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MASS REARING OF THE GREATER WAX MOTH, *Galleria mellonella* L.

Sabreen G. El-Gohary*, S.I. Yousif-Khalil, M.M.A. El-Maghraby and Sh.M. Abd-Alla

Plant Prot. Dept., Fac. Agric., Zagazig Univ., Egypt

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ABSTRACT: This study was conducted to investigate the suitability of three suggested artificial diets prepared for mass rearing of the greater wax moth, *Galleria mellonella* L. to meet with the higher demand for mass multiplication of entomopathogenic nematodes as well as many parasitoids for insect biological control purposes. The suggested diets were compared to the natural food (*i.e.* honey bee wax) under laboratory conditions. The effect of such feeding on some biological aspects of eggs, larvae, pupae and adults of *G. mellonella* was studied. Summarized results revealed diet D3 (4 parts scratched bee wax and 1 part bee collected clover pollen) proved the best followed by natural (control) feeding. The superiority of diet D3 was represented by: shortened larval duration, induced significantly larval survival (88.33%) and weight (186 mg/mature larva) and pupal weight, minimized pupal mortality (6.7%), induced female moth fecundity (1056.6 eggs per female) and egg fertility and hatchability (97.81%).

Key words: *Galleria mellonella*, artificial diet, larval survival, female fecundity, pupation, sex ratio, male longevity, pupal duration, larval duration periods.

INTRODUCTION

Wax moths are the most destructive pests of honeybee wax combs in storage and in working weak colonies allover the world (**Caron, 1992; Candel *et al*., 2003; Singh *et al*., 2014**). The gross symptoms of the infestation of wax moth are the tunnels made in the wax combs with white silk secretions, larval faeces and different stages linning of the pest. The greater wax moth *Galleria mellonella* L. is the main and the most destructive pest of bee wax combs, especially elder ones (**Jindra and Sehnal, 1989; Scoot-Dupree, 1999; Mohamed *et al*., 2014**). Adult and larvae of wax moth can also transfer the pathogens of serious honeybee diseases (**Charriere and Imdorf, 1997**).

However, mass rearing of wax moth had received (paid) greater attention in the recent years due to its importance as model insect greatly demanded (requested) as substrate for mass multiplication of the entomopathogenic nematodes (**Cardoso *et al*., 2007; Birah *et al*., 2008; Kulkarni *et al*., 2012; Metwally *et al*., 2012; Singh *et al*., 2014; Vanzyl and Malan, 2015**). In addition, wax moth larvae are needed for rearing many insect natural enemies such as *Microplitis* spp., *Archytas* spp., *Apanteles* spp. and *Bracon hepetor* L. (**Ashfaq *et al*., 2005**). Generally, wax moth nowadays are used for insect physiology and biochemistry insecticide screening and insect toxicology (**Wu *et al*., 2010**). Morever, **Ellis *et al*. (2013)** used it as model organism for studies in insect physiology, genomics and proteomic.

From this stand point, this work is an attempt to mass rear wax moth with low expenses as possible, to produce the highest possible number of healthy wax moth larvae with no side effects on the target insect model.

MATERIALS AND METHODS

The present investigation was carried out in Apiculture and Sericulture Research Laboratory of Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt during the period extended from 2014-2016.

* **Corresponding author: Tel. :** +201141956559
E-mail address: sabreenelgohary178@gmail.com

Source of the Greater Wax Moth and Culturing

Samples of honey bee wax combs infested with different stages of *Galleria mellonella* L. were collected from a private apiary located at El-Moullak locality, Abu Hammad district, Sharkia Governorate, Egypt. The collected samples were transferred in suitable black plastic bags to the laboratory. Infested wax combs were cut into suitable pieces then placed in clean glass jars covered with perforated covers (lid) and let so as culture to supply female and male adult moth for egg production.

Rearing of the Greater Wax Moth

During summer season, newly emerged adult moths (male and female) were kept in plastic jars of 5×30 cm provided with perforated plastic covers. A piece of suitable size of black muslin cloth was placed covering the bottom of each jar for egg laying under laboratory conditions (22-35^{o}C, 40- 65% RH). Every 24 hours, eggs laid were collected and transferred into another clean jar and left until egg hatching under the same conditions. Newly hatched larvae were counted every 20 neonates together as replicates and placed each in clean jar (5x15 cm) and provided with 40 g of one of the tested diets. These replicates were divided into four groups of three replicates each to investigate the efficiency of the tested diets for mass rearing of the greater wax moth.

Testing the Efficiency of the Diets

Three tested diets were suggested and prepared for rearing *G. mellonella* larvae. Control larvae were fed on honeybee wax comb. A piece of 7 × 8 cm of median age (3 years old) wax comb that weighed about 40 g was used. The test diets composition were presented in Table 1. Three replicates of 20 neonates each as previously mentioned were considered for each diet (D1, D2, D3). The biological parameters of different stages of *G. mellonella* were studied and recorded.

After adult emergence, one pair (male and female) were separated and kept in clean plastic jar to study the female fecundity, egg fertility and (%) hatching eggs.

Statistical Analysis

Data obtained were statistically analysed using ANOVA test (**Snedecor and Cochran, 1967**). The Computer Program **SAS (1999)** was followed for performing statistical analysis.

RESULTS AND DISCUSSION

The efficiency of three artificial diets and natural feeding on mass rearing of the greater wax moth, *G. mellonella* was studied. Results in detail are as follows:

Larval Stage

Larval duration period (day)

Results presented in Table 2 indicate that the larval duration period of the greater wax moth larvae reared on the diets D1, D2, D3 and bee wax comb (control) recorded 36.33, 36.60, 33.60 and 29.30 days, respectively. Analysis of variance indicated that the control larvae manifested the shortest significant mean larval duration. However the differences between the test diets were insignificant. Results of the present work are in general accordance with those of **Cardoso *et al.* (2007)** who reported that larval stage duration was in the range of 23.4-40.4 days according to rearing temperature. In addition, **Jun (2010)** found that, the larval stage duration recorded 38.60 days. The shorter duration period of the greater wax moth larvae could be attributed to the existenece of honey bee wax in their diets (D3 and control). Bee wax may contain in addition to the natural organic food materials, exuvae, remainders of larval food (bee bread), honey bee pheromone that enhance larval development.

In this respect, **Eischen and Dietz (1990)** reported that larval duration of wax moth larvae fed on a diet contained 5% pollen or combination with honey and bee wax became shorter by 2-5 days. The same trend was also reported by **Liu *et al.* (1997)** who recorded 6.8 days reduction in larval duration, where larvae were fed on Chinese honey bee combs as compared to those fed on natural feeding. However, **Swamy *et al.* (2009)** recorded longer larval duration period, when the greater wax moth larvae were arised on honey bee combs of *Apis cerana* L., *Apis dorsata* L., *Apis mellifera* L. and *Apis florae* L., recording 49.3, 54.2, 110.65 and 58.6 days, respectively. On the contrary, **Huang *et al.* (2010)** found that different formulas of artificial feed did not affect the developmental period.

Table 1. Ingredients of suggested artificial diets for mass rearing of the greater wax moth, *Galleria mellonella* L. under laboratory conditions (22- 35°C, 40- 65% RH and 24 hr. darkness)

Ingredient (g or ml)	Tested diet			
	D1	D2	D3	Bee wax (control)
Wheat flour	100	200	-	-
Corn flour	50	100	-	-
Wheat germ	50	-	-	-
Wheat bran	250	170	-	-
Dried brewers yeast	20	10	-	-
Milk powder	100	150	-	-
Clover pollen	-	-	100	-
Bee honey	180	200	-	-
Glycerine	120	200	-	-
Bee wax comb	-	-	-	40 g (8 x 7 cm)
Macerated bee wax comb	20	-	400	-

Table 2. Effect of the tested diets on some biological parameters of the greater wax moth, *Galleria melouella* L. larvae

Character	Initial Number	Duration period of larval stage (day)		Larval survival		Weight of mature larva (mg)	Cocooning (%)
Diet		Range	Mean	No.	(%)		
D1	60	32-40	36.33^{a}	36^{a}	60.00	140^{b}	91.9
D2	60	32-42	36.60^{a}	43^{b}	71.66	142^{b}	93.9
D3	60	28-39	33.60^{a}	53^{c}	88.33	186^{a}	97.4
Control	60	25-35	29.30^{b}	50^{c}	83.33	170^{a}	95.8
$LSD_{0.05}$			3.14	5.2		18.7	NS

Means in the same column not sharing letter are statistically different (P<0.05).

Larval Survival

Results in Table 2 clear that, the number of survived larvae from the 1st instar to maturity recorded 36, 43, 53 and 50 larvae for wax moth larvae reared on diets D1, D2, D3 and bee wax (control), respectively. The respective percent survival attained 60.00, 71.66, 88.33 and 83.33%. Analysis of variance revealed significant differences between means, except that between diet D3 and the control. Generally D1 showed the least survival, whereas D3 induced the highest survival. The higher larval survival could be attributed to the adequate supply of the main nutrients as well as pheromones and hormones necessary for good and rapid larval growth and development found in honey bee comb (control) in addition to clover pollens added to diet D3. In this respect, **Marston and Campbell (1973)** found that larval survival from the 1st instar larvae to maturity ranged between 28.7 – 94.2% according to the type of feeding. The same trend was also reported by **Mohamed and Coppel (1983)** recording percent mortalities of 48 in egg and early larval stage and 10% in later larvae of wax moth *G. mellonella.*

Weight of Mature Larvae (mg)

As shown in Table 2, the mean weight of mature wax moth larva fed on diets D1, D2, D3 and bee wax (control) recorded 140, 142, 186 and 170 mg, respectively. Statistical analysis revealed significant differences between means. Larvae fed on diet D3 showed the heaviest significant mean weight, whereas those fed on diet D1 revealed the lightest significant one. The higher weights of wax mature larvae could be attributed to the protein rich food (diet 3 and control). This statement is in accordance with that of **Singh *et al.* (2014)** who found that the diet contains 500 g bee wax comb out of 1000 g diet resulted in better biological parameters and that diet could be success fully used an even for commercial rearing of *G. mellonella*.

Percentage of Cocooning

Obtained results cleared that, the mean percentage of cocooning (cocoon spinning) recorded 91.9, 93.9, 97.4 and 95.8% for the greater wax moth larvae arised on diets D1, D2, D3 and bee wax (control), respectively, without any significant difference between means (Table 2).

Pupal Stage

Pupal percentage

Obtained results indicated that percent pupation recorded 88.88, 93.02, 96.22 and 94.00% for wax moth larvae arised on diets D1, D2, D3 and honey bee wax (control), respectively (Table 3). Statistical analysis revealed insignificant differences between means.

Pupal duration period (day)

As shown in Table 3, it is obvions that pupal duration period of the greater wax moth pupae arised from larvae fed on diets D1, D2 , D3 and bee wax comb ranged 7-12 , 8-14 , 8-13 and 9-15 days and averaged as much as 9, 10, 10.3 and 11 days, respectively. Statistical analysis revealed insignificant differences between the means. Obtained results are in accordance with those of **Swamy (2008) and Jun (2010)** who recorded 8.6 and 8.8 days for pupal duration, respectively. However, **Cardoso *et al.* (2007)** reported that longer pupal duration in relation to rearing temperature, recording 18, 15 and 12.2 days at 22, 27 and 32^{o}C, successively. In addition, **Huang *et al.* (2010)** reported that the tested different food formulas did not affect the developmental period of *G. mellonella*.

Pupal weight (mg)

Results presented in Table 3 indicate that, the mean weight of the greater wax moth cocoon spun by larvae arised on the diets D1, D2, D3 and the control (bee wax comb) averaged 154, 146, 193 and 197 mg, respectively.

Statistical analysis of data revealed significant differences between the weight of control and diet D3 pupae when compared to the pupae resulted from wax moth larvae fed on diets D1 and D2. However, insignificant differences were detected between diets D1 and D2 and between diet D3 and the control in this respect. The same trend was also reported by **Coskun *et al.* (2006), Birah *et al.* (2008) and Huang *et al.* (2010)**.

Pupal mortality

Results presented in Table 3 clear that, percent pupal mortality recorded 17.5, 14.0 , 6.7 and 7.3% for the greater wax moth pupae resulted from larvae arised on the diets D1, D2 , D3 and bee wax (control), respectively. Diets D1 and D2 caused highest significant means of pupal mortality percentage.

Table 3. Effect of the tested diets on some biological parameters of the greater wax moth, *Galleria melouella* L. pupae

Character	Pupation (%)		Pupal duration		Pupal weight (mg)	Pupal mortality (%)
Diet	No.	(%)	Range	Mean		
D1	32	88.88	7-12	9.0	154[a]	17.5[b]
D2	40	93.02	8-14	10.0	146[a]	14.0[b]
D3	51	96.22	8-13	10.3	193[b]	6.7[a]
Control	47	94.00	9-15	11.0	197[b]	7.3[a]
LSD $_{0.05}$	NS	NS		NS	22.6	6.1

Means in the same column not sharing letter are statistically different (P<0.05).

Adult Stage

Female fecundity (Number of eggs/ female)

As shown in Table 4, the mean number of eggs laid per one female moth of *G. mellonella* resulted from larvae arised on the diets D1, D2, D3 and traditional diet (bee wax) recorded 293.2, 847.6, 1056.6 and 610.0 eggs, respectively. Statistical analysis revealed significant differences between means. Diet D1 showed the least significant fecundity, whereas D3 manifested the highest significant one.

Egg fertility

Obtained results cleard that, the mean numbers of fertile eggs per female *G. mellonella* moth resulted from larvae arised on the diets D1, D2, D3 and control diet were 173.2, 613.2, 753.8 and 273.6 fertile eggs, respectively (Table 4). The corresponding percent fertilities were 59.07, 72.35, 71.34 and 44.85%. Analysis of variance demonstrated that D2 and D3 caused significantly higher number of fertile eggs/ female as compared to D1 and control diet (bee wax).

Egg hatchability

Results obtained indicated that, the mean number of hatched eggs recorded 75.2, 579.2, 737.3 and 261.7 eggs for eggs laid by female moth resulted from larvae arised on the diets D1, D2, D3 and bee wax (control), respectively. The respective percentage of hatchability recorded 43.42, 94.46, 97.81 and 95.65%. Analysis of variance of data cleared that D2 and D3 resulted in insignificant increase in the number of hatched eggs as compared to D1 and control. However, D3 manifested superiority in this respect (Table 4).

Egg incubation period (day)

Results obtained indicate that, the mean egg incubation period attained 6.3, 6.7, 6.1 and 6.5 days for the greater wax moth eggs laid per female moth arised from larvae fed on the diets D1, D2, D3 and honey bee wax (control), respectively (Table 4). Analysis of variance revealed insignificant differences between means.

Results reported in the literature are in parallel with the results of the present work. For instance, **Swamy (2008)** recorded incubation period means of 8.6, 8.6, 9.2 and 8.7 days when larvae were arised on honey bee wax combs of *A. cerana, A. dorsata*, *A. mellifera* and *A. florae*, respectively. Similarly, **Jun (2010)** recorded incubation period of 7.9 days. On the other hand, **Cardoso *et al.* (2007)** found that, rearing temperature has significant effect on egg incubation period recording 13.4, 8.4 and 6.8 days at 22, 27 and 32^{o}C, respectively. The total numbers of laid eggs in the present work are in partial accordance with those reported by other authors. For instance, **Mohamed and Coppel (1983)** recorded fecundity range of 650 – 1120 eggs per female. Similar trend was also reported by **Swamy (2008) and Jun (2010)** who recorded a mean of 790.90 ± 169.78 and 864.55 ± 98.6 eggs per female, successively. The varied results could be attributed to the varied food, rearing condition, the season of the year and the varied region with its varied ecological variables. As the author, is aware the unique interpretation to increasing or decreasing of the fecundity and egg fertility is the constituent of the diets of vital biological materials that may be pheromones, hormones, minor elements or vitamins that enhanced egg production in the ovary or and increasing the performance of the ovaries.

Table 4. Effect of the tested diets on fecundity, fertility, hatchability and egg incubation period of the greater wax moth, *Galleria mellonella* L. adult female

Character	Fecundity	Fertility		Hatchability		Egg incubation period (day)
Diet	No. of eggs/female	No. of fertile eggs/female	Fertile eggs (%)	No. of hatched eggs	Hatched eggs (%)	
D1	293.2 [c]	173.2[b]	59.07	75.2[b]	43.42	6.3
D2	847.6[b]	613.2[a]	72.35	579.2[a]	94.46	6.7
D3	1056.6[a]	753.8[a]	71.34	737.3[a]	97.81	6.1
Control	610 [b]	273.6[b]	44.85	261.7[b]	95.65	6.5
LSD $_{0.05}$	277.87	176.71		177.13		NS

Means in the same column not sharing letter are statistically different (P<0.05).

Sex ratio

As shown in Table 5, the mean sex ratio expressed as the percentage of male/female moth recorded 0.78, 0.76, 1.03 and 0.87% for the adult moth resulted from wax moth larvae reared on diets D1, D2, D3 and bee (control), respectively. The differences between means were insignificant. The same trend was also reported by **Marston and Campbell (1973)** who tested 9 diets for rearing *G. mellonella* indicating that the differences between the tested diets in respect of adult sex ratio were not significant.

Male Moth Longevity

Obtained results indicated that the mean longevity of male moth resulted from wax moth larvae reared on the diets D1, D2, D3 and bee wax (control) recorded 6.0, 7.3, 10.1 and 9.6 days, respectively (Table 5). Analysis of variance revealed significant differences between the means, where diet D1 showed the shortest significant male longevity. In this respect, **Swamy (2008)** studied in biology of *G. mellonella*, and indicated that the adult male lived for 16.4 ± 2.69 days. Also, **Jun (2010)** found that *G. mellonella* male moth longevity attained 11.2 days.

Female Moth Longevity

Pre-oviposition period

Obtained results cleared that the mean pre-oviposition period of female moth resulted from wax moth larvae fed on the diets D1, D2, D3 and bee wax (control) recorded 1.3, 2.0, 3.3 and 2.6 days, respectively (Table 5). Significant differences were noticed between the means.

Oviposition period

The mean oviposition period of female moth resulted from wax moth larvae fed on the diets D1, D2, D3 and bee wax (control) attained 2.3, 5.0, 6.6 and 7.0 days, respectively (Table 5). Analysis of variance indicated that diet D1 resulted in the shortest significant mean oviposition period, and the other differences were insignificant.

Post-oviposition period

The mean post-oviposition period reached 11.3, 13.0, 15.0 and 18.6 days for wax moth females resulted from the larvae arised on diets D1, D2, D3 and bee wax (control), respectively. Statistical analysis revealed significant differences between means where the longest significant post-oviposition period was recorded for control female while the shortest significant one was recorded for those arised on diet D1.

Total female moth longevity

Results presented in Table 5 indicate that the mean total female moth longevity attained 14.9, 20.0, 24.9 and 28.2 days for the moth arised from wax moth larvae reared on diets D1, D2, D3 and bee wax (control), respectively. Statistical analysis cleared that diet D1 caused

Table 5. Effect of the tested diets on sex ratio and longevity male and female moth of the greater was moth, *Galleria mellonella* L.

Character / Diet	Sex ratio	Male longevity (day)	Female longevity (day)			
			Pre-oviposition period	Oviposition period	Post-oviposition period	Total
D1	0.78	6.0^{b}	1.3^{b}	2.3^{b}	11.3^{c}	14.9^{b}
D2	0.76	7.3^{a}	2.0^{bc}	5.0^{a}	13.0^{b}	20.0^{a}
D3	1.03	10.1^{a}	3.3^{a}	6.6^{a}	15.0^{b}	24.9^{a}
Control	0.87	9.6^{a}	2.6^{ab}	7.0^{a}	18.6^{a}	28.2^{a}
LSD $_{0.05}$	NS	2.89	1.03	2.35	2.89	4.87

Means in the same column not sharing letter are statistically different (P<0.05).

the least significant mean female moth longevity, and the other differences were insignificant. In this connection, **Swamy (2008)** recorded noticeable shorter female moth longevity (6.9 ± 0.7 days), he added that, the pre-oviposition, oviposition and post-oviposition period were 1.1 ± 0.3, 4.6 ± 0.66 and 1.2 ± 0.4 days, respectively. **Jun (2010)** came to the same female moth longevity recording 6.7 days. These variations could be attributed to the type of food and rearing conditions that may be different.

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التـــربيـــة المكثفـــة لـــدودة الشـــمع الكبيـــــرة

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محمد مصطفى احمد المغربي - شوقي محمود عبدالله

قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق - مصر

أُجرى هذا البحث لدراسة مدى ملائمة ثلاث وجبات مختلفة لتغذية دودة الشمع الكبيرة في تربية مكثفة لمجابهة الاحتياجات المتزايدة للتربية الموسعة للنيماتودا المتطفلة على الحشرات وبعض الطفيليات الحشرية لأغراض المكافحة البيولوجية للحشرات، تم مقارنة الوجبات المقترحة بالتغذية الطبيعية (شمع النحل) تحت ظروف المعمل، وتم دراسة بعض النواحي البيولوجية للبيض واليرقات والعذارى والحشرات الكاملة لدودة الشمع الكبيرة التي تم تربيتها على الوجبات المقترحة، ويمكن تلخيص النتائج كما يلي: أوضحت النتائج أن الوجبة المكونة من ٤ أجزاء فتات شمع نحل و١ جزء حبوب لقاح برسيم من جمع النحل كانت الأفضل من بين الوجبات تليها في الأفضلية الكنترول (شمع النحل) وتمثلت الأفضلية فيما يلي: قصر فترة الطور اليرقي، زيادة معنوية نسبة بقاء اليرقات (٨٨,٣٣%) ووزن اليرقة البالغة (١٨٦ مجم/يرقة بالغة) ووزن العذراء، وقللت نسبة الموت في العذراء للحد الأدنى (٦,٧%)، وأدت إلى زيادة إنتاجية الفراشة الأنثى من البيض (١٠٥٦,٦ بيضة لكل أنثى) ونسبة خصوبة البيض ونسبة فقسه (٩٧,٨١%).

المحكمـــــون:

١- أ.د. عبدالبديع عبدالحميد غـــانـم أستاذ الحشرات الاقتصادية المتفرغ – كلية الزراعة – جامعة المنصورة.

٢- أ.د. محمد علي محمد عبده الديب أستاذ الحشرات الاقتصادية المتفرغ – كلية لزراعة – جامعة الزقازيق.