

INTER-RELATIONSHIP BETWEEN *Galleria mellonella* L. (LEPIDOPTERA: GALLERIDAE), *Apanteles galleriae* Wilk. (HYMENOPTERA: BRACONIDE) AND THE FUNGUS *Beauveria bassiana* (Bals.) VUILL.

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ABSTRACT

The parasitoid females of *Apanteles galleriae* Wilk. laid its eggs in both healthy and fungus-infected *Galleria mellonella* larvae. The percentage of parasitism and the number of eggs laid per parasitoid female were not affected by the fungus *Beauveria bassiana* infection. The highest mortality of the host (*G. mellonella*) and the least number of parasitoids (*A. galleriae*) emerged when the host larvae were infected with *B. bassiana* immediately after parasitization. When *G. mellonella* was infected by the fungus, this caused mortality of different stages of the parasitoid inside the host, being 45.3, 8.6 and 2.5% for larval, pupal and adult stages, respectively. Developmental durations were 1.44, 12.50, 1.48 and 4.60 days in healthy hosts and 1.99, 12.50, 1.85 and 7.00 days in fungus infected ones for eggs, larvae, prepupae and pupae, respectively. Longevity was 7.48 and 4.21 days, and life cycle amounted 27.50 and 23.00 days for adult female reared from healthy and infected hosts, respectively.

INTRODUCTION

The endoparasitoid, *Apanteles galleriae* Wilk. (Hymenoptera: Braconidae) has been recorded as the most common and efficient natural enemy of wax moths, *Galleria mellonella* L. and *Achroia grisella* larvae; in Egypt (El-Hemaesy, 1983 and Gamal El-Din, 1985), in Argentine (Blanchard, 1936), in France (Wilkinson, 1932), in USA (Anonymous, 1959), in Mauritius (Wilkinson, 1934).

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuill. was used as a microbial control agent against larval and pupal stages of the greater wax moth, *G. mellonella* and lesser wax moth, *A. grisella* in Czechoslovakia (Boczkowska, 1935), in Cuba (Broche, 1986), in USA (Shimanuki, 1981 and Gupta *et al.*, 1994), in Egypt (El-Sufty, 1983; Ibrahim, 1996 and Mansour, 1999 & 2003). The use of this fungus as a microbial control agent against *G. mellonella* may reduce the natural parasitism by *A. galleriae*.

The present work aimed to study the effect of *Galleria mellonella* infection with *Beauveria bassiana* on the biology and developmental stages of the parasitoid *Apanteles galleriae*.

MATERIALS AND METHODS

This investigation was conducted in the laboratory to evaluate the effect of diseased *Galleria mellonella* L. larvae by the fungus *Beauveria*

Cultures preparation:

Larvae of *G. mellonella* (second or third instars) were obtained from infested old bee wax. The larval stock was kept feeding on bee wax till the beginning of tests. The endo-parasitoid, *Apanteles galleriae* Wilkinson was obtained from parasitized *G. mellonella* larvae. Conidia of the fungus *Beauveria bassiana* were obtained, through personal communication, from the Egyptian fungal strain isolated by El-Sufty and Boraei (1987). To ensure active fungus throughout the study, the fungus strain was maintained in the laboratory by culturing on full grown *G. mellonella* larvae, according to Mansour (1999).

Both parasitoid and insect host cultures were maintained under $25 \pm 2^{\circ}\text{C}$ and $75 \pm 5\%$ R.H. The host larvae were confined in Petri dishes containing old bee wax as a food source for the wax moth. A piece of cotton was saturated with sugar solution as a food for the parasitoid, and then the parasitoid was introduced (1 host: 2 parasitoid females) for two hours.

G. mellonella larvae were infected with *Beauveria bassiana* when dipped in a fungal suspension containing 5×10^7 conidia/ml and 0.1% Tween 80. The other larvae which served as a check were treated with tap water containing 0.1% Tween 80. Both treated and untreated *G. mellonella* larvae were kept into Petri dishes at 25°C and 80% R.H.

Biological studies on *Apanteles galleriae*:

Effect of infection by the fungus on the percentage of parasitism:

The rate of parasitism by *A. galleriae* oviposition was investigated by using batches of healthy and fungus infected larvae 1, 2, 3, 4 and 5 days old, each batch consisted of 15 insects. Larvae were randomly arranged on a paper sheet and introduced to the parasitoids inside a wooden frame cage covered with cheese cloth (15 x 15 x 50 cm). Host larvae were individually dissected two hours later and the parasitized ones were recorded.

Effect of the parasitism by *A. galleriae* on the fungus infection:

To determine the effect of parasitism on viability of *B. bassiana* against *G. mellonella*, parasitized larvae were treated with the fungus immediately after parasitization and 1, 2, 3 and 4 days after parasitization in lots of 12 larvae and incubated until the emergence of parasitoid adult. Batches of unparasitized larvae (each of 12 larvae) were treated with the fungus parallel with the parasitized larvae to serve as a check. Dead parasitized and unparasitized larvae, and hosts producing parasitoids were recorded.

Effect of insect host infection on some biological aspects of the parasitoid *A. galleriae*:

Effect of host infection on development of the parasitoid was studied on batches of healthy and infected hosts, each of 5 larvae. Infected hosts were treated with the fungus immediately after parasitization. A batch of healthy and infected hosts were dissected every day to record the

development of the parasitoid. Mean durations of the parasite immatures were recorded.

Twenty adult parasitoids (10 males and 10 females) emerging from healthy and infected hosts were randomly chosen. Each pair of the parasitoid was introduced into Petri dish containing 3 healthy larvae which were daily renewed by a new set until death of parasitoids. Host larvae were daily dissected and eggs laid by each female were counted. Preoviposition, oviposition and postoviposition periods and longevity were determined.

Effect of host infection on mortality of *A. galleriae* stages:

Twenty five healthy and infected hosts were maintained until parasitoid emergence, host cadavers were dissected and dead individuals (larvae, pupae and adult) were counted. Emerging parasitoids were counted and sexed.

RESULTS AND DISCUSSION

Effect of infection by the fungus on the percent of parasitism:

The parasitoid female *A. galleriae* laid its eggs in both healthy and fungus infected *G. mellonella* larvae 1-5 days old, while larvae older than 5 days were not oviposited (Table 1). Young larvae were generally preferred. Parasitism was statistically the same on both healthy and fungus infected larvae.

Table (1): Percentage of the parasitism by *A. galleriae* in healthy and fungus infected larvae of *G. mellonella*.

| Larval status | Days after fungus infection | | | | | |
|---------------|-----------------------------|---------|---------|--------|--------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Healthy | 95.3 b | 100.0 a | 100.0 a | 85.7 c | 40.0 d | 0.0 e |
| Infected | 95.9 b | 100.0 a | 98.0 ab | 81.3 c | 35.3 d | 0.0 e |

| Comparison | S.E.D. | L.S.D. 5% | L.S.D. % 1 |
|------------|--------|-----------|------------|
| S.M means | 1.88 | 3.89 | 5.29 |
| M means | 0.77 | 1.59 | 2.16 |

These results indicate that rate of *A. galleriae* parasitism seems to be slightly affected by host infection with *B. bassiana* (Table 1). These values were 95.3 & 95.9, 100 & 100, 100 & 98, 85.7 & 81.3 and 40 & 35.3 for healthy and infected larvae aging 1, 2, 3, 4 and 5 days, respectively.

Similarly, parasitoid females of the braconid, *Microplitis croceipes* laid their eggs in host larvae (*Heliothis zea* Hb.) infected with the fungus *Nomuraea rileyi* without noticeable differences between healthy and diseased larvae (King and Bell, 1978).

Effect of the parasitism by *A. galleriae* on the fungus infection:

Data presented in Table (2) indicated that parasitized larvae of *G. mellonella* were less susceptible to *B. bassiana* infection than unparasitized

ones. The highest mortality of parasitized larvae occurred when hosts were infected immediately after parasitization. Mortality distinctly decreased with the increase of duration between parasitization and fungus infection. In infected hosts, the parasitoid was able to complete its development particularly in hosts infected with the fungus after 2nd day of parasitism.

These results indicated that the parasitoid *A. galleriae* reduced the host susceptibility to *B. bassiana* infection. It seems that the parasitoid inhibits the fungus development inside the host, so a considerable number of parasitoid individuals could complete their development.

Table (2): Effect of the parasitism by *A. galleriae* on the percentage of mortality caused by the fungus infection.

| Days after parasitism | Parasitized larvae | | Unparasitized larvae |
|-----------------------|--------------------|------------------------|----------------------|
| | % mortality * | % produced parasitoids | % mortality |
| 0 | 79.0 | 21.0 | 100.0 |
| 1 | 68.3 | 31.7 | 100.0 |
| 2 | 57.7 | 42.3 | 95.00 |
| 3 | 57.0 | 43.0 | 80.0 |
| 4 | 20.7 | 79.3 | 71.7 |
| 5 | 00.0 | 100.0 | 70.3 |

Rate of parasitism for uninfected parasitized larvae was 100%.

* Hosts produced no parasitoids.

These results are in agreement with those of King and Bell (1978) who found that the highest mortality of parasitized larvae infected with the fungus *Nomuraea rileyi* occurred when the host larvae were infected with the fungus prior to or within one day after parasitization.

Influence of the insect host infection by *B. bassiana* on some biological aspects of the parasitoid *A. galleriae*:

Table (3) presents durations of *A. galleriae* stages and total life cycle for both sexes reared in healthy and infected hosts. Larval and pupal durations were longer and longevity of female and male was shorter for parasitoids reared in infected hosts than those reared in healthy ones. These results revealed that the pre- and post-oviposition periods for female resulting from infected hosts were longer than those of female resulted from healthy hosts (0.12 ± 0.01 & 0.13 ± 0.01 and 0.08 ± 0.01 & 0.04 ± 0.002), respectively. The oviposition period was distinctly reduced for females reared in infected hosts as compared with those reared in healthy ones. Longevity of parasitoid females emerged from healthy and infected hosts revealed that all females resulted from healthy hosts survived up to 7.48 ± 1.89 days while those emerged from infected ones survived up to 4.21 ± 0.12 days. The total life cycle amounted to 27.50 ± 2.91 and 24.73 ± 1.28 days for parasitoids produced from healthy hosts and 23.0 ± 1.5 and 20.0 ± 2.31 days for those produced from infected ones for female and male, respectively. Mean number of eggs laid by females emerged from infected hosts was about half that laid by females resulted from healthy ones; 45 ± 15.7 and 25 ± 12.9 , respectively.

Effect of host infection by *Beauveria bassiana* on mortality of *A. galleria* stages:

Data presented in Table (4) revealed that infection of host larvae with *B. bassiana* caused mortality among larvae, pupae and adults of parasitoid inside the host and the highest mortality occurred in the larval stage (56.4%). Mortality of the different stages inside healthy hosts only amounted to 1.1%. These results are in agreement with those reported by El-Sufty and Führer (1981) for *Pieris brassicae* larvae parasitized by the braconid *Apanteles glomeratus* and infected with the fungus *B. bassiana*. They reported that the duration of larval stage of the parasitoid was 3 days longer in the infected hosts than in healthy ones. They added that mortality of different stages of the parasitoid caused by the fungus was 38% (23% in larval, 14% in pupal and 1% in adult stages).

Table (3): Some biological aspects of the parasitoid *A. galleriae* reared on fungus infected *G. mellonella* larvae and healthy ones.

| Parasitoid stage | Duration (day) | | | | | |
|--------------------------|----------------|------|---------------|---------------|------|---------------|
| | Healthy host | | | Infected host | | |
| | Range | | Average + S.D | Range | | Average + S.D |
| | Min. | Max. | | Min. | Max. | |
| Egg | 1 | 2 | 1.44 ± 0.2 | 1 | 3 | 1.99 ± 0.01 |
| Larva | 10 | 13 | 12.50 ± 2.44 | 11 | 15 | 12.50 ± 2.45 |
| Prepupa | 1 | 2 | 1.48 ± 0.30 | 1 | 3 | 1.85 ± 0.13 |
| Pupa | 4 | 7 | 4.60 ± 0.33 | 5 | 8 | 6.00 ± 0.02 |
| Pre-oviposition | 0.07 | 0.10 | 0.08 ± 0.005 | 0.01 | 0.22 | 0.12 ± 0.01 |
| Oviposition | 5 | 7 | 6 ± 0.9 | 4 | 6 | 4.0 ± 1.9 |
| Post-oviposition | 0.01 | 0.08 | 0.04 ± 0.002 | 0.11 | 0.15 | 0.13 ± 0.01 |
| Longevity | | | | | | |
| Female | 6 | 10 | 7.48 ± 1.89 | 3 | 7 | 4.21 ± 0.12 |
| Male | 4 | 6 | 4.71 ± 1.71 | 2 | 3 | 2.50 ± 0.10 |
| Life cycle | | | | | | |
| Female | 22.0 | 34.0 | 27.50 ± 2.91 | 21.0 | 32.0 | 23 ± 1.50 |
| Male | 28.0 | 30.0 | 24.73 ± 1.28 | 20.0 | 25.0 | 20 ± 2.31 |
| No. of egg/female | 40.0 | 72.0 | 45 ± 15.7 | 23.0 | 36.0 | 25 ± 12.9 |
| Sex ratio (male: female) | 1.0 | 1.5 | - | 1.0 | 1.5 | - |

Table (4): The percentage of mortality of different stages of *A. galleriae* as affected by healthy and infected host larvae.

| Host status | % mortality | | | |
|-------------|--------------|-------------|-------------|-------|
| | Larval stage | Pupal stage | Adult stage | Total |
| Healthy | 0.3 | 0.7 | 0.1 | 1.1 |
| Infected | 45.3 | 8.6 | 2.5 | 56.4 |

The inter-relationship between endoparasitoids and entomopathogenic microorganisms were discussed by various authors. Tanada (1956) and Steinhaus (1963) reported that when the bacterium and virus infect young cabbage worms, the parasitoids developing in the larvae were generally unable to complete their development. However, when older hosts are infected by these microorganisms, the parasitoids can sometimes complete their development. El-Sufty and Führer (1981 & 1985) found that in host-parasitoid systems, such as *Pieris brassicae*-*Apanteles glomeratus* and

Cydia pomonella-*Ascoqaster quadridentatus*, growth and dissemination of the fungus *B. bassiana* within parasitized host larvae were strongly inhibited. They concluded that the parasitoid larvae cause the incidence of a fungistatic materials, presumably originating from terstocytes. As for pupal parasitoids, Führer et al. (1978) reported that the larvae of *Pimpla turionellae*, as an endoparasitoid of lepidopterous pupae, produces a clear liquid from its proctodaeum, which acts fungi statistically against *B. bassiana* and *Penicillium* sp.

The present study indicate that *A. galleriae*, which is the most common parasitoid of *G. mellonella* and *A. grisella* larvae, seemed to be not drastically affected by the infection of its host larvae with *B. bassiana*. Deleterious effect would occur only in parasitized larvae infected within 2 days after parasitization. It may be suggested that the fungus *B. bassiana* could be used against *G. mellonella* without effective reduction of *A. galleriae* parasitism.

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تداخل العلاقة بين دودة الشمع *Galleria mellonella* وطفيل *Apanteles galleriae* والفطر *Beauveria bassiana*

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يهدف هذا البحث الى دراسة تأثير اصابة الفطر بيوفاريا باسيانا ليرقات حشرة دودة الشمع الكبيرة على تطفل الطفيل ابانتيليس جاليري وتطوره داخل يرقات الحشرة ، وكذا دراسة الصفات البيولوجية الهامة لأفراد الطفيل التي تتجح في الخروج من العائل المصاب.

وتتلخص النتائج في الآتي:

- 1- لم تميز أنثى الطفيل بين اليرقات المصابة بالفطر والأخرى السليمة ولم تتأثر نسبة التطفل ولا عند البيض السدى تضعه كل انثى من إناث الطفيل.
- 2- كانت حساسية اليرقات المتطفل عليها للإصابة بالفطر أقل منها في اليرقات الغير متطفل عليها.
- 3- أدت الإصابة بالفطر بعد التطفل مباشرة الى موت أعداد أقل من أطوار الطفيل حيث سجلت 79% موت في اليرقات المتطفل عليها بينما سجلت 100% موت في اليرقات الغير متطفل عليها.
- 4- قصرت فترة حياة أنثى الطفيل الناتج من العوائل المصابة بالفطر عن تلك الناتجة من العوائل السليمة.
- 5- لم تؤثر إصابة العائل بالفطر على فترتي ما قبل وما بعد وضع البيض ولا النسبة الجنسية للطفيل.