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BIOLOGY OF GREATER WAX MOTH, *GALLERIA MELLONELLA* L. DURING DEARTH PERIOD

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ABSTRACT

Eggs of greater wax moth, *Galleria mellonella* L. are small sized, spherical, whitish at early stage and creamish to light brownish colour at later stages. Instar wise average length (mm), width (mm) and head capsule size (mm) respectively were 1.10 ± 0.10 mm, 0.23 ± 0.06 mm and 0.20 ± 0.02 mm (1st instar); 1.96 ± 0.02mm, 0.33 ± 0.06 mm and 0.33 ± 0.02 mm (2nd instar); 4.46 ± 0.35 mm, 0.37 ± 0.14 mm and 0.37 ± 0.01 mm (3rd instar); 6.33 ± 0.55 mm, 0.68 ± 0.31 mm and 0.66 ± 0.02 mm (4th instar); 7.57 ± 0.45 mm, 2.33 ± 0.58 mm and 2.13 ± 0.12 mm (5th instar); 15.47 ± 0.60 mm, 2.73 ± 0.81 mm and 2.70 ± 0.02 mm (6th instar) and 20.97 ± 0.57 mm, 2.73 ± 1.27 mm and 2.71 ± 0.01 mm (7th instar). Female pupal developmental period was 10.67 ± 0.58 days as compared to 9.33 ± 0.58 days in male. Adult male moth was observed to be smaller in size than the female. Fore wings of male moths were light grey in colour while slightly deeper grey colour in female moths, and had the filiform type long antenna. Length of adult female was 18.03 ± 2.00 mm with 27.23 ± 2.54 mm wing span as compared to 12.77 ± 0.80 mm length and 16.03 ± 0.95 mm wings span in male. Female lifespan was 11.33 ± 0.58 days and male was 23.67 ± 0.58 days. A female laid 1063.33 ± 74.89 eggs in her life-span where 196.60 ± 45.19 eggs were laid per day.

Key words: Greater wax moth, *Galleria mellonella*, Morphometrics, Biology, *Apis mellifera*

Beekeeping is an additional occupation and source of income for farm-families (Anonymous, 2019a). Out of total honey production in India, Bihar produces 10,800 metric tonnes honey that contributes 9.52% (2018-19) (Anonymous, 2019b). But, this industry is incessantly affected by a number of diseases and enemy problems. Few become more devastating such as wax moths, wasps, mites, etc. (Gulati and Kaushik, 2004; Vishwakarma et al., 2012). Based on the survey of 30 beekeepers from different corners of Bihar, the infestation of wax moth and varroa mite were ranked 1st and 2nd on seriousness order (Kumar, 2018). However, there are two different genera of wax moth, greater (*Galleria mellonella* L., Pyralidae: Lepidoptera) and lesser (*Achroia grisella* F., Pyralidae: Lepidoptera) most disturbing and economically important pests of combs and hive products of *Apis mellifera* in tropical and subtropical regions (Nagaraja and Rajagopal, 2009). In Bihar, the peak incidence of greater wax moth was observed during August, September and October (Mandal and Vishwakarma, 2016). The wax moth caterpillars feed on combs, pollen, larval exuviae and other proteinaceous substances (Caron, 1999). Besides these, adult larvae and adult wax moths play an important role in transfer of pathogens of honey bee foulbrood disease (Owayss and Abd-Elgayed, 2007).

For minimizing the infestation and losses from the greater wax moth under stored combs and in live bee colonies, use of synthetic chemicals resulted in harmful effects on honey bees, environment and human health (Singh, 1975), whereas, the use of plant products resulted in 48% mortality of *G. mellonella* in stored combs (Mandal and Vishwakarma, 2018). Still, there is need to develop some eco-friendly approaches to manage wax moth infestation in live bee colonies. Though, only few attempts have been made on morphometric and biology of greater wax moth (*G. mellonella*) infesting *A. mellifera* colonies from southern Bihar. Therefore, the present investigation was aimed to know the biology of *G. mellonella* in changing environmental conditions that may enormously be helpful for beekeepers to take care against wax moth infestation in honeybee colonies.

MATERIALS AND METHODS

Experiments were carried out at the Beekeeping-cum-Honey Production Unit and Laboratory of the

Department of Entomology, Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur, Bihar during 2018-19, located at 25º15' N to know the biology of *G. mellonella* during changing environmental conditions. For conducting the experiments mass cultures of different life stages of greater wax moth were required. To initiate the mass rearing of greater wax moth under laboratory conditions, culture of infested combs (without bees) along with larvae, pupae and adults of wax moth were collected from the bee hives of the college apiary. The collected larvae were fed with the artificial diets in plastic containers of size - 21 x 15 x 10 cm, with holes in the covers. The artificial diets containing wheat bran (500 g), ground honey bee comb (200 g), glycerine (300 ml), tap water (150 ml) and honey (150 ml) were mixed and fed to the immature stages of wax moth. However, the ingredients incorporated in the taken artificial diets with slight modifications as suggested by Coskun et al. (2006).

Different larval instars were separately reared in plastic containers on artificial diets which were covered with muslin cloths. Fully grown larvae were placed in empty bee hives along with sufficient quantity of artificial diets for pupation. After adult emergence, a pair of male and female moth was released separately into widened mouth plastic jars of size 20 x 50 cm for mating and further laying of eggs. The method was followed as suggested by Mohamed et al. (2014) and Ellis et al. (2013). Few microscopic slides were pasted at the top of plastic jars for facilitating egg laying activity (Adeline L. Jorjao et al., 2018).

The shape, colour and size of eggs, larval instar, prepupa, pupa, cocoon and adults of wax moth were observed using ocular micrometer, stage micrometer and general scale with the help of binocular stereoscopic microscope for identification. The egg laying patterns and fecundity rate per female were observed. Observations on hatching period of eggs, larval, prepupal, pupal, oviposition period were also recorded to know the developmental period of *G. mellonella*. Observed data were subjected to analysis of the standard mean and standard deviation at 5% level of probability and simple correlation using standard procedure as given by Gomez and Gomez (1984) and as per procedure given for data analysis in Microsoft Office Excel 2007 version.

RESULTS AND DISCUSSION

Egg: Eggs are very small, spherical and whitish in colour at early stage and later turn in creamish light to brownish colour when exposed into normal atmosphere. Eggs are laid in form of clusters at the top of jars on microscopic slides as provided for laying purpose and fixed there with excreted of glued materials. On the other hand, observations on egg laying pattern in bee hives were also taken, where eggs are deposited at different places such as edge of frames, cracks and crevices present inside the bee hives. The present finding is agreed with the findings of Hanumanthaswamy (2008) who reported that the eggs of greater wax moth were small, elliptical and pearly white in colour and laid in mass having glued materials in cracks and crevices of hives under nested conditions. The average egg length and width was 0.41 ± 0.01 mm and 0.23 ± 0.02 mm, respectively (Table 1), which is almost similar with the findings of Hanumanthaswamy (2008) i.e. 0.44 ± 0.02 mm long, 0.29 ±0.02 mm broad. The egg diameter may be influenced by provided diet and weather parameters. Hatching period was observed as 3.17 ± 0.29 days and normally occurred in morning hours (Table 1), while Hanumanthaswamy (2008) reported that the incubation period was 8.6 ±0.48 days. The oviposition period was also observed that occurred during night hours. Hanumanthaswamy (2008) reported that the oviposition occurred during night hours between 19.00 to 03.00 h and hatching occurred during morning hours between 8-11 AM, while an average egg length and width varied from 0.478 mm and 0.394 mm, respectively (Kwadha et al., 2017).

Larva: Newly hatched larvae appear whitish in colour that gradually turn dirty coloured, and start moving in search of food inside the bee wax combs. They usually move towards the mid rib or base of wax comb and start feeding from there. The wax combs are badly destroyed by the developing larvae through making of tunnels and formation of web like structures by mixing of excreta with excreted proteineous substances. There were seven larval instars observed during the investigation. Findings of the present worker is corroborated with results as reported by Venkatesh Hosamani et al. (2017) and Hanumanthaswamy (2008) in respect of body colour and feeding behaviour of greater wax moths. The growth rate of the larvae depends upon the temperature and the nature of food.

Instar wise average length (mm), width (mm) and head capsule size (mm) were respectively 1.10 ± 0.10 mm, 0.23 ± 0.06 mm and 0.20 ± 0.02 mm for 1st instar; 1.96 ± 0.02 mm, 0.33 ± 0.06 mm and 0.33 ± 0.02 mm for 2nd instar; 4.46 ± 0.35 mm, 0.37 ± 0.14 mm and 0.37 ± 0.01 mm for 3rd instar; 6.33 ± 0.55 mm, 0.68 ± 0.31 mm

and 0.66 ± 0.02 mm for 4th instar; 7.57 ± 0.45 mm, 2.33 ± 0.58 mm and 2.13 ± 0.12 mm for 5th instar; 15.47 ± 0.60 mm, 2.73 ± 0.81 mm and 2.70 ± 0.02 mm for 6th instar and 20.97 ± 0.57 mm, 2.73 ± 1.27 mm and 2.71 ± 0.01 mm for 7th instar larvae (Table 1). After hatching, the wax moth larvae were approximately 1–3 mm in length and 0.12–0.15 mm width in diameter as per the findings of Paddock (1918) and Smith (1965), while final instar larvae were about 25–30 mm in length and 5–7 mm in width diameter, respectively. They also reported that the larva was creamy white in colour, with its sclerotized body parts which darken with each moulting.

Average body length (mm) and width of head capsule (mm) of seven different larval instars of *G. mellonella* was significantly positively correlated with the width of head capsule (Table 2). However, the mean body length of different instars and width of head capsule although were positively correlated with the developmental period of the mentioned instars but the correlation values were not statistically significant. The body length of third, sixth and seventh instars larvae were observed quite high as compared to others. However, massive increase in the body size of third, sixth and seventh larvae indicate as more devastating

Table 1. Morphometrics and developmental periods of life stages of *G. mellonella*

Life stages	Length (mm) Mean ± SD	Width (mm) Mean ± SD	Head capsule (mm) Mean ± SD	Wing expanse (mm) Mean ± SD	Duration (Days) Mean ± SD
Egg	0.41 ± 0.01	0.23 ± 0.02	-	-	3.17 ± 0.29
Larva					
1st Instar	1.10 ± 0.10	0.23 ± 0.06	0.20 ± 0.02	-	2.83 ± 0.58
2nd Instar	1.96 ± 0.02	0.33 ± 0.06	0.33 ± 0.02	-	2.17 ± 0.29
3rd Instar	4.46 ± 0.35	0.37 ± 0.14	0.37 ± 0.01	-	4.17 ± 0.29
4th Instar	6.33 ± 0.55	0.68 ± 0.31	0.66 ± 0.02	-	8.33 ± 0.58
5th Instar	7.57 ± 0.45	2.33 ± 0.58	2.13 ± 0.12	-	7.00 ± 0.50
6th Instar	15.47 ± 0.60	2.73 ± 0.81	2.70 ± 0.02	-	6.33 ± 0.58
7th Instar	20.97 ± 0.57	2.73 ± 1.27	2.71 ± 0.01	-	5.00 ± 0.50
Prepupa	17.43 ± 1.50	3.93 ± 1.21	-	-	4.83 ± 0.29
Female pupa with cocoon	20.60 ± 2.51	4.73 ± 1.21	-	-	10.67 ± 0.58
Male pupa with cocoon	12.53 ± 2.50	2.93 ± 0.90	-	-	9.33 ± 0.58
Adult					
Female	18.03 ± 2.00	-	-	27.23 ± 2.54	11.33 ± 0.58
Male	12.77 ± 0.80	-	-	16.03 ± 0.95	23.67 ± 0.58
Fecundity rate (eggs/day/female)	-	-	-	-	196.60 ± 45.19
Fecundity rate (eggs/female)	-	-	-	-	1063.33 ± 74.89

Table 2. Correlation coefficients- body length, width of head capsule, developmental period of *G. mellonella*

Instars	Mean length (mm)	Mean width of head capsule (mm)	Developmental period (days)	Correlation values
1st	1.00	0.20	2.83	0.78* (Between length and width)
2nd	1.96	0.30	2.17	
3rd	4.49	0.31	4.17	
4th	7.61	0.51	8.33	0.47* (Between length and developmental period)
5th	6.30	2.00	7.00	
6th	6.30	2.00	6.33	
7th	15.40	2.60	5.00	0.43* (Between width and developmental period)

* Significant at p= <0.05

as compared to other stages/instars. The duration of 1st, 2nd, 3rd, 4th, 5th, 6th and 7th larval instars was 2.83 ± 0.58, 2.17 ± 0.29, 4.17 ± 0.29, 8.33 ± 0.58, 7.00 ± 0.50, 6.33 ± 0.58 and 5.00 ± 0.50 days, respectively. A total of seven successive larval instars were observed and developmental period was observed for 36 days (Table 1).

Though, Sehnal (1966), Anderson and Mignat (1970), and Hanumanthaswamy (2008) reported that there were seven successive larval instars occupied and the developmental period was 4.40 ± 0.48, 5.20 ±0.4, 6.5 ±0.67, 7.3 ±0.45, 8.3 ±0.45, 8.4 ±0.66 and 9.2 ±0.4 days, respectively, eventually total larval duration was 49.3 ±1.62 days. The observed results are in agreement with the findings of Venkatesh Hosamani et al. (2017) that the seven successive larval instars had duration of 4.50± 0.49, 5.30± 0.50, 6.60± 0.68, 7.30± 0.50, 8.30± 0.45, 8.50± 0.67 and 9.30± 0.40 days, respectively, however total larval duration was 50.3± 3.40 days. Finally, total larval period was observed for 36 days which is quite similar with findings of Fasasi and Malaka (2006) i.e. 34.9- 37.6 days, Elsawaf (1950) i.e. 39-62 days and Sehnal (1966) i.e. 22-27 days.

Prepupa: The mature larvae start moving in search of suitable place for pupation and scrape the chosen surface slightly prior pupation. They prefer to enter into cracks and crevices or corners inside hive for pupation. Fully developed larva shrinks in size up to some extent and builds a cocoon by mixing of secreted silk like structures and excreta for pupation. The cocoon was spindle shaped, extended, hard and fibrous. Prepupal length (mm) and width (mm) was observed to be 17.43 ± 1.50 and 3.93 ± 1.21, respectively, with developmental period of 4.83 ± 0.29 days (Table 1). Correspondingly, the duration of prepupa of greater wax moth was 2.10 ± 0.53 days; mean length was 22.85 ± 1.95 mm and mean breadth was 8.05± 0.92 mm as reported by Hanumanthaswamy (2008) and 2.20 ± 0.53 days as reported by Venkatesh Hosamani et al. (2017).

Pupa: The pupa was observed to be obtect type; initially pale in colour later turning dark brown in colour when seen without cocoon. A row of small spines were observed at the back of head and extended to abdominal segment. Female pupa have a cloven sternum on its eighth abdominal segment while it is absent in male and have a small pair of external rounded knobs on the ventral side of ninth abdominal segment. The average length was observed to be 20.60 ± 2.51 mm and 12.53 ± 2.50 mm and breadth was 4.73 ± 1.21 mm and 2.93 ± 0.90 mm of female and male pupa with cocoon, respectively (Table 1). The male pupae were normally found smaller than the female.

Hanumanthaswamy (2008) reported the pupa was obtect type and pale coloured which gradually attained dark brown colour towards its maturity, and the mean length (mm) and width (mm) of pupae was 13.97 ± 0.58 and 4.25 ± 0.29, respectively. The greater wax moth pupa was observed to be 12–20 mm in length and 5–7mm in diameter as reported by Ellis et al. (2013). The female pupal development period was 10.67 ± 0.58 days while for male it was 9.33 ± 0.58 days (Table 1). The results observed by the present worker may likely be similar as reported by Elsawaf (1950), who reported that the pupal duration lasts for 8-11 days during May-June generations and 12-49 days during overwintering generations, whereas Opoosun and Odebiyi (2009) reported that the pupal period for 25.4 days, Hanumanthaswamy (2008) for 8.6 ± 0.73 days, Sehnal (1966) for 6.5- 8 days and Kannagara (1940) for 8-9 days, respectively. The minor differences could be attributed to the change in the weather parameters and provided diets.

Adult: Emergence of adult moths from the pupae occurred during late evening or night hours. The perkiness of moths was observed quite well after two days of emergence. The fore wings of the both sexes were observed to be grey coloured at early stage while it was little deep grey coloured as observed in female. The hind wings of both sexes were observed to be bronze coloured and usually concealed. The size and body colour of adults can differ according to the nature and quantity of food ingested during larval stage. The antenna was observed to be filiform type. Length of female moth varied from 18.03 ± 2.00 mm with a range of 27.23 ± 2.54 mm wing expanse, while it was 12.77 ± 0.80 mm with 16.03 ± 0.95 mm wing expanses in male moth (Table 1). The findings of the present worker is quite similar with the report of Venkatesh Hosamani et al. (2017), who reported wing expanse of female wax moth was 25.20±1.22 mm, wherever 24.60±0.53 mm in male wax moth.

The body length of female wax moth was 15-20 mm with 31 mm wingspan and 169 mg body weight as reported by Williams (1997) and Ellis et al. (2013). The life span of female and male wax moth was observed to be 11.33 ± 0.58 days and 23.67 ± 0.58 days, respectively (Table 1). As a result, the male lifespan is two times more than the female, which may be due to the change

in quality and quantity of food consumed during the larval stage alongside the weather parameters. The present result correlates with the findings of Venkatesh Hosamani et al. (2017), who reported that the male and female wax moth lived for 16.50 ± 2.70 days and 6.88 ± 0.7 days, respectively, while it was 21 days and 12 days as reported by Warren and Huddleston (1962).

Fecundity: After their emergence, adult male and female moths were placed into a plastic container for facilitating mating which took place within two days. The female started laying eggs in clusters after mating for 24 hours. Eggs were deposited at the top and darker portion inside the plastic jars. The egg laying generally occurred during evening or night hours. A single female laid 1063.33 ± 74.89 eggs in cluster in her life-span of 11 days. On an average, a single female laid 196.60 ± 45.19 eggs per day per cluster with at least 5 to 7 egg clusters in her life span (Table 1).

The fecundity rates of female moth may be influenced by weather parameters and quality of diets ingested during developmental periods at different life stages. The achieved results are corroborated with the findings of Venkatesh Hosamani et al. (2017), who reported that a greater wax moth female lays 760.9±168.88 eggs in her lifespan with 161.80±45.87 eggs / female / day. A wax moth female laid 705 eggs in her lifespan as reported by Warren and Huddleston (1962), while 236.0 - 304.3 eggs/ female/ day laid either singly or in batches of 2-80 as reported by Chandel et al. (2003). A female greater wax moth laid 107-297 creamy eggs per day (Fasasi and Malaka, 2006) and 312 eggs per day (Viraktamath, 1989).

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