae	M1	1.0 X10 ⁶	40 ^f		1.48 × 10 ¹²	0.273 ± 0.159
		1.0 X10 ⁷	43.3 ^{ef}	2.98×10^{7}		
		1.0 X10 ⁸	63.3 ^b			
	M2	1.0 X10 ⁶	50 ^{de}		5.74 × 10 ¹³	0.051 ± 0.122
		1.0 X10 ⁷	53.3 ^{cd}	4.56×10^{7}		
		1.0 X10 ⁸	53.3 ^{cd}			
	МЗ	1.0 X10 ⁶	40 ^f		1.48×10^{14}	0.273 ± 0.159
		1.0 X10 ⁷	53.3 ^{cd}	3.48×10^{7}		
		1.0 X10 ⁸	60 ^{bc}			
	M4	1.0 X10 ⁶	23.3 ^g		5.74 × 10 ¹⁵	0.34 ± 0.164
		1.0 X10 ⁷	36.7 ^f	8.24 × 107		
		1.0 X10 ⁸	63.3 ^b			
Untreated			10 ^h			
р			0.0001 ***			
L.S.D. _{0.05}			7.403			

Numbers in the same column followed by the same latter (s) are not significantly different.

***means highly significant.

B. Competence of isolated fungi against newly hatched larvae of P. gossypiella.

The exhibited results in Table (2) illustrated that the impact of the tested isolates on larval mortality and pupation percentages of *P. gossypiella*. The larval mortality percentages of pink bollworm were significantly affected after treated with fungi isolates. The 1×10^8 spores/ml of *M. anisopliae isolates* M1 and M4 caused the highest larval mortality percentage (86.7%). Also, isolates M2, M1 induced a mortality (83.3, 80.00%) for 1×10^8 and 1×10^7 spores/ml, respectively. Furthermore, isolate M3 showed significant differences between the concentrations of 1×10^7 and 1×10^8 spores/ ml. It could be said that M3 isolate with the concentration of 1×10^8 spores/ ml was more virulent than M3 with the concentration of 1×10^7 spores/ ml by the highest mortalities recorded on newly hatched larvae compared to 3.33% in the untreated larvae. Whereas the fungal isolate *B. bassiana* (AUMC 9896) showed less mortality percent (60.0, 63.3 and 66.7%) for $(1\times10^6, 1\times10^7 \text{ and } 1\times10^8 \text{ Spores/ml})$ concentrations, respectively Table (2). These results gave a high reduction percentage in pupation in those larvae were treated with *M. anisopliae* M1 and M4 with concentration $(1\times10^8 \text{ spores/ml})$ vs. 96.7 % pupation in untreated larvae.

 LC_{50} values of isolates are represented in Table (2). These results cleared the dose-response of newly hatched larvae when treated with different concentrations of *M. anisopliae* and *B. bassiana* (AUMC 9896), the LC_{50} value of *B. bassiana* (AUMC 9896) was appeared to be lowest one 3.60×10^4 spores/ml compared with isolate *M. anisopliae* (M4) was 2.24×10^6 spores/ml.

Isolates		Conc. (spores/ml)	Larval mortality%	pupation%	LC₅₀ (Spores/ml)	LC90 (Spores/ml)	Slope ± SE
. bassianaB (AUMC 9896)		1.0 X10 ⁶	60 ^{efg}	40 bcd		1.47 × 10 ¹⁰	0.087 ± 0.159
		1.0 X10 ⁷	63.3 defg	36.7 ^{cde}	3.60×10^{4}		
		1.0 X10 ⁸	66.7 ^{def}	33.3 def			
M. anisopliae	M1	1.0 X10 ⁶	66.7 ^{def}	33.3 ^{def}	6.12 × 10 ³	3.85 × 10 ⁹	0.337 ± 0.179
		1.0 X10 ⁷	80 ^{abc}	20 ^{gh}			
		1.0 X10 ⁸	86.7ª	13.3 ^h			
	M2	1.0 X10 ⁶	53.3 ^g	46.7 ^b	1.37× 10 ⁸	1.45 × 10 ¹³	0.424 ± 0.167
		1.0 X10 ⁷	60 efg	40 bcd			
		1.0 X10 ⁸	83.3 ^{ab}	16.7 ^h			
	M3	1.0 X10 ⁶	53.3 ^g	46.7 ^b	4.55 × 10⁵	2.76 × 10 ¹⁰	0.268 ± 0.163
		1.0 X10 ⁷	70 ^{cde}	30 ^{ef}			
		1.0 X10 ⁸	73.3 bcd	26.7 ^{fg}			
	M4	1.0 X10 ⁶	56.7 ^{fg}	43.3 ^{bc}	2.24 × 10 ⁶	6.24 × 10 ⁸	0.524 ± 0.170
		1.0 X10 ⁷	56.7 ^{fg}	43.3 ^{bc}			
		1.0 X10 ⁸	86.7ª	13.3 ^h			
Untreated		3.33 ^h	96.7ª				
Р		0.0001 ***	0.0001 ***				
L.S.D.0.05		9.804	8.305				

Table 2. Larval mortality, Pupation percentage, LC₅₀ and LC₉₀ values of *P. gossypiella* newly hatched larvae after treated with three concentrations of five isolates of entomopathogenic fungi.

Numbers in the same column followed by the same latter (s) are not significantly different. ***means highly significant.



Fig 1. The larval durations after treated of newly hatched larvae with the different concentrations of fungi isolates compared to the untreated.

The data in Fig. (1) Showed that, larval duration increased in all the isolates compared to the untreated. These finding observed that the concentration 1×10^8 spores/ ml give the longest larval duration in all treatments, and the longest of them ever was 22 days were recorded for the isolate *M. anisopliae* (M1), compared to 15.7 days in the untreated.

C- Competence of isolated fungi against a pupal stage of *P. gossypiella*.

The demonstrated results in showed the virulence of certain isolates against the *P. gossypiella* pupal stage. Pupal mortality, adult emergence, adult malformation percentage and pupal duration after treated the pupal of pink bollworm with the fungal isolates were counted.

As shown in Table (3), the high pupal mortality percentage estimated by (80.00%) resulted from *B. bassiana* (AUMC 9896) concentration (1×10^8 Spores/ml), compared to the untreated pupae (0.0%). While in case of *M. anisopliae* M4, the lowest concentration (1×10^6 Spores/ml) was recorded (46.7%) pupal mortality. Statistical analysis showed that there are significant differences in adult emergence percentage between all concentrations in every isolate Table (3). Also, with the comparison between those isolates for every

concentration, it is found that, *B. bassiana* (AUMC 9896) with concentration $(1 \times 10^7 \text{ Spores/ml})$ caused highly significant decrease in adult emergence percent (6.7%), whereas the untreated was 100%. The data in (Table 3) indicated that *M. anisopliae* spore suspensions caused high increased in malformation of adult emergence than *B. bassiana* (AUMC 9896), this malformation recorded by 23.3% compared to the untreated pupae (0.0%). *B. bassiana* (AUMC 9896) showed a similar effect of *M. anisopliae* isolates on pupal duration, all isolates prolonged pupal duration, and data indicated that there were insignificant Table (3)

Isolates		Concentrations (spores/ml)	Pupal mortality%	Adult emergence%	Adult malformation%	Pupal Duration (days)
B. bassiana (AUMC 9896)		1.0 X10 ⁶	66.7 ^{bc} *	20 ^{bc}	13.3 ^{ab}	11
		1.0 X10 ⁷	73.3 ^{ab}	6.7 ^c	20 ^{ab}	11
		1.0 X10 ⁸	80 ª	13.3 ^{bc}	6.7 ^{ab}	10.7
	M1	1.0 X10 ⁶	53.3 ^{de}	26.7 ^{bc}	20 ^{ab}	12
		1.0 X10 ⁷	53.3 ^{de}	23.3 ^{bc}	23.3 ª	12
		1.0 X10 ⁸	60 ^{cd}	23.3 ^{bc}	16.7 ^{ab}	11.7
	M2	1.0 X10 ⁶	66.7 ^{bc}	13.3 ^{bc}	20 ^{ab}	11.7
M. anisopliae		1.0 X10 ⁷	70 ^{abc}	13.3 ^{bc}	16.7 ^{ab}	10.7
		1.0 X10 ⁸	73.3 ^{ab}	16.7 ^{bc}	10 ^{ab}	11.3
	М3	1.0 X10 ⁶	50 ^{de}	26.7 ^{bc}	23.3 ª	11
		1.0 X10 ⁷	53.3 ^{de}	23.3 ^{bc}	23.3 ^a	11
		1.0 X10 ⁸	66.7 ^{bc}	20 bc	13.3 ^{ab}	11.3
	M4	1.0 X10 ⁶	46.6 ^e	36.7 ^b	16.7 ^{ab}	11.7
		1.0 X10 ⁷	60 ^{cd}	23.3 ^{bc}	16.7 ^{ab}	11.3
		1.0 X10 ⁸	70 ^{abc}	16.7 ^{bc}	13.3 ^{ab}	11.3
Untreated			0 f	100 a	0	9.33
P			0.0001	0.0001	0.0207	0.3319
L.S.D.0.05			9.251	10.05	1.697	ris

Table 3. Effects of five isolates of entomopathogenic fungi on pupal stage of P. Gossypiella.

Numbers in the same column followed by the same latter (s) are not significantly different. Significant at * (0.05), *** (0.001) ns = not significant.

DISCUSSION

The entomopathogenic fungi *B.bassiana* and *M. anisopliae* Sorokin from the order Hypocreales (Ascomycota) are natural enemies of a wide scope of insects and arachnids as reviewed by (Rehner, 2005). It is noticed that *B. bassiana* was the most effective isolate than *T. harzianum* and *P. violacea* to control the most destructive cotton pest, the pink bollworm (Abd-El Azeem *et al.*, 2020).

The results showed increasing in mortality rates with increasing in the concentration of all fungal isolates on eggs, larva, and pupa stages of *P. gossypiella*. The increase in mortality percentage may be due to the quantity of conidia received by the host insect. As to the above illustrated results from treated eggs, newly hatched larvae and pupa of *P. gossypiella* with three concentrations of *B. bassiana* and *M. anisopliae* proved that *B. bassiana* more effective mortality percentage on the eggs and pupal stages than all *M. anisopliae* isolates, whereas on larval stages the *M. anisopliae* isolate (M1) caused high mortality than the rest of the isolates, this results in agreement with (de Souza *et al.*, 2020), when study the sub-lethal concentrations of different fungal isolates on *Helicoverpa armigera* they found that the mortality percentage of *2*nd caterpillars due to the fungi isolates of *B. bassiana* and *M. anisopliae* were various values. The isolates of *M. anisopliae* (IBCB 425 and ESALQ 860) and *B. bassiana* (IBCB 1363 and IBCB 36) with the highest means and the use of sub-lethal concentrations of the fungi isolates of *B. bassiana* and *M. anisopliae* was caused a negative effect on the biology of (*H. armigera*. Also, Farooq *et al.*, 2020), found that when at different exposure intervals, concentrations of *M. anisopliae* and *B. bassiana* gave high mortality percentage in the treated 2nd instar larvae of *P. gossypiella*. (Hegab and Zaki, 2012), showed that the isolate of *B. bassiana* cause reduced larval mortality against *Earias insulana*.

(El-Akad *et al.*, 2016), studied that, the fungal isolate, *B. bassiana* proved to be the lowest LC_{50} value than *M. anisopliae* on all stages of *P. gossypiella*. (Aly, 2002), studied the effect of various concentrations of *B. bassiana* on different stages of *Agrotis ipsilon* and found that the LC_{50} values were 2.01×10³ spores/ml for eggs and 4.74×10³ spores/ml for larvae. The current results agree partially with the results obtained by (Abd-El Azeem

et al., 2020), who showed that the *B. bassiana* was the most effective isolate than *T. harzianum* and *P. violacea* to control the pink bollworm. The hatchability percentages were decreased by *B. bassiana* spore suspension and the filtrate (70.06, 73.91%), respectively. Our results give a decrease in the pupation percentage when treated with *B. bassiana* and *M. anisopliae*, and decrease in adult emergence percent and increased adult malformation compared to the untreated, these results were matched with (Farooq *et al.*, 2020) who mentioned that *P. gossypiella* pupation and the adult emergence was significantly affected by *M. anisopliae* and *B. bassiana* concentrations. The results of (El-Akad *et al.*, 2016), are in line with our results, which reported that when treated a newly hatched larvae of *P. gossypilla* by *M. anisopliae* and *B. bassiana*, the adult malformation percentage was (16.11, 20.00%), respectively, while in untreated there was no malformation percentage. Also, our results showed increases in larval duration when treated with LC₅₀ of (*B. bassiana* and *M. anisopliae*). On other hand, (Dannon *et al.*, 2021), reported that *B. bassiana* as an entomopathogenic fungal species has been suggested to be used for controlling *H.armigera* as a biopesticide.

CONCLUSION

In summary, all immature stages of *P. gossypilla* were susceptible to all tested isolates of *B. bassiana* and *M. anisopliae*. However, significant differences found among these isolates with different fungal concentrations. These fungal isolates would be useful for the development of biopesticide and in devising a proper integrated pest management strategy against this pest.

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القدرة الامراضية لفطريات ممرضة للحشرات على الاطوار غير الكاملة لدودة اللوز القرنفلية

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