

Full Length Research Paper

Effect of food types of *Galleria mellonella* L. (Lepidoptera: Pyralidae) on biological aspects and life table of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae)

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In the present work, laboratory and field strains of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) were reared on two food types (artificial diet for laboratory strain and wax frames for field strain) to study some biological aspects of the developmental stages of braconid wasp, *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) the parasitoid of *G. mellonella*. Results of total developmental period, percentages of adults' emergence and longevity of females of the parasitoid are different when reared on two strains of *G. mellonella*. Total developmental period of the immature stages was shorter on field strain (47.26 days) than that reared on laboratory strain of *G. mellonella* recording 57.5 days. In contrast, no significant differences were observed between the longevity of adult females reared on the two strains, where the longevity period was 25.33 and 26.3 days in laboratory and field strains, respectively. Also, life cycle of immature stages, longevity of adults and reproductive parameters of the parasitoid *A. galleriae* were determined. The fecundity of *A. galleria* decreased when reared on laboratory strain of *G. mellonella*; it was 140 progeny/females and increased to 177.3 progeny/females when *A. galleria* was reared on *G. mellonella* field strain. On the other hand, the sex ratio of the parasitoid and longevity are different between the two strains. Results of the life tables for *G. mellonella* and the parasitoid, *A. galleria* strains, showed shortage in generation periods for that reared on the field strain. The present study was conducted to explore the effect of food types on some biological parameters of *G. mellonella* and its parasitoid, *A. galleria*.

Key words: Parasitoid, greater wax moth, biology, life table, host preference.

INTRODUCTION

The greater wax moth, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) is a severe pest of field-based

honeybee colonies and is stored in combs causing high losses to honey bee colonies in different countries. This

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pest has received more attention as a model organism for toxicological and biological control investigations. Wax is an important product for the honeybee, which is used in some medicines, dental as well as cosmetic industries. Wax contains many nutrients, such as pollen and honey bee, and is therefore attacked by *G. mellonella*. The larvae of this insect feed on wax combs stored especially in the dark. They cause damage to active combs and severe economic damage. It is important for beekeepers to worry about controlling this serious pest, because it is very destructive and weakens honey bee colonies. Many studies have been performed to find ways to control this dangerous insect pest, especially biological control agents (Hanumanthaswamy and Rajagopal, 2017; Kwadha et al., 2017). The rearing of *G. mellonella* larvae was studied by some authors. Kwadha et al. (2017) showed that diet quality affects larva development. Abiotic factors such as temperature and relative humidity affect the entire life cycle. It has been shown that temperature averages of 29 and 33°C are optimum for development and 29 to 33% relative humidity (RH) appears to be appropriate for survival. El-Gohary et al. (2018) and Vanzyl and Malan (2015) investigated the suitability of different suggested artificial diets for mass rearing multiplication of the greater wax moth, *G. mellonella* L. using entomopathogenic nematodes as well as many parasitoids for insect biological control purposes.

The braconid wasp, *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) is a solitary endoparasitoid and effective parasitoids on the greater and lesser wax moths, *G. mellonella* and *Achroia greissella*. This parasitoid attacks the early-instar larvae of wax moths *G. mellonella* and emerges to spin its cocoon and pupate well before the host larvae reach full size (Abo Abdalla and Gadelhak, 2016). Hegazi et al. (2017) reported that *A. galleriae* may be host specific for *Galleria mellonella*; 4th and 5th instars are the preferred hosts. The female lays its eggs singly inside the caterpillar, and the life cycle takes 16 to 22 days. *A. galleriae* parasitoid and its host larvae, *G. mellonella* live in near-continuous darkness in the beehives or wax comb stores. These species are with no known diapauses in Egypt. It has been shown that *A. galleriae* has the characteristics of a biological control agent. The present study was designed to investigate the effect of food types on some biological parameters of *G. mellonella* and its parasitoid, *A. galleriae*.

MATERIALS AND METHODS

Field strain of *G. mellonella* (reared on old wax comb)

Stock cultures of greater wax moth, *G. mellonella* (L.) were established from larvae. Adults were collected from beekeeping of Faculty of Agriculture, Menoufia Governorate in June 2018 and reared on old wax comb which was collected from the same location and kept at 32±1°C and 65 - 70% RH; it was reared for several generations. Ten newly emerged females and males were used for each replicate; there were three replicates. Males and

females were kept in glass jar (2 kg) and covered by cloth of muslin under the previously mentioned rearing conditions, then kept in 18 h dark and 6 h light. The glass jars were examined daily until eggs were laid; and hatching was observed and collected. Incubation period of eggs, larval and pupal duration, and adults' longevity were estimated.

Laboratory strain of *G. mellonella* reared on artificial diet

The susceptible laboratory strain of the wax moth, *G. mellonella* (L.) was reared for several generations on an artificial diet that included wheat corn, glycerin, soya flour, milk powder, inactive dry yeast and honeybee wax. It was done in a glass jar, under the laboratory conditions at 32±1°C and 65-70% R.H. at Economic Entomology Department, Faculty of Agriculture, Menoufia Governorate.

Rearing of *A. galleriae*

Stock cultures of the parasitoid, *A. galleriae* were established from pupae and adults were collected from beekeeping of the Faculty of Agriculture at Menoufia Governorate during June 2018. They were reared on field strain of *G. mellonella* for several generations.

Laboratory colonies of *A. galleriae* were reared on early larval instars of laboratory and field strains of *G. mellonella*. Different stages (from 2nd to 4th instars larvae) of *G. mellonella* (field and laboratory strain) were exposed to parasitoid which reared at 25±1°C and 65 - 70% RH. Ten newly emerged females of the parasitoid were used for each replicate in a glass cage under previous rearing condition. Adults of *A. galleriae* were fed on 30% honey solution and kept at the same conditions with host larvae of *G. mellonella*. Life cycle from eggs to pupae and longevity of newly emerged adult female and male *A. galleriae* for laboratory and field strain of *G. mellonella* host was estimated in the same rearing conditions with the host species. All experiments were repeated three times.

Statistical analysis

All experiments contained three replicates. The results were analyzed by one – way analysis of variance (ANOVA) using COSTAT statistical software (Cohort Software, Berkeley). When the ANOVA statistics were significant (P < 0.01), means were compared by the Duncan's multiple range test. Life table parameters were calculated according to Birch (1948) using Life 48 basic computer program (Abou-Setta et al., 1986) and Euler-Lotka equation:

$$\sum_0^{\infty} e^{-rm} L_x m_x dx = 1,$$

Where: (X) Age in days, (L_x) Age specific survival rates, (M_x) Female fecundity, (R₀) Net reproductive rate (R₀ = $\sum l_x m_x$), (T) Generation time (T = $(\sum l_x m_x x) / R_0$), (r_m) Intrinsic rate of increase (r_m = log R₀ / T), (λ) Finite rates of increase (λ = exp. r_m) and (Dt) Doubling time (Dt = log(e(2))/r_m).

RESULTS AND DISCUSSION

Effect of food types on *G. mellonella* development

Data in Table 1 show the effect of food types on different stages of *G. mellonella*. The data show that there are no significant effects of food kind on egg incubation period of

Table 1. Effect of food types on life duration of different stages of laboratory and field strains of *G. mellonella*.

Strain	Incubation period/egg	Larval stage	Pupal stage	Total immature stages	Adult stage	
		Duration	Duration time/days		Longevity female	Emergence %
Laboratory	8.0±0.1 ^a	37.10±1.4 ^a	12.41±1.7 ^a	57.5±2.3 ^a	25.33±2.8 ^a	83.0±1.25 ^b
Field	7.3±0.2 ^a	29.66 ±1.3 ^b	10.3±1.3 ^b	47.26±2.8 ^b	26.3±0.66 ^a	94.0±2.81 ^a

Means followed by same letter in column are not significantly different at 5% level.

Table 2. Effect of food types on life table parameters of *G. mellonella*.

Parameter	<i>G. mellonella</i> (field strain)	<i>G. mellonella</i> (laboratory strain)
Survival to maturity	0.90	0.82
Sex ratio (females/total)	0.67	0.51
The net reproductive rate (R_0)	49.4	31.57
Mean generation time (T)	57.4	68.4
The intrinsic rate of increase (r_m)	0.07	0.05
The finite rate of increase (λ)	1.07	1.05
Time of population doubling (DT)	10.5	14.7

both laboratory and field strains, but larval and pupal periods were significantly affected by the food type. In the laboratory strains were fed on artificial diet; the larval and pupal periods were prolonged than in field strain fed on honeycomb. These periods were 37.10 days/larvae, 12.41 days /pupa, 29.66 days/larvae and 10.3 days/ pupa for laboratory and field strains, respectively.

Also, the total immature stages period of *G. mellonella* laboratory strain highly elongated to 57.5 days (from eggs to pupae), compared to 47.26 days in field strain. In addition, adult emergence% was also significantly affected by the food type where it was 83 and 93% for laboratory and field strain respectively. No significant effect occurred in female longevity, which was 25.33 and 26.3 days /female for laboratory and field strain, respectively.

Htet and Ueno (2019) reported that the impacts of the larval diet were in relation to protein and cholesterol. However, the present study showed the influence of larval diets on the development and survival of all stages of *G. mellonella*. Also larvae obtain nutrients from honey, pupal skins, pollen, wax, and other impurities found in the beeswax; beeswax contains a lot of important elements for growth and development such as protein and vitamins.

Effect of food types on life table of *G. mellonella*

Results obtained on life table parameters are presented in Table 2 and Figures 1 and 2 Tested food types affected survival rates of immature stages to adult stage.

Survival rates as 0.90 and 0.82 were obtained when larvae of *G. mellonella* fed on honeybee comb (field strain) and artificial diet (laboratory strain), respectively.

Sex ratio was also affected by tested food kind. Sex ratios (female / total) as 0.67 and 0.51 were obtained for field strain and laboratory strain larvae of *G. mellonella*, respectively. The net reproductive rate (R_0) varied according to the used food kind. Respective record of 49.4 females/ female was recorded for field strain compared to 31.57 females/ female was recorded for laboratory strain larvae of *G. mellonella*.

Accordingly, it seems that feeding on natural food of honey bee comb is the most favorable food kind to field strain larvae of *G. mellonella* for reproduction; showing the highest value of net reproductive rate. From the mean generation time (T), evident differences were observed between the two food types. This period decreased with the feeding on natural food of honey bee comb in field strain of *G. mellonella*, but increase with the feeding on artificial diet in laboratory strain; the average values were 57.4 and 68.4 days, respectively.

Associating with the two tested food kind, the intrinsic rates of increase (r_m) were in respective 0.07 and 0.05 for field and laboratory strains. When these were converted into finite rates of increase (λ), the population of *G. mellonella* had the capacity to increase by the respective values 1.07 and 1.05 times/female/day when *G. mellonella* larvae were reared on honey bee comb and artificial diet; they had the highest capacity to increase in field strain. Population doubling time (DT) was estimated to be 10.5 and 14.7 days for field and laboratory strains, respectively. Obtained results indicated that wax comb

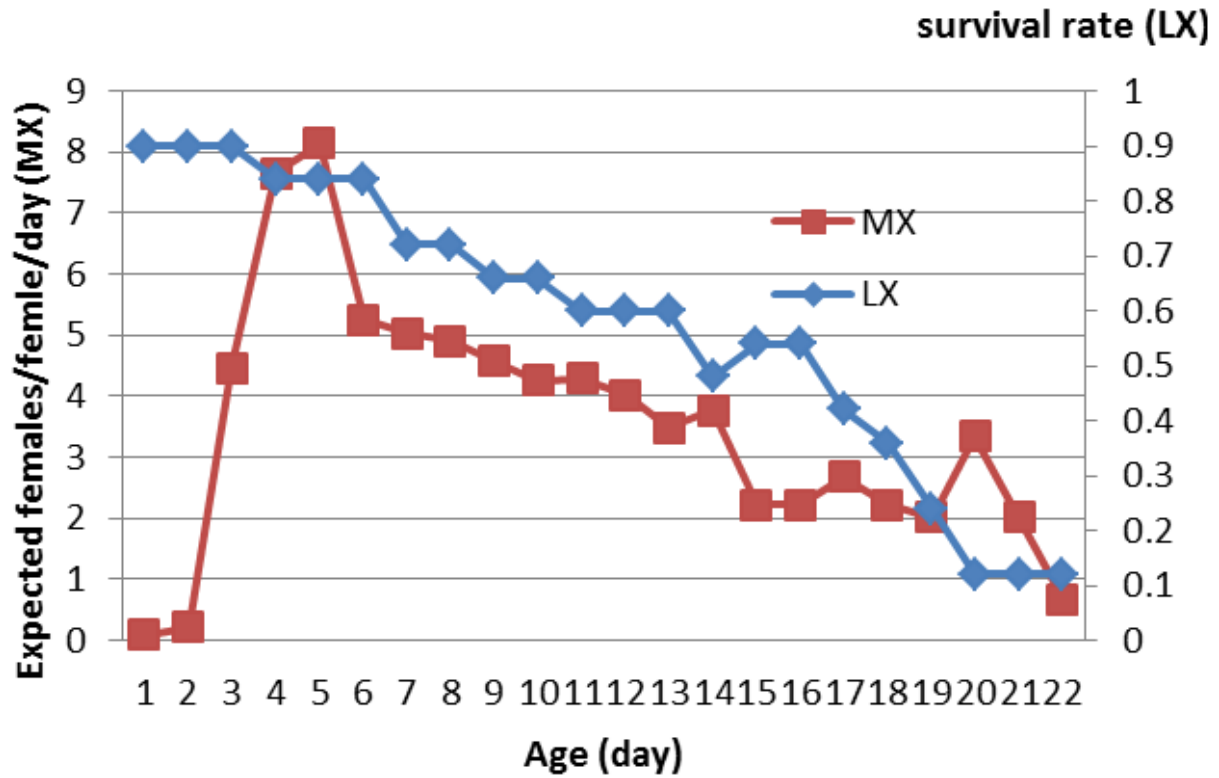


Figure 1. Age- specific fecundity and survival rate of *G. mellonella* field strain feeding on old wax comb (natural food). LX: Survival rate; MX: Female fecundity.

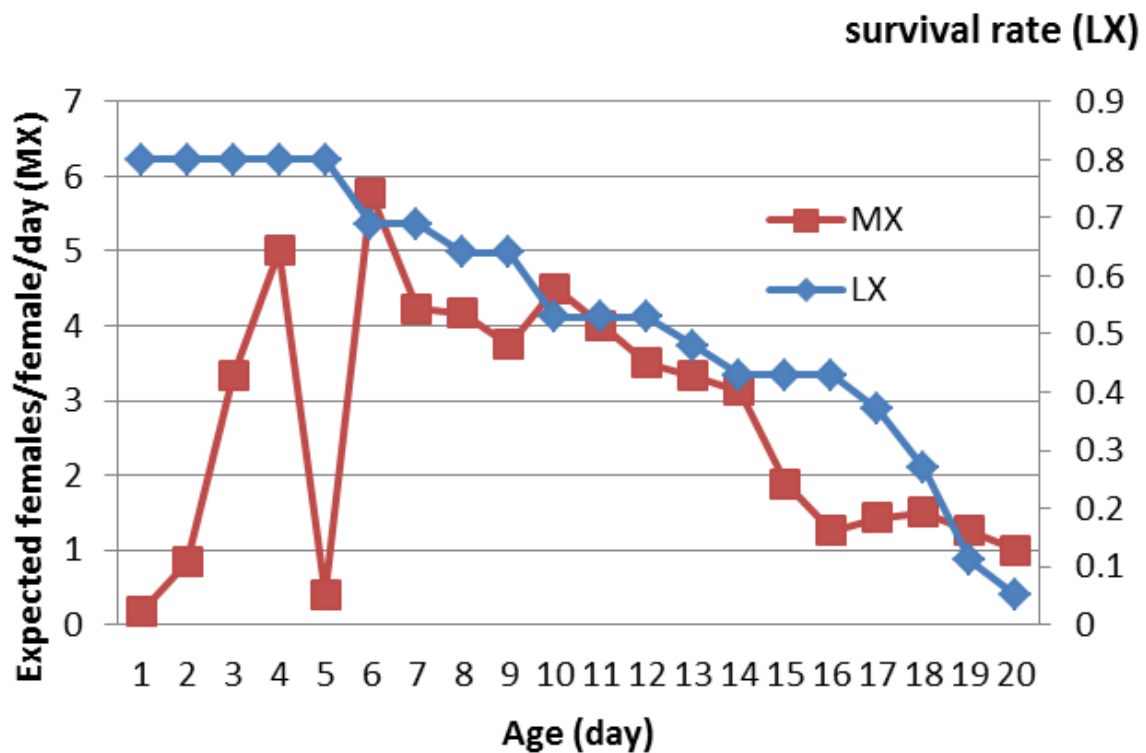


Figure 2. Age- specific fecundity and survival rate of *G. mellonella* laboratory strain fed on artificial diet. Where LX: Survival rate and MX: Female fecundity.

Table 3. Rearing of *A. galleriae* on laboratory and field strains of *Galleria mellonella*.

Strain	% Parasitism	Eggs to pupal stage duration (days)		Pupal duration (day)	Pupal dead%	Adult emergence %
		pupation%	Duration			
Laboratory	66.0±2.6 ^b	78.6 ^b	14.33±0.3 ^a	6.3±0.5 ^a	13.4 ^a	87.6±2.3 ^b
Field	86.66±2.4 ^a	89.3 ^a	13.36±0.88 ^a	5.4±0.32 ^a	7.7 ^b	92.3±1.6 ^a

Means followed by same letter in column are not significantly different at 5% level.

Table 4. Sex ratio, fecundity and longevity of parasitoid *A. galleriae* reared on laboratory and field strain of *G. mellonella*.

Strain	Sex ratio	Fecundity	Longevity	
			♂	♀
Laboratory	48.66±1.1 ^b	141.0±3.2 ^b	28.3±0.9 ^a	30.0±0.4 ^a
Field	50.33±0.68 ^a	177.3±2.6 ^a	28.6±0.5 ^a	31.3±0.9 ^a

*Means followed by same letter in column are not significantly different at 5% level.

was more favorable for feeding *G. mellonella* larvae. These results agree with Abedelsalam et al. (2014); they studied the effects of five natural diet materials on the biology of the greater wax moth (Tables 1 and 2).

Effect of host food kind types on the biology of the parasitoid *A. galleriae*

Data in Table 3 show the significant difference in the percentages of parasitism between laboratory and field strains of *A. galleriae* reared on laboratory and field strains of *G. mellonella*; the percentages of parasitism were 66 and 86.66%, respectively. Duration of immature stages from eggs to pupae was significantly affected by type of host strain. Duration of immature stages of *A. galleriae* reared on laboratory strain of *G. mellonella* was longer than which reared on field strain. These durations were 20.63 days and 18.76 days, respectively (Figures 1 and 2).

Also data presented in Table 3 summarize the effect of type of host strain on percentages of pupation and adults' emergence of *A. galleriae*. It is clearly obvious that pupation percentages and adults' emergence of *A. galleriae* reared on field strain of *G. mellonella* significantly increased (89.3% for pupation percentage and 92.3% for adults emergence); while these percentages significantly decreased when *A. galleriae* was reared on laboratory strain of *G. mellonella* (78.6% for pupation percentage and 87.6% for adults' emergence). The fecundity (Total number of progeny produced by a single mated female), sex ratio and time longevity in days of *A. galleriae* reared on either *G. mellonella* laboratory and field strain are given in Table 4. The fecundity of *A. galleriae* reared on *G. mellonella* field strain was slightly higher than *A. galleriae* reared on *G.*

mellonella laboratory strain. There was significant difference between field strain (177.3 progeny/female) and 144.0 progeny/female on laboratory strain.

The sex ratio of *A. galleriae* obtained from strains reared on *G. mellonella* laboratory and field strains were 50.33 female /female when *A. galleriae* was reared on *G. mellonella* field strain compared to 48.66 on *G. mellonella* laboratory strain. Also longevity of female and male were significantly affected by host kind strain; it was 25.9 days/female and 30 days/male in laboratory strain while it was 28.4 days/female and 27.3 days/male in field strain. Present results agree with Hegazi et al. (2019) who found that the period from egg to adult for both sexes of *A. galleriae* changed according to the diet ingredients of the host on which the parasitoid completed its immature stages of development.

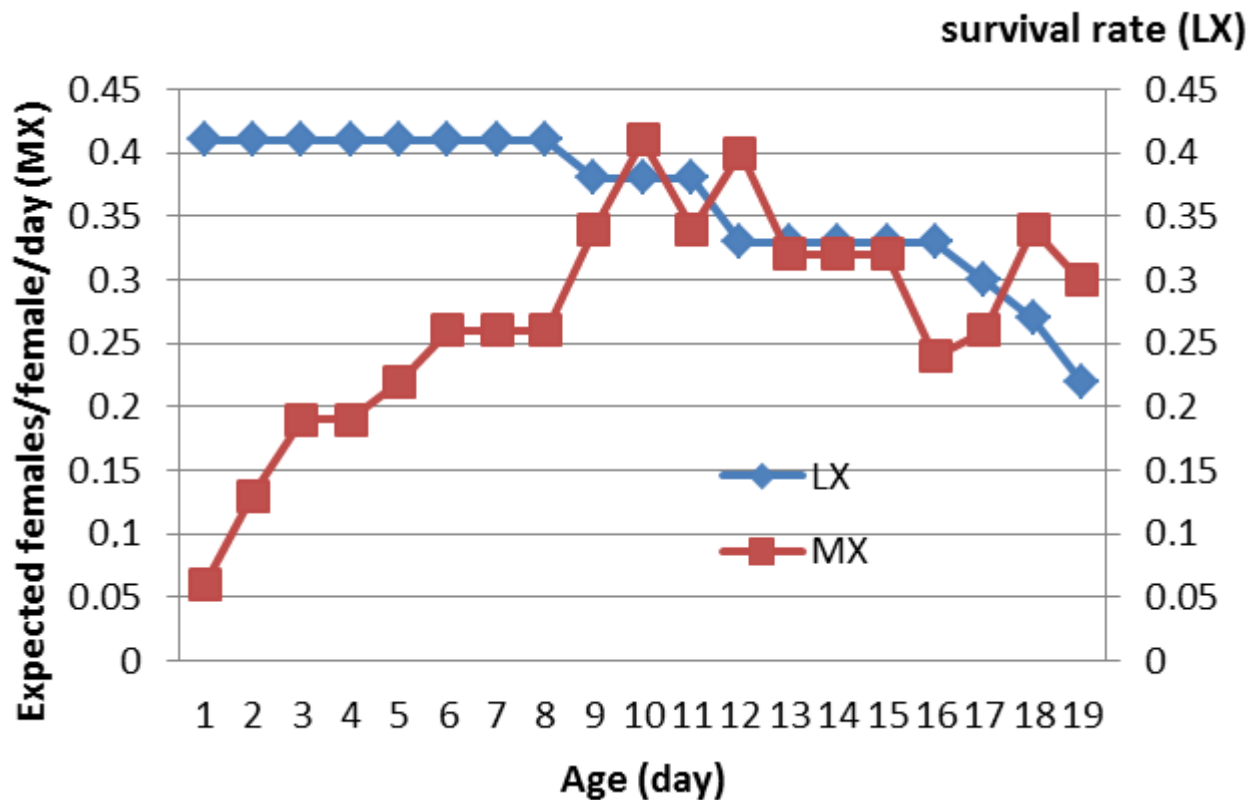
Effect of food types on life table parameters of parasitoid *A. galleriae*

Results obtained from life table parameters are presented in Table 5 and Figures 3 and 4. Tested food types affected survival rates of immature stages to adult stage. Low survival rate of 0.41 was obtained when parasitoid feeds on laboratory strain larvae of *G. mellonella* while 0.82 was obtained when parasitoid feeds on field strain larvae of *G. mellonella*.

Sex ratio (female / total) of 0.53 and 0.48 was obtained when parasitoid feeds on field and laboratory strain larvae of *G. mellonella*, respectively. The net reproductive rate (R_0) varied according to the food types used. (R_0) was 5.31 and 1.82 females/female when parasitoid fed on field and laboratory strain larvae of *G. mellonella*, respectively. The results showed that feeding on field strain larvae of *G. mellonella* is the most favorable food

Table 5. Effect of food types on life table parameters of parasitoid *A. galleria*.

Parameter	Feeding on field strain larvae of <i>G. mellonella</i>	Feeding on laboratory strain larvae of <i>G. mellonella</i>
Survival to maturity	0.82	0.41
Sex ratio (females/total)	0.52	0.48
The net reproductive rate (R_0)	5.31	1.82
Mean generation time (T)	28.0	29.7
The intrinsic rate of increase (r_m)	0.05	0.04
The finite rate of increase (λ)	1.05	1.04
Time of population doubling (DT)	13.83	14.33

**Figure 3.** Age- specific fecundity and survival rate of *A. galleria* fed on laboratory strain of *G. mellonella*. Where LX: Survival rate and MX: Female fecundity.

kind for reproduction of parasitoid; it has the highest value of net reproductive rate.

Evident differences were observed in the mean generation time (T) between the two food types. This period decreased as *G. mellonella* fed on field strain, but increased with the feeding on laboratory strain; the average values were 28.0 and 29.7 days, respectively. The intrinsic rates of increase (r_m) were 0.05 and 0.04 for field and laboratory strains, respectively. When these were converted into finite rates of increase (λ), the population capacity of *G. mellonella* increased. The values

were 1.05 and 1.04 times/female/day when *G. mellonella* larvae were reared on wax comb and artificial diet, respectively; showing the highest capacity to increase in field strain. Population doubling times (DT) were 13.83 and 14.33 days for field and laboratory strains, respectively. Results obtained indicated that wax comb was more favorable for feeding *G. mellonella* larvae. We note from the previous results that the feeding of the host (*G. mellonella* larvae) also affects the feeding of the parasitoid and therefore some biological aspects of this parasitoid. Also the nutrition type promotes the growth

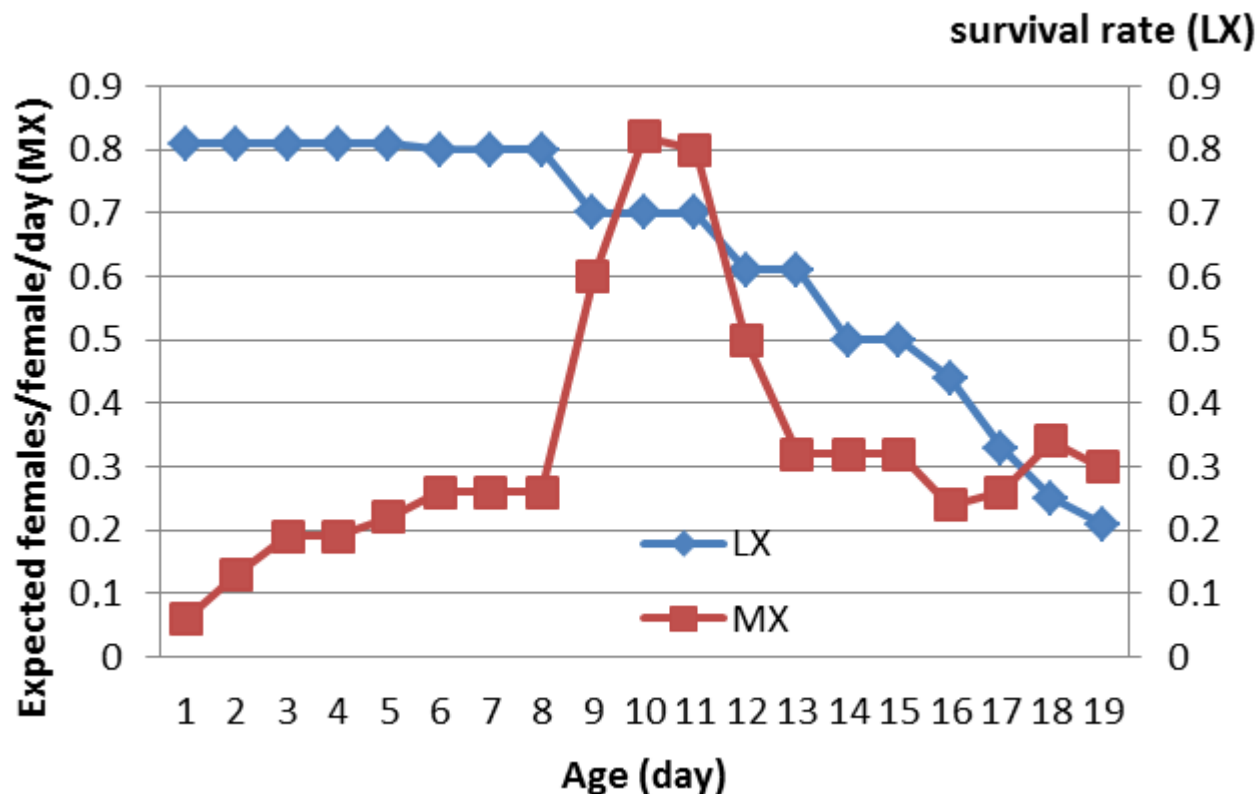


Figure 4. Age- specific fecundity and survival rate of *A. galleria* fed on field strain of *G. mellonella*. Where LX: Survival rate and MX: Female fecundity.

of both host and its parasitoid, where we find that nutrition of the old wax containing abundant quantities of good food ingredients promoted the growth of the host positively. This reflected on the sexual ratio. The net reproductive rate (R_0) reduced mean generation time and time of population doubling (DT) for its parasitoid.

These results agree with Adly and Marzouk (2019) who studied the biology of parasitoid *Bracon hebetor* Say. (Hymenoptera: Braconidae), on *G. mellonella* in laboratory, honeybee colonies, and stored wax combs. In addition, the results confirm those of Abo Abdalla and Gadelhak (2016) and Farag et al (2015) who studied the effects of natural food additives on the protein content of adult and immature stages of *A. galleriae*; it was shown that fecundity, sex ratio, progeny and parasitization ability vary according to parasitoid age. In addition, it has been shown that fecundity and sex ratio are affected by nutritional factors of host species. They explained that the differences in the fecundity, female sex ratio and longevity are affected by feeding on different host diets.

Conclusion

Generally, the positively differences in all biological parameters in rearing of the parasitoid *A. galleriae* on

laboratory and field strain of *G. mellonella*, may be related to the host nutrition of field strain and laboratory strain of *G. mellonella*. This relation was also found in behavior of parasitism in host insects. These effects include development, sex ration and longevity adults of parasitoid *G. mellonella* larvae feed on the old wax. Perhaps the components of the old wax, its pollen and its high protein contents may have a positive effect on the growth of the developmental stages of both wax larvae, and the parasitoids. These conclusions can be used in laboratory education and mass rearing of both *G. mellonella* and its parasitoid, *A. galleriae* that feeds on them.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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