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Effect of entomopathogenic fungi on *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and *Earias insulana* (Lepidoptera: Noctuidae) and their predators

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Abstract:

Entomopathogenic fungi infect and kill insect pests in the green house and used as agents for biological control. The aim of this work is to study the toxicity of serial concentrations of fungal spore suspension of both *Metarhizium anisopliae* and *Paceilomyces lilicanus* against the newly hatched larvae of pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) and spiny bollworm *Earias insulana* (Boisduval (Lepidoptera: Noctuidae) in addition to the effect of the two fungi on green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) eggs and larvae and on ladybird beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) eggs. Results showed that the toxicity of *P. lilicanus* was higher on *P. gossypiella* treatment; wheras the toxicity of *M. anisopliae* was higher in case of *E. insulana* treatment. On the other hand, the effect of the two fungi on *C. carnea* eggs was obvious effective, whereas, *C. septempunctata* was not affected after the same egg's treatment.

Introduction

Cotton bollworms included the pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) and spiny bollworm (SBW) *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae) are considered the key insect pests infested each of squares, flowers as well as the green bolls causing destruction of the cotton plants resulting increasing qualities and quantities of the cotton yield. Using bioinsecticides proved to be harmless to predators and parasitoids in cotton field (Tillman and Mulrooney, 2000) and in laboratory conditions, it was harmless to *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) eggs and pupae stages irrespective of concentrations or method of treatments (Mandour, 2009).

Family Chrysopidae have high predatory capacity to different ecosystems (Costa *et al*. 2003). Importance of *C. carnea* as biological control agent for cotton pests; whereas insecticides influence different species of the natural enemy, it is important to evaluate the effects of the tested fungicides as biological control agents.

Coccinellidae is a widespread family of small beetles (Seago *et al*., 2011). The ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) mainly free-living predatory species. Therefore, it is considered to be useful insects, because many species prey on herbivorous homopterans (Liu and Stansly, 1996). Entomopathogenic fungi were successfully applied worldwide as biological control agents since 1880's (Krassilstschik, 1888). They were used for insect pests control programs (Herlinda *et al*., 2010 and for instance *Metarhizium anisopliae* and *Paceilomyces lilicanus* as well as the entomopathogenic bacteria, *Bacillus thuringiensis* were tested against the insect pests (the diamondback moth, the cabbage worm and beet armyworm) in the green house and field (Sabbour and Sahab, 2005). *M. anisopliae* is considered one of the most common entomopathogenic fungal species used as biological control agent against insect pests (Barra *et al*., 2013). In view of the importance of *C. carnea* and *C. septempunctata* as the biological control agents for cotton pests compared to insecticides effects on those species of natural enemy. This work aimed to evaluate the efficacy of two entomopathogenic fungi on the predators; *C. carnea* and *C. septempunctata* and their prey included *P. gossypiella* and *E. insulana*.

Materials and methods

1.Insect used:

First instar larvae of pink bollworm *P. gossypiella* was reared for several generations on modified artificial diet as described by Abd El-Hafez *et al*. (1982) under laboratory conditions at 27+1°C and 75+5% R.H. and spiny bollworm *E. insulana* was reared in Cotton Bollworm Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki. Giza, Egypt, on artificial diet described by (Amer, 2015).

Eggs of *C. carnea* and *C. septempunctata* were obtained from predators and parasitoids unit, Plant Protection Research Institute.

2.Fungus culture:

Isolates of *M. anisopliae* (Metschnikoff) Sorokin and *P. lilicanus* (Thom) Samson, were obtained from Assiut University, Mycological center Faculty of Science. The isolates were cultured on Sabouraud Dextrose Yeast Agar (SDYA) medium g/l (Sabouraud, 1892) containing 40 g glucose, 20 g peptone, 20 g agar, 2 gm Yeast extract and 1000ml of distilled water in flasks autoclaved at 21ºC for 15-20 min.

3.Inoclum preparations:

Fungal cultures were grown on (SDYA) medium g/l and incubated at 25±2ºC in darkness for 14 days. Conidial suspensions were prepared by scraping cultures with a sterile objective glass and transferred to 10 ml of sterile water containing 0.05% Tween 80 in a laminar flow chamber. The conidia were harvested by scraping the surface of the culture with inoculation needle. The mixture was stirred for 10 minute the hyphal was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using Haemocytometer. Serial dilutions were prepared in distilled water containing 0.1% tween- 80 and preserved at 5ºC until used. In vitro entomopathogenicity tests were applied to evaluate efficacy of the fungal isolates against the newly hatched larvae of *P. gossypiella* and *E. insulana*. A volume of 1 ml of the adjustable concentrations 10^8, $5x10^7$, $2.5x10^6$, $1.25x10^5$ and $0.625x10^4$ spores/ml viable conidia was directly applied to the larvae by feeding. Three replicates per treatment with per replicate were made.

4. Bioassay of treated *Pectinophora gossypiella* and *Earias insulana* larvae:

Response of the newly hatched larvae of *P. gossypiella* and *E. insulana* was studied. Serial concentrations of 10^8, $5x10^7$, $2.5x10^6$, $1.25x10^5$ and $0.625x10^4$ spores/ml were prepared. Thirty newly hatched larvae were transferred individually to the surface

of the treated diet, each concentration replicated three times, after 24 hours transferred the alive larvae on untreated diet and kept in glass tubes (2 x 7.5 cm) caped with cotton stopper. The same procedure was done with untreated diet exposed to newly hatched larvae and used as control. All tubes were incubated at 26±2ºc and 70-85±5% RH and inspected daily.

5. Bioassay of treated *Chrysoperla carnea* eggs and larvae:

The eggs were sprayed directly with 10^8 of *P. lilicanus* and *M. anisopliae* through laboratory bioassays then placed into glass tubes and observed daily for the number of hatched larvae in each treatment. The obtained larvae were maintained in the same tubes and fed with *P. gossypiella* eggs. Control replicates were treated by water only. thirty healthy starved larvae of the 2nd instar larvae of *C. carnea* were kept individually in glass tubes (2x7 cm) in each replicate and fed on treated *P. gossypiella* eggs by spraying the eggs cards with *P. lilicanus* and *M. anisopliae* solutions and dried them then complete nutrition on *P. gossypiella* eggs free from insecticidal treatment till formation of cocoons. Control replicates were treated by water only then incubated all tubes at 26 ± 1°C and 70 ±5% RH and evaluated daily until emergence of the insects. Mortality was recorded at intervals after 2, 3, 5,7,10 days after larval feeding.

6. Bioassay of treated *Coccinella septempunctata* eggs:

The eggs were sprayed directly with 10^8 of *P. lilicanus* and *M. anisopliae* through laboratory bioassays then placed into glass jars and observed daily for the number of hatched larvae in each treatment.

7.Data analysis:

Corrected cumulative mortalities were reported for both isolates from treatment and corrected according to Abbott´s (1925) as follows:

Corrected percent mortality = [(T – C)/(100-C)] x 100

The median lethal concentration (LC_{50}) values were determined using Finney (1952).

Results and discussion

LC_{50} values and % expected mortality after treated the newly hatched larvae of *P. gossypiella* with different concentrations of *P. lilicanus* after 2, 3, 5, 7 and 10 days of treatments are shown in Table (1). The corresponding LC_{50} values were $5.60x10^7$, $4.87x10^6$, $4.85x10^6$, $3.76x10^6$ and $2.14x10^5$spores/ml.

Expected mortality percentages of newly hatched larvae of *E. insulana* after treatment with different concentrations of *P. lilicanus* shown in Table (2). The LC_{50} values were 6.40 $x10^7$, $5.18x10^7$, 5.02 $x10^7$, 4.05 $x10^6$ and 2.67 $x10^6$ spores/ml after 2, 3, 5, 7 and 10 days of treatment respectively. While after twelve days of treatment, all the treated larvae dead in contrary with the untreated larvae.

Response of newly hatched larvae of *P. gossypiella* after treatment with different concentrations of *M. anisopliae* is shown in Table (3). LC_{50} values were 3.92 $x10^6$, 2.91 $x10^6$ and 1.82 $x10^5$ after 2, 3, 5 days but after 7 and 10 days of treatment LC_{50} was $1.70x10^5$ spores/ml.

Response of newly hatched larvae of *E. insulana* after treatment with different concentrations of *M. anisopliae* is shown in Table (4). LC_{50} values were 2.68 $x10^7$ after 2 days and 2.63 $x10^7$ after 3 and 5 days and 1.56 $x10^5$ after 7 days of treatment, respectively.

Table (1): Toxicity of *Paceilomyces lilicanus* against newly hatched larvae of *Pectinophora gossypiella* under laboratory conditions.

Conc.	%Expected Mortality values				
	After 2 days	After 3 days	After 5 days	After 7 days	After 10 days
10^8	60.41	64.20	84.07	88.90	96.82
$5X10^7$	50.82	52.94	78.27	83.48	93.67
$2.5X10^6$	41.11	41.65	71.43	76.64	88.54
$1.25X10^5$	32.20	32.20	63.75	68.51	81.00
Slope value	0.87±0.31	1.00±0.31	0.73±0.32	0.84±0.34	1.10±0.44
LC_{50}	$5.60 x10^7$	$4.87 x10^6$	$4.85 x10^6$	$3.76 x10^6$	$2.14 x10^5$

Table (2): Toxicity of *Paceilomyces lilicanus* against newly hatched larvae of *Earias insulana* under laboratory conditions.

Conc.	% Expected Mortality values				
	After 2 days	After 3 days	After 5 days	After 7 days	After 10 days
10^8	62.20	66.10	68.96	94.62	98.03
$5X10^7$	46.80	51.71	55.81	89.82	95.28
$2.5X10^6$	32.16	36.60	42.24	82.50	90.11
$1.25X10^5$	20.41	23.94	29.94	72.56	81.72
$0.625X10^4$	12.62	14.95	20.14	60.57	69.99
Slope value	1.39±0.25	1.37±0.24	1.03±0.23	1.14±0.26	1.29±0.32
LC_{50}	$6.40 x10^7$	$5.18 x10^7$	$5.02 x10^7$	$4.05 x10^6$	$2.67 x10^6$

Table (3): Toxicity of *Metarhizium anisopliae* against newly hatched larvae of *Pectinophora gossypiella* under laboratory conditions.

Conc.	%Expected Mortality values				
	After 2 days	After 3 days	After 5 days	After 7 days	After 10 days
10^8	65.36	65.95	89.09	93.21	93.21
$5X10^7$	54.09	60.00	76.74	81.79	81.79
$2.5X10^6$	42.47	47.53	59.07	62.69	62.69
$1.25X10^5$	31.48	33.42	39.29	39.74	39.74
Slope value	0.97±0.35	0.03±0.35	1.66±0.39	194±0.42	194±0.42
LC_{50}	$3.92 x10^6$	$2.91 x10^6$	$1.82 x10^5$	$1.70 x10^5$	$1.70 x10^5$

Table (4): Toxicity of *Metarhizium anisopliae* against newly hatched larvae of *Earias insulana* under laboratory conditions.

Conc.	%Expected Mortality values			
	After 2 days	After 3 days	After 5 days	After 7 days
10^8	96.46	97.05	97.05	99.48
$5X10^7$	93.40	93.78	93.78	98.37
$2.5X10^6$	55.57	88.22	88.22	95.71
$1.25X10^5$	25.16	79.86	79.86	90.31
$0.625X10^4$	8.14	68.79	68.79	81.13
Slope value	2.80±0.32	1.18±0.30	1.18±0.30	1.41±0.42
LC_{50}	$2.68 x10^7$	$2.63 x10^7$	$2.63 x10^7$	$1.56 x10^5$

In this field of study, El- Massry *et al*. (2016) found that *E. insulana* exhibited higher effective to *Trichoderma harzianum* treatment than *P. gossypiella*. There was a highly significant difference between the all tested concentrations comparing with the untreated one in case of the two larvae species. Also, Hegab and Zaki (2012) evaluate the effect of *B. bassiana* against *E. insulana*. The accumulated mortalities of both insects' larvae, after six days of treatment, were represented as the acute toxicity. Also, results cleared that the effect of Biover® fungi attained decreasing in all biological aspects.

Other authors observed the same finding in case of Tables (1, 2 and 3) that the treated larvae dead after 10 days of treatment as found by, Venugopal *et al*. (2017). They found that larval mortality observed after 3-7 -10 days of fungal treatment. With entomophthoralean fungi, unicellular yeast-like cells with chitinous walls (hyphal bodies) spread throughout the insect obtaining nutrients, leading to the death of the host by physiological starvation about 3-7 days after infection (Ritu *et al*., 2012) found the same different concentration of *B. basiansa* (62.98),(60.58),(59.67),(58.32).

Effect of the tested *P. lilicanus* and *M. anisopliae* using high concentrations (108 spores/ml) against *C. carnea* and *C. septempunctata* eggs presented in Table (5). After treating *C. carnea* eggs, the hatchibility percentage of eggs recorded 73.30% when treated with *P. lilicanus* with 21.43% reduction, while it was 86.7% in case of *M. anisopliae* treatment with 7.14% reduction, compared with 93.3% in control. While, after treating *C. septempunctata* eggs the hatchability percentage was 100% in case of *P. lilicanus* and *M. anisopliae* treatment.

The corrected mortality percentages of *C. carnea* 2nd instar larvae were recorded after 2, 3, 5 and 7 days of treatment with *P. lilicanus* and *M. anisopliae* are shown in Table (6). The results showed that the high concentration of the *P. lilicanus* and *M. anisopliae* affected on larvae predator significantly at 2, 3, 5 and 7 days respectively, the corresponding mortality percentages were 15, 15, 20 and 30%. *M. anisopliae* was more toxic to second instar larvae than *P. lilicanus*.

Table (5): Effect of *Metarhizium anisopliae* and *Paceilomyces lilicanus* on *Chrysoperla carnea* and *Coccinella septempunctata* eggs.

Tested fungi	*C. carnea*		*C. septempunctata*	
	%egg	%reduction	%egg	%reduction
P. lilicanus	73.30	21.43	100	0.00
M. anisopliae	86.70	7.14	100	0.00
Control	93.30	0.00	100	0.00

Table (6): Corrected mortality percentages of 2nd instar larvae of *Chrysoperla carnea* caused by *Metarhizium anisopliae* and *Paceilomyces lilicanus*.

Insecticides	% corrected Mortality				Larval Duration	%pupation	Pupal Duration	% adult emergence	
	2 days	3 days	5 days	7 days				♀	♂
P. lilicanus	15	15	20	30	20.83	66.67	9.29	0.00	50
M. anisopliae	25	55	80	85	17.83	92.86	8.71	30.7	61.5
Control	0.00	0.00	0.00	0.00	13.33	100	8.33	60	40

The immature stages (Larval and pupal duration) was recorded after treating the 2nd instar larvae of *C. carnea* with the two entomopathogenic fungi, results showed that *P. lilicanus* prolonged larval duration than control. Moreover, slight increase in pupal duration after treatment at 9.29 days compared with 8.33 days in control. So, the emerged adults from this treatment resulted malformed adults.

Figure (1) shows the malformed adults resulted from 2nd instar larvae of *C. carnea* treated with *P. lilicanus* compared with the normal one.

In this respect, Ayubi *et al*. (2013) studied the lethal effects of four compounds, imidacloprid, lufenuron, thiametoxam and thiodicarb, on the eggs and 1st instar larvae of *C. carnea* in laboratory conditions. Dipping bioassay tests were used for eggs and the residual contact method for larvae. Thiodicarb had no effect on eggs. On larvae, thiametoxam was the most toxic and lufenuron proved to be the least toxic. Also, Moustafa (2016) found that biocides were harmless on *c. carnea* eggs than conventional insecticides. Recently, Fernando (2018) the use of fungal entomopathogens as environmentally friendly alternatives for management of insect pests. Unfortunately, their effectiveness continues to be limited by their susceptibility to ultraviolet (UV) light and low moisture. In addition, this review presents several areas that should receive focused attention to increase the probability of success for making fungal entomopathogens an effective alternative to chemical control.

1 2 3 4

Figure (1): Normal and malformed adults after *Chrysoperla carnea* larvae treatment to insecticides

1: Normal adult **2,3,4:** Malformed adults after treatment with ***Paceilomyces lilicanus.***

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