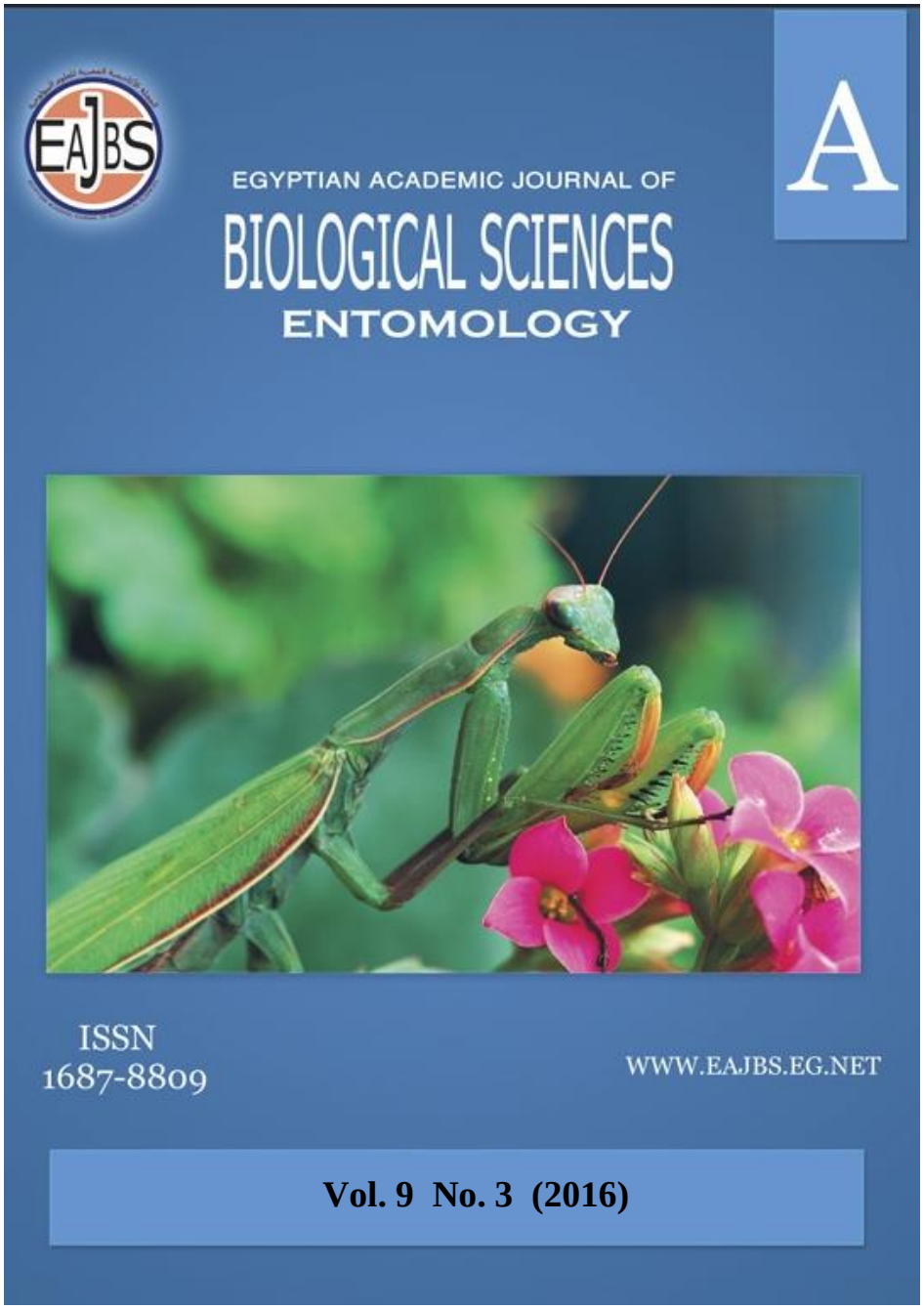

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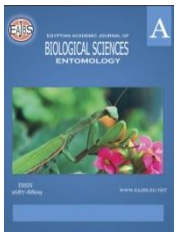

Effect of *Beauveria bassiana* and *Metarhizium anisoplae* on some biological aspect of *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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ABSTRACT

This experiment were carried out to study the effect of treatment of newly hatched (neonate) larvae and pupae of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) with different entomopathogenic fungi; Biover (*Beauveria bassiana*) and Bioranza (*Metarhizium anisoplae*) was investigated under laboratory conditions of 27 ± 1º C and 65 % ± 10 % R.H.

The results show that *P. Gossypiella* was susceptible to both fungi. LC_{50} values obtained were1.42 and 0.98 g\lfor *P. Gossypiella* larvae treated with different concentrations of *B.bassiana* and *M.anisopilae*, respectively.

The biological aspects of *P. gossypiella* were affected when treating the newly hatched larvae with the LC_{50}'s of both fungi. Results generally revealed changes in the different biological aspects (larval duration, pupation percentage, pupal duration and the percentage of adult emergence).

Determine the effect of *B. bassiana* and *M. anisopliae* on the prey consumption rate of *Oriusa lbidipennis* to pink bollworm eggs.

INTRODUCTION

In Egypt, many economic pests infest cotton through the season. Lepidopterous insects are the most destructive pests of the cotton plant namely the pink bollworm, *Pectinophora gossypiella*. The insecticides used in agricultural pest control may cause several problems, such as the selection of resistant lineages (Metcalf 1980). Entomopathogenic fungi, specifically the anamorphic taxa *Beauveria bassiana* and *Metarhizium anisopliae*, Hypocreales (Ascomycota), are among the natural enemies of pests in agroeco systems and the fungi are candidates (Meyling and Eilenberg 2007).

MATERIAL AND METHODS

The culture of *P. gossypiella* was maintained in the Plant Protection Research Institute, Cairo. Larvae of the pink bollworm were reared under laboratory conditions of 27±2ºC and 70±5% RH. on semi- artificial kidney bean diet as described by (Rashad *et al.* 1993a).

***B. bassiana*a** (**Biover)** and ***M. anisoplae*a (Bioranza)** are commercial product formulation. Locally produced by the Insect Pathogen Unit, Plant Protection Research Institute, Ministry Of Agriculture, Egypt.The international unit was 32,000 viable spores per mg. The active ingredient was 10% W.P. and the recommended application rate was 200 gm. /100 liter water/Faddan.

Pathogenecity tests of *B. Bassiana*a and *M. anisoplae*a against the pink bollworm, *P. gossypiella*:

Pathogenecity tests with *B. bassiana* and *M.anisoplae* were carried out at five concentrations (0.5, 1, 2, 4 and 6gm/ 1litter) against the newly hatched larvae of *P. gossypiella* by add 5ml from each concentration on surface of diet in petri-dishes.

Three replicates for each concentration were made; each containing fifteen of the newly hatched larvae. The mortality was recorded 48h post treatment. Surviving larvae were separated in small tubes and the data were recorded daily till pupation.

The larval mortality percentages were recorded after 48 hours to 12 days and corrected against those of the control by Abbott's formula (Abbott, 1925) as follows:

$$\text{Corrected mortality} = \frac{\text{Observed mortality\% - Control mortality\%}}{\text{100 - Control mortality\%}} \times 100$$

The control was sprayed with distilled water only.

Calculation of lethal concentration values:

Five concentrations of the entomopathogenic fungus *B. bassiana* and *M. anisoplae* were prepared. The lethal concentration values as log/probit regression lines were calculated according to the method described by (Finney, 1971). The computer program Sigma Plots for Windows (version 2) and the slope of the probit line was also indicated.

Effect of the different entomopathogenic fungi treatments on newly hatched larvae of *P. gossypiella:*

The newly hatched larvae of *P. gossypiella* we treated by the lethal concentration values of different entomopathogenic fungi.

Newly hatched larvae were fed on media sprayed by the lethal concentration of *B. bassiana* and *M. anisoplae* and left until dried in petri-dishes. Three replicates for each treatment were made; each replicate contained five newly hatched larvae. The mortality was recorded 48h post treatment.

Surviving larvae were separated in small tubes till pupation and the data were recorded daily till adult emergence.

Effect of Biover (*Beauveriabassiana*) and Bioranza (*Metarhiziumanisoplae*) on the prey consumption of *Oriusalbidipennis* to pink bollworm eggs:

B. bassiana and *M. anisoplae* were carried out at the lethal concentration values against the eggs cards of *P. gossypiella*by spay each the lethal concentration value on surface of the eggs cards. Three replicates for each one were made; each containing ten eggs on each card and one of nymph of *O. albidipennis*. The data was recorded daily until adult eclosion or mortality. The control contain card of eggs sprayed with distilled water only and *O. albidipennis*.

Statistical analysis:

All data obtained from above experiments were analyzed statistically using complete randomized blocks design. Student t-test as statistical analysis of the obtained data was used by COSTAT program.

RESULTS AND DISCUSSION

Biological effects of tested entomopathogens against *P. gossypiella*:

Susceptibility of *P. gossypiella* to *B. bassiana*:

The mortality percentage of the newly hatched larvae of *P. gossypiella* treated with the different concentration of *B. Bassiana* increased gradually with increasing the time elapsed after treatment **(Table1**), the mortality percentage ranged from 35.71 and 75.00% after 48h and twelve days post treatment, respectively. The total mortality percentage recorded 35.71, 42.86, 53.57, 64.29 and 75.00% at the concentrations (0.5, 1, 2, 4 and 6gm/ 1litter) respectively.

Table 1: Effect of different concentrations of *B. bassiana* on mortalities percentage of *P. gossypiella*:

Conc.g/l	Days of treatment					Total mortality%
	2nd	4th	6th	9th	12th	
0.5	14.28	24.99	28.57	35.71	35.71	**35.71**
1.0	21.43	28.57	35.71	42.86	42.86	**42.86**
2.0	24.99	35.71	46.43	49.99	53.57	**53.57**
4.0	28.57	39.28	46.43	57.14	64.29	**64.29**
6.0	35.71	53.57	64.29	71.42	75.00	**75.00**

The entomopathogenic fungi were able to penetrate directly the outer cuticle of the insects, which contains chitin fibrils within a protein matrix together with lipids, waxes and small quantities of phenols and pigments. Generally, Deuteromycetes fungi produce conidia (spores) which are distributed randomly by wind and water. Fungal entomopathogens such as *Lecanicillium* (formerly *Verlicillium*) spp., *B. bassiana, M. anispoliae*, *Isaria farinose* (formerly *Paecilomyces farinosus)* and *Isaria fumosorosea (formerly Paecilomyces fumosoroseus)* play on important role in the regulation of insect populations (Zimmermann, 2008 and Gurulingappa *et al.* 2011). The fungal pathogen penetrated the insect cuticle, spread throughout the insect host, developing a network of hyphae structures. Boucias *et al*. (1993) studied conidia of most taxa of entomopathogenic fungi, once demonstrated that in contact to insect cuticle, it may germinate and produce penetration structures from which penetrative hyphae are formed.

The results obtained from exposing new hatched larvae of *P. gossypiella* to different concentrations of *B. bassiana*, clearly indicated that there was an increase in the mortality percentage with an increasing in the percentage of spore concentrations, this is in agreement with Aly (2002) who detected the laboratory bioassay on the effect of the fungus, *B. bassiana* with different concentrations on various stages of *Agrotis ipsilon*. The LC_{50} values for eggs were 2.01×10^3 spores/ml and for larvae, it was 4.74×10^3 spores/ml.

Susceptibility of *P. Gossypiella* to *M. anisopliae*:

The mortalities percent of the newly hatched larvae of *P. gossypiella* treated with the different concentration of *M. Anisopliae* increased gradually with increasing the time elapsed after treatment (Table 2).

For studying the toxicity of *M. anisopliae*, an aqueous suspension was used. Mortalities were concentration dependent.

Data in table 2 show, the mortality percentage ranged from 42.86 and 82.14% after 48h and twelve days post treatment, respectively. The total mortality percentage recorded42.86, 46.43, 60.71, 67.71 and 82.14% at the concentrations (0.5, 1, 2, 4 and 6gm/ 1litter) respectively.

Table 2: Effect of different concentrations of *M. anisopliae* on the mortalities percentage of *P. gossypiella*:

Conc. g/l	Days of treatment					Total mortality%
	2nd	4th	6th	9th	12th	
0.5	17.85	28.57	35.71	39.28	42.86	**42.86**
1.0	28.57	32.14	39.28	42.86	46.43	**46.43**
2.0	24.99	39.28	49.99	57.14	60.71	**60.71**
4.0	32.14	42.86	53.57	60.71	67.71	**67.71**
6.0	42.86	60.71	67.86	75.00	82.14	**82.14**

The present studies show that increasing in the applied concentrations to newly hatched larvae of *P. gossypiella* gave an increase in the mortality percentage and these results are in agreement with Filho *et al.* (2002) evaluated *M. anisopliae* and *B. bassiana* isolates and screened for pathogenicity against *Alabama argillaceae* in Brazil at 27 ± 2°C, RH 70 ± 5% and a photophase of 12 hours. They found that the isolate 645 of *B. bassiana* caused the highest mortality at the highest concentration, followed by isolates 634, 604, and IPA 198. The lowest lethal time for *B. bassiana* and *M. anisopliae*, was achieved by the isolates 483 (4.1 days) and 1189 (2.0 days), respectively.

Determination of LC_{50} value:

Five concentrations of the entomopathogenic fungus were prepared (*B. bassiana* and *M. anisopliae*) to calculated lethal concentration values.

B. bassiana proved to be effective on larvae of *P. gossypiella* as depicted by the calculated LC values. The LC_{50} values for the newly hatched larvae were 1.42g\l [Table3]. The slope values were 0.93 for newly hatched larvae, proving the homogenecity of the tested individuals, [Fig. 1]

Table 3:LC_{50} value of microbial control against *P. gossypiella*:

Microbial control:	LC_{50}	95% (fiducial limit)		Slop
		Lower	Upper	
Beauveria bassiana	1.42	1.02	1.88	0.93
Metarhizium anisopliae	0.98	0.65	1.31	0.95

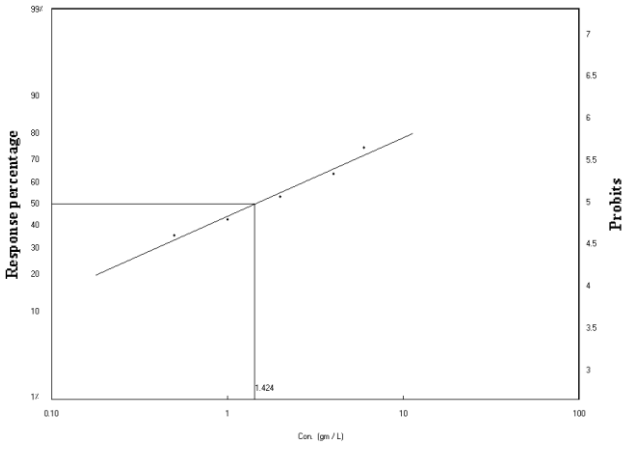

Fig. 1: Toxicity regression line of *B. bassiana* on *P. gossypiella*:

Our result agree with Aly (2002) stated that treatment the 2nd instar larvae of *S. exigua* with theLC_{50} and LC_{90} of *B. bassiana* gave some changes in the stages of the test insect and caused a reduction in total protein.

M. anisopliae proved to be effective on larvae of *P. gossypiella* as depicted by the calculated LC values. The LC_{50} values for the new hatched larvae were 0.98g\l [Table 3]. The slope values were 0.95 for new hatched larvae, proving the homogenecity of the tested individuals, [Fig. 2].

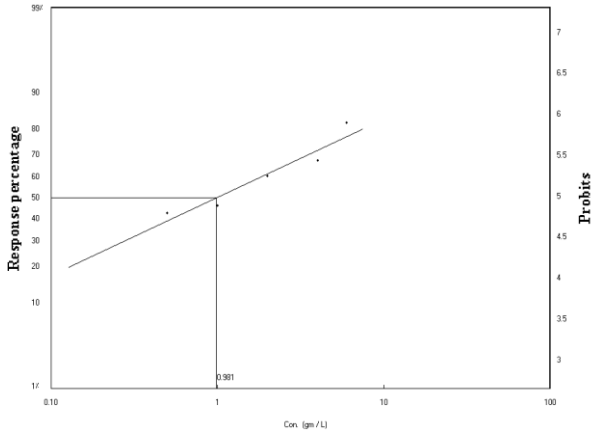

Fig. 2: Toxicity regression line of *M. anisopliae* on *P. gossypiella*:

The result of Chienyan *et al*. (1998) are harmony with our result who recorded that the LC_{50} of *M. anisopliae* var. *anisopliae* extract against *Spodopteraexigua* and obtained in case of 3rd instars; 1.41 x 10^5 ppm, 7.07 x 10^4 ppm, and 5.18 x 10^4 ppm, after 1 days', and 3 days' of infection, respectively.

Effect of the different entomopathogenic fungi treatments on newly hatched larvae of *P. gossypiella:*

Treatment of both newly hatched larvae of *P. gossypiella* by used of the lethal concentration value of different entomopathogenic fungi.

Media were sprayed byLC_{50} value of *B. bassiana* and *M. anisopliae*.

Effect of LC_{50} of different entomopathogenic fungi on larval duration of *P. gossypiella*:

Application of LC_{50} of *B. bassiana* and *M. anisopliae* resulted in a mean larval duration 14.04 and 15.07 days, respectively for *P. Gossypiella* pretreated as newly hatched larvae compared to 12.63days forthe control [Table4].

Effect of LC_{50} of different entomopathogenic fungi on pupal duration of *P. gossypiella*:

The mean pupal duration of *P. gossypilla* pretreated of newly hatched larvae with *B. bassiana*and *M. anisopliae* were 9.22 and 9.36 days respectively, treated with LC_{50}, compared 7.32 daysin control [Table4].

Effect of LC_{50} of different entomopathogenic fungi on Pupation of *P. gossypiella*:

The percentage of newly hatched larvae of *P. gossypilla* which succeeded in reaching pupation was 48.33 and 50%when treated with the LC_{50} of *B. bassiana* and *M. anisopliae* respectively [Table4] compared to 93.33% in control.

Effect of LC_{50} of different entomopathogenic fungi on Adult emergence of *P. gossypiella*:

The percentage of adult emergence after treatment of newly hatched larvae of *P. gossypilla* with LC_{50} of *B. bassiana* and *M. anisopliae* were 31.67 and 33.33%, respectively [Table 4] compared to 93.33% in control.

Effect of LC_{50} of different entomopathogenic fungi on Deformation percentage of *P. gossypiella*:

The treating of newly hatched larvae of *P. gossypilla*by the used entomopathogenic fungi as LC_{50}'s resulted in adult deformations as showed in Table (4). That means the pupae failed to emergence as adult.

In case of treating newly hatched larvae of *P. gossypilla* by *B. bassiana* and *M. anisopliae*, the adult deformation percentage recorded 16.11and 20.00%respectively, while in control there was no deformation percentage. [Table4].Results showed an increased in larval duration when treating with LC_{50} of (*B. bassiana*and *M. anisopliae*). Similarly Elham F. M. Abdel-Rahim 2011, The toxic effect of larval treatment of 2nd instar of *Spodoptera littoralis* with the biotic insecticides alone (1st test) or in mixtures of *Bacillus* (B) + *Beauveria* (Br), *Bacillus* (B) + Bioranza (Bz) and *Beauveria* (Br) + Bioranza (Bz) at the entire, half and quarter doses (2nd test) was assay in the laboratory tests. The larval duration was prolonged, and *Beauveria* + Bioranza mixture had the most potent in this respect, it increased the larval period to 29 and 27.7d at the half and quarter doses, respectively, as compared to12.2d of control.

Table 4: Effect of LC_{50} of different entomopathogenic fungi on larval duration, Pupal duration, Pupation%, Adult emergence% and Deformation % *P. gossypiella* : treated as new hatched larvae.

Treatments	larval duration (days ± S.E)	°Pupati on %	Pupal duration (days ± S.E)	Adultem emergence %	Deformation %
Beauveriabassiana	14.04^{b}±0.20	48.33	9.22^{a}±0.17	31.67	16.11
Metarhiziumanisopliae	15.07^{a}±0.067	50.00	9.36^{a}±0.22	33.33	20.00
Control	12.63^{c}±0.197	93.33	7.32^{b}±0.05	93.33	-
LSD	0.7186	-	0.555	-	-

° Pupation% based on the number of treated larvae.
•Deformation was pupal-adult deformation.
ns**:** not significant, (student-t test).

It is quite clear from the obtained results that the pupal duration was increased when treatments adapted with LC_{50} of {*B. bassiana*, and *M. anisopliae*}. These results agrees with Hafez *et al*. (1997), who investigated the effect of the entomopathogenic fungus *B. bassiana* on the various developmental stages of the potato tuber moth, *Phthorimae aoperculella* and realized an obvious increase in the pupal duration. That agreement with Elham F. M. Abdel-Rahim 2011, The effect of larval treatment of 2nd instar of *Spodoptera littoralis* with the biotic insecticides alone (1st test) or in mixtures of *Bacillus* (B) + *Beauveria* (Br), *Bacillus* (B) + Bioranza (Bz) and *Beauveria* (Br) + Bioranza (Bz) at the entire, half and quarter doses (2nd test) was assay in the laboratory tests the larval treatment with the biocides product alone recorded the longest period of pupal duration than that of biotic-products mixtures. The pupal weight was reduced, and the larval treatment with mixtures gave the highest effect inthis respect, as compared with that of biotic-products alone. Also, Reda and El-Nemaky 2008, the biological parameters of the pink bollworm, *P. gossypiella* (Saund.) were affected when treated as newly hatched larvae with LC_{50}s of the biocides; Protecto (*Bacillus thuringiensis*), Biover (*B. bassiana*) and Protecto + Biover. There was prolongation in both pupal duration and female adult longevity especially in oviposition period except for with Protecto + Biover treatment. Also, the biocides decreased hatchability and increased sterility (observed and corrected)

addition, larval duration, male adult longevity, egg laying rate, mating frequency and percentages of egg hatchability, Fecundity and mating ability were decreased as compared with the control.

Our results show a reduction in pupation percent in treating with LC_{50} of {*B. bassiana* and *M. anisopliae*}, and it gives a reduction in adult emergence percentage compared to the control. This is agreement with Hafez *et al*. (1997), when investigating the effect of the entomopathogenic fungus *B. bassiana* on *Phthorima eaoperculella*. They found that decrease in the emergence, longevity of moths, deposited eggs, and egg hatchability were dependent on the applied treatments. The same authors (1997) also studied the effect of the entomopathogenic fungus *B. bassiana* on the various developmental stages of the potato tuber moth, *Phthorima eaoperculella*. They recorded adults exhibiting malformed characteristics.

Similarly Angel-Sahagún *et al*. (2005), when applied the entomopathogenic fungi *M. anisopliae* on *Paecilomyces fumosoroseus* and *B. bassiana* on *Haematobia irritans* and found a reduction in adult emergence when applied to eggs and pupae, producing mortality when applied to adults.

An increase in the pupal duration was observed and the emergence of malformed adult was recorded. Similarly, Abd-Allah, A. (2005) studied the effect of *B. bassiana* and *M. anisopliae* on PTM developmental stages. The result indicated that 1st instar larvae more susceptible the 4th instar larvae and *B. bassiana* less effective than *M. anisopliae*. Increase in larvae mortality, caused decreases the percentage of pupation, increase in the pupation period and decrease adult emergence.

Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the prey consumption rate of *Oriusa lbidipennis* to pink bollworm eggs:

The *B. bassiana* and *M. anisopliae* were carried out at the lethal concentration values (1.42and0.98 g\L) respectively, against the eggs cards of *P. gossypiella* as prey of *Oriusa lbidipennis.*

Efficiency of the prey consumption rate of *Oriusa lbidipennis* by using the entomopathogenic fungi *Beauveria bassiana*, on pink bollworm eggs:

Results showed that the infected prey affected the duration of most nymphal stages of *O. albidipennis* (Table 5). Nymphal duration was significantly longer for *O. albidipennis* nymphs feeding on *B. bassiana* infected eggs of *P. gossypiella* (12.00 days) than for nymphs feeding on control eggs was 11.00 days.

Table 5: The effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the developmental time, adult longevity and number of daily prey consumption rate of *Oriusa lbidipennis* on *Pectinophora gossypiella* eggs:

Prey treatments	Nymphal duration (days)	Adult longevity (days)	No. of consumed (egg/days)
Beauveria bassiana	12.00±0.58	8.67±0.33	6.05±0.24
Metarhizium anisopliae	12.33±0.88	7.67±0.33	5.85±0.19
Control	11.00±0.58	9.33±0.33	7.86±0.14

In addition to the effect on Nymphal duration, adult longevity of *O. Albidipennis* nymphs feeding on *B. bassiana* infected eggs of *P. gossypiella* was significantly affected (8.67 days), while adult longevity in control was 9.33 days. Those *O. albidipennis* fed infected *P. gossypiella* eggs had significantly shorter adult lifespan by approximately 10-15% than those fed control prey.

B. bassiana also had a significant effect on the number of prey consumed by *O. albidipennis* nymphs (6.05 eggs/days), while the prey consumption rate in control was 7.86eggs/days.

Efficiency of the prey consumption rate of *Oriusalbidipennis* by using the entomopathogenicfungi *Metarhiziumanisopliae*, on pink bollworm eggs:

Data in (Table 5) showed that the nymphal duration was12.33 days, significantly longer for *O. albidipennis* nymphs feeding on *M. anisopliae* infected eggs of *P. gossypiella* than for nymphs feeding on control eggs was 11.00 days.

The adult longevity of *O. albidipennis* nymphs feeding on *M. anisopliae* infected eggs of *P. gossypiella* was decreased (7.67 days); compared with the adult longevity in control was 9.33 days.

*M.anisopliae*had a significant effect on the number of prey consumed by *O. albidipennis* nymphs (5.85 eggs/days), while the prey consumption rate in control was 7.86 eggs/days.

The predatory bug *Oriusa lbidipennis* (Reuter) (Hemiptera: Anthocoridae) has tremendous potential as a biological control agent, especially in its native range around the Mediterranean Basin and East Africa (Islam S. Sobhy *et al.* 2010).

Although generalist predatory insects are capable of attacking a diverse spectrum of prey species, the results of our study show that the prey infected by entomopathogenic fungi can impact a wide range of characteristics related to the performance of *O. albidipennis*, including developmental time, adult longevity and number of daily prey consumption which have important implications for population dynamics. Because the quality of food for predatory insects can be measured by growth (Arijs and De Clercq 2004).Also Pourian, H. R. *et al.* 2010 indicated that the susceptibility of female adults and 5th nymphs of *Oriusa lbidipennis* (Reuter) was tested against this isolate of *M. anisopliae* without attendance of host. Results indicated that two stages of predator; female adults and 5th instar nymphs had similarly low susceptibilities to fungal infection. In general, our results revealed that the anthocorid predator, *O. albidipennis* was less affected by this isolate of fungus.

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ARABIC SUMMERY

تأثير فطر *Beauveriabassiana* و*Metarhiziumanisopliae* على بعض الجوانب الحيويه لدودة اللوز القرنفليه.

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اجريت هذه التجربه لدراسة تاثيربعض الفطريات الممرضه وهى مركب Biover وBioranza على دودة اللوز القرنفليه تحت ظروف المعمل على درجة حرارة 27 ± 1º C ورطوبة نسبيه 10 % ± 65 %.
وقد اظهرت النتائج حساسية يرقات دودة اللوز القرنفليه لكلا المركبين حيث كانت ال LC_{50}هى 1.42 و 0.98 جم/ل للمركبين*Beauveriabassiana* و *Metarhiziumanisopliae*على الترتيب.
تأثيرات الجوانب الحيويه لدودة اللوز القرنفليه عند معامله اليرقات حديثة الفقس ب LC_{50} لكلا المركبين.
كما اوضحت النتائج تغيرات فى الجوانب الحيويه المختلفه (مدة الطور اليرقى- نسبة خروج العذارى- مدة طور العذراء- نسبة خروج الفراشات)