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Post embryonic development of *Galleria mellonella* L. and its management strategy

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Abstract

The present investigation has been carried out to study the post embryonic development of *Galleria mellonella* L. and its management strategy at apiary Laboratory of Department of Entomology, Assam Agricultural University, Jorhat during 2001-02. The results showed that the egg measured to be 0.39 ± 0.4 mm and 0.5 ± 0.02 mm in length and width respectively and the duration was found to be 3-10 days. The larval and prepupal duration were recorded to be 22-69 days and of 3-12 days respectively. The pupa were found to be 14.32 ± 0.15 mm in length and 4.75 ± 0.08 mm in width lasting for 8-10 days. The adult longevity was 11.0 ± 1.15 days for male and 22.0 ± 0.35 days for female. The effective management strategy was found to be with the treatment of *Bt*. formulation var. *Karstaki* @1 percent per hive followed by neem oil 3% and sulphur fumigation 28 g/hive.

Keywords: *Galleria mellonella*, post embryonic development, management strategy

1. Introduction

Honey bees are the integral part of ecosystem and have an impact on both forest and agricultural ecosystem. About one third of the human diet comes from insect pollinated plants and honey bees account for 80 percent of pollination [1]. Honey bees are subjected for infestation by many pest and natural enemies. Greater wax moth, *Galleria mellonella* L. has been reported to be most serious pests of honey bees. The extent of wax moth infestation is reported to be as high as 48.7 percent [2]. The wax moth is generally found throughout the year with a peak population during May to August which is coincided with the dearth period for honeybee [3].

Post embryonic development of insects involved different life stages from egg eclosion to the adult emergence which is characterised by conspicuous increase in size and more or less change in form [4]. *Galleria mellonella* is a holometabolous pest undergoing all stages of metamorphosis. For the management of this pest, various management strategies have been adopted such as chemical and non-chemical methods have been developed including freezing, heating and CO₂ fumigation. The chemical includes fumigants such as phostoxin and sulphur were found as effective against larvae and pupae of *Galleria mellonella* [5]. The information on post embryonic development of *G. mellonella* is scanty hence the present investigation has been carried out to study the post embryonic development as well as to evolve effective management strategy of this pest.

2. Materials and Methods

Post embryonic development of *Galleria mellonella* L., a serious pest of honey bee has been carried out in the Apiary and apiculture Laboratory of Department of Entomology, Assam Agricultural University, Jorhat during 2001-02 and validated in 2015-16.

2.1 Larval and pupal period

To study the larval period of *Galleria mellonella*, the technique of Gupta [6] was followed. Five numbers of freshly hatched larvae were transferred with the help of camel hair brush to the petridishes and were provided with pieces of comb. The young larvae entered inside the comb and start feeding. The piece of comb was changed every alternate day and careful examination of the comb was made. To determine the pupal period, atleast ten numbers of sixth instar larvae were kept undisturbed in the petridishes of 6cm x 15cm size. Each larva was kept singly in separate petridishes. The adult emergence was recorded from the puparium. Morphometry was worked out by measuring different stages of the pest and expressed in millimeter (mm)

In the management strategy, various plant products such as *Azadirachta indica*, *Melia azadirachta*, neem oil, neem seed kernel extract (NSKE) and *Karanj* oil were treated to the comb as described by Haq *et al.*, with certain modifications [7]. *Azadirachta indica* at a concentration of 15g and 20g per hive, *Melia azedarach* at a concentration of 15g and 20 g; neem oil at a concentration of 2% and 3%, NSKE at a concentration of 3% and 5% and *Karanj* oil at a concentration of 2% and 3%, were sprayed over the entire surface of the comb by using an atomizer. The technique of Battu and Singh was followed to study the efficacy of bio agent Bt. var *Karstaki* against wax moth. *Karstaki* at a concentration of 0.5 percent and 1 percent which was sprayed over the entire surface of the comb by using atomizer [8]. For providing artificial cold treatment technique of Gulati and Kaushik, was followed. The frames of honey combs were kept in deep freezer at a temperature of -7 °C and 12 °C for 5 hours. The treated combs were then kept inside the boxes and observations were taken [9]

2.2 Statistical analysis

The data were statistically analysed and significance or non significance was judged with the help of 'F' test and critical difference at 5% level was worked out.

3. Results

The post embryonic development of *Galleria mellonella* involved egg eclosion to the emergence of adult and was studied from March to November (2015-16). The results were presented for developmental period of various stages of *G. mellonella* in Table 1 and measurements of different stages such as larvae, pupa are presented in Table 2.

3.1 Egg

The eggs of *Galleria mellonella* were spherical or slightly oblong and smooth. The freshly laid eggs were creamy white and eggs were measured to be 0.32 to 0.47mm (0.39±0.04mm and 0.45 to 0.55mm (0.50±0.02mm) in width Table 2. The colour of the egg changed from white to brownish after hatching.

3.2 Larva

The larvae were eruciform, freshly hatched larvae were white and later turned to dirty grey with brownish head. The larval period in various generations were found to be 30-34 days with an average of 33.34±2.86 days in the first generation, 24 to 27 days with an average of 25.34±1.48 days in second generation, 22 to 25 days with an average of 23.34±1.28 days in third generation, 25-27 days with an average of 26.84±2.66 in fourth generation and 50 -69 days with an average of 56.99±2.00 on fifth generation. The length of first instar larvae varied from 1.90 to 2.30mm (2.04±0.04 mm) and the width of the body varied from 0.36 to 0.39 mm (0.37±0.004 mm). The length of the head capsule varied from 0.43 to 0.46mm (0.43±0.003 mm) and width from 0.33 to 0.36mm (0.34 ±0.004 mm). In the second instar the length and width were 6.50 to 8.50 mm (8.01±0.21 mm) and 0.05 to 1.12 mm (1.07±0.03 mm), while the head capsule varied from 0.54 to 0.57 mm (0.56±0.007 mm in length and 0.46 to 0.54 mm (0.51±0.001 mm) in width. The length of third instar larvae was 9.50 to 11.50(10.80±0.19mm) and the width varied from 1.20 to 1.30mm(1.24±0.01 mm) and the length and width of head capsule were 0.79 to 0.93mm(0.86±0.03mm) and 0.64 to 0.86mm (0.77±0.02 mm). In fourth instar, length increased to 12.00 to 12.60mm (12.41±0.07mm) and width 1.40 to 1.55 mm (1.49±0.01 mm) and head capsule increased to 1.08 to

1.18 mm (1.12±0.01 mm) in length and 0.93 to 1.04 mm (0.99±0.01mm) in width. Again, in fifth instar the length was found to 18.00 to 19.50 mm(19.00±0.15mm), width 2.00 to 2.16(2.06±0.01 mm) with head capsule 1.33 to 1.51 (1.43±0.02mm) length and width of 1.15 to 1.36mm(1.24±0.02mm). In the sixth instar the larvae varied from 21.00 to 24.10 mm(22.60±0.14 mm) and the width varied from 4.00 to 5.00 mm(4.50±0.16 mm) with the head capsule length of 1.67 to 2.00 mm(1.97±0.00 mm) and width 1.33 to 1.57 mm(1.39±0.05 mm). The various measurements of head capsule indicated the growth of status of the pest (Table 2).

3.3 Pre-pupa

The pre pupal period was found to vary from 5-6 days with an average of 5.5±0.55 days in the first generation, 3 to 4 days with an average of 4.00±0.15 days in the second generation, 3 to 4 days with an average of 3.83±0.41 days in third generation, 4 to 5 days in fourth generation and 9 to 12 days in fifth generation. In the pre pupal days there was reduction in the body size of the larvae and the larvae were smoother. The larvae remained inside a web, stopped feeding and become inactive. The length varied from 19.00 to 23.00mm (21.45±0.15 mm) and the width varied from 3.50 to 4.50 mm (4.00±0.05 mm)

3.4 Pupa

Pupal period was found to vary from 9 to 11 days with an average of 11.00±0.57 days in the first generation, 9 to 10 days with an average of 9.67±0.52 days in the second generation, 8 to 9 days with an average 8.67±0.22 days in the third generation, 9 to 10 days with an average of 10.11±0.32 days in the fourth generation and 16 to 20 days with an average of 18.67±0.17 days in the fifth generation (Table 2) The length of the pupa varied from 11.00 to 15.00 mm (14.32±0.15 mm) and the width varied from 3.50 to 4.50 mm (4.75±0.08 mm).

3.5 Adult

The life span of male waxmoth ranged between 6 to 14 days with an average of 11.00±1.15 days and the female waxmoth lived for 12 to 23 days with an average of 21.00±0.35 days under laboratory condition. The adult female was larger than the male. The outer margins of the forewings of the male had a semilunar notch whereas that of the female was smooth. The abdominal tip of the female is pointed but the male is flat. The size and colour of the adults were influenced by the diet. The length of the male varied from 16.15 to 18.30 mm (17.32±0.05 mm) and the wingspan varied from 18.00 to 20.00 mm (18.35±0.08 mm). The length of the female varied from 18.00 to 20.00 mm (19.15±0.22) and the wing span varied from 19.50-22.00 mm (22.00±1.15mm).

4. Discussion

The incubation period of egg was found to vary from 3-10 days (Table 2). Similar observations were made by Mishra, Khanbash and Oshan [10, 11]. The length and width of different larval instars were 2.04 ±0.04 and width 0.37±0.04mm in first instar, 8.01±0.2 and 1.07±0.03mm in second instar, 10.8±0.19 and 1.24±0.01mm in third, 12.41±0.07 in fourth, 19.00±0.5mm in fifth and 22.6±0.14 and 4.50±0.16mm in sixth instar. The findings were in agreement with Brar, Khanbash and Oshan [12, 11]. The total larval period varied from 22 to 69 days with an average of 31.17±2.05 days. There was reduction in body size of the larvae in pre-pupal stage with 21.45±0.15mm length and 4.00±0.05 mm in width

having duration of 3-12 days. The pupae measured to be 14.32 ± 0.15 mm in length and 4.75 ± 0.08 mm in width having the duration 8-10 days (Table 2). The adult longevity was found to vary from 6 to 14 days for male and 23 days for female. Similar observations were made by Tompkins and Griffith [13].

5. Management strategy of *Galleria Mellonella*

Different management strategy such as use of plant products viz. *Azadiracta indica* (15 g and 20 g/ hive), *Melia azadirachta* (15 g and 20 g), neem oil (2% and 3%), neem seed kernel extract (NSKE) at 3% and 5% and *kuraj* oil at 25 and 3% concentration were treated in the infested comb by *Galleria mellonella*. Similarly, *Bt var. Karstaki* @ 0.5% and 1% was also sprayed in the infested comb; mechanical device such as keeping the comb in deep freezer at -7°C and -12°C for 5 hrs were used. Post embryonic development of the pest was studied in order to find out the most injurious stage of the pest to the honey comb. Accordingly, third instar *Galleria mellonella* larvae were found to be most motile and injurious to the honey comb. There was significant difference within the treatments and between the treatments in different concentrations of various ingredients as shown in table 3. Among the treatments *Bt. var Karstaki* at 1 percent concentration was found to be most effective in minimizing the percent comb infestation and cumulative mortality was

found to be 93.32% followed by sulphur fumigation @ 28 g/hive mortality of 89.98%, neem oil at 3% concentration gave mortality of 79.99% (table 3). Basedow *et al.* [14] reported similar results while evaluating *Bacillus thuringiensis aizawai* and neem for controlling the larvae of *G. Mellonella*. Effectiveness of sulphur fumigation on *G. mellonella* was tested by Ahmed *et al.* and Calderone and observed that sulfur fumigation can be used for controlling wax moth [4]. [15]. Swamy [16] recorded that there were seven successive larval instars. The total larval period was observed to be 49.3 ± 1.62 . The duration of prepupa and pupa were 2.1 ± 0.53 and 8.6 ± 0.73 days respectively in Karnataka condition but there is dissimilarity in the findings as our present finding six larval instars under Assam condition which might be due to allopatry of population in two different geographical location

6. Conclusion

Post embryonic development of *Galleria mellonella* L. revealed that the egg duration, larval, prepupal and pupal were found to be 3-10 days, 22 to 69 days, of 3-12 days and 8-10 days respectively. The adult longevity was recorded to be 6-14 days for male and 23 days for female. The effective management strategy was found to be with the treatment of *Bt. formulation var. Karstaki* @1 percent per hive followed by neem oil 3% and sulphur fumigation 28 g/hive.

Table 1: Duration of different life stages of *Galleria mellonella*

Period of observation	Generation	Incubation period (days)	Larval period (days)	Prepupal period (days)	Pupal period (days)	Total life cycle (days)
March 23 to May 28, 2015	I	5.00±0.55 (4-5)	33.34±2.86 (30-34)	5.50±0.55 (5-6)	11.00±0.75 (9-11)	55.00±0.57 (54-59)
May 29 to July, 2015	II	4.50±0.75 (4-5)	25.34±1.48 (24-27)	4.00±0.15 (3-4)	9.67±0.52 (9-10)	44.00±0.65 (43-46)
July 20 to Sept, 11 2015	III	4.00±0.01 (3-4)	23.34±1.28 (22-25)	3.83±0.41 (3-4)	8.67±0.22 (8-9)	40.00±0.85 (40-43)
Sept 12 to Nov 9, 2015	IV	4.50±0.32 (4-3)	26.84±2.66	5.00±0.00	10.00±0.32	47.00±1.20
Nov 2015 to March, 2016	V	8.83±0.35 (8-10)	56.99±2.00 (50-69)	9.53±0.32 (9-16)	18.00±0.17 (16-20)	103.00±2.00 (100-110)

Data based on 6 observations

Figures in the parentheses showing range

Table 2: Measurement of head capsule of different instars and different stages of *Galleria mellonella*.

Measurement of head capsule of different instar			Measurement of different stages of <i>Galleria mellonella</i>		
Stages	Length(mm)	Width(mm)	Stages	Length(mm)	Width(mm)
Instar I	0.43±0.003 (0.43-0.46)	0.34±0.004 (0.33-0.36)	Egg	0.39±0.04 (0.32-0.47)	0.50±0.02 (0.45-0.55)
Instar II	0.56±0.007 (0.54-0.57)	0.51±0.001 (0.46-0.54)	Larval stages Instar I	2.04±0.04 (1.90-2.30)	0.37±0.004 (0.36-0.39)
Instar III	0.86±0.03 (0.79-0.93)	0.77±0.02 (0.64-0.86)	Instar II	8.01±0.21 (6.50-8.50)	1.07±0.03 (0.05-1.12)
Instar IV	1.12±0.01 (1.08-1.18)	0.99±0.01 (0.93-1.04)	Instar III	10.80±0.19 (9.50-11.50)	1.241±0.01 1.20-1.30
Instar V	1.43±0.02 (1.33-1.51)	1.24±0.02 (1.15-1.36)	Instar IV	12.41±0.07 (12.00-12.60)	1.49±0.01 (1.40-1.55)
Instar VI	1.97±0.08 (1.67-2.00)	1.99±0.00 (1.33-1.57)	Instar V	19.00±0.15 (18.00-19.50)	2.06±0.01 (2.00-2.16)
			Instar VI	22.60±0.14 (21.00-24.10)	4.50±0.16 (4.00-5.00)
			Pre pupa	21.45±0.15 (19.00-23.00)	4.00±0.05 (3.50-4.50)
			Pupa	14.32±0.15 (11.00-15.00)	4.75±0.08 (3.50-4.50)
			Adult ♂	17.32±0.05 (16.15-18.30)	18.35±0.08 (18.00-20.00)
			♀	19.15±0.22 (18.00-20.00)	22.00±1.15 (19.50-22.00)

Data based on 6 observations

Figures in the parentheses showing range

Table 3: Effect of different treatments on mortality of third instar *Galleria mellonella* larvae at different time interval

Treatments	Concentrations	Mean percent mortality(hrs after treatment)				Cumulative mortality (%)
		24 hrs	48hrs	72hrs	96hrs	
1. Sulphur fumigation	14g/hive	3.33 (6.44)	20.00 (26.06)	23.33 (28.77)	30.00 (33.00)	76.66
	28g/hive	6.66 (12.43)	23.33 (28.77)	26.66 (30.99)	33.33 (35.21)	89.98
2. <i>Azadirachta indica</i>	15g/hive	0.00 (4.45)	10.00 (18.43)	10.00 (18.43)	23.33 (30.99)	43.33
	20g/hive	0.00 (0.45)	13.33 (21.14)	16.66 (23.85)	36.66 (37.22)	66.65
3. <i>Melia azadarach</i>	15g/hive	0.00(0.45)	3.33(6.44)	10.00(18.43)	23.33(28.77)	36.66
	20g/hive	0.00(0.45)	6.66(12.43)	20.00 (26.56)	30.00(32.21)	56.66
4. Neem oil	2%	0.00(0.45)	16.66(26.06)	20.00(26.56)	30.00(32.21)	66.66
	3%	3.33(6.44)	20.00(26.56)	23.33(28.77)	33.33(35.21)	79.99
5. <i>Bacillus thuringiensis</i> var. kurstaki	0.5%	0.00(0.45)	23.33(28.77)	43.33(41.15)	16.66(23.85)	83.32
	1%	0.00(0.45)	26.66(30.99)	46.66(43.07)	20.00(26.56)	93.32
6. NSKE	3%	0.00(0.45)	13.33(21.14)	16.66(23.85)	20.00(26.56)	49.99
	5%	0.00(0.45)	16.66(23.85)	20.00(26.06)	26.66(30.99)	63.32
7. Karanj oil	2%	0.00(0.45)	10.00(18.43)	13.33(21.14)	23.33(28.77)	46.66
	3%	0.00(0.45)	13.33(12.14)	20.00(26.56)	33.33(35.41)	66.66
8. Keeping frames in deep freezer	-7°C	0.00(0.45)	0.00(0.45)	13.33(21.14)	23.33(28.77)	36.66
	12 °C	0.00(0.45)	0.00(0.45)	10.00(15.14)	13.33(21.14)	23.33
9. Control	Distilled water spray	0.00(0.45)	0.00(0.45)	0.00(0.45)	0.00(0.45)	0.00
S. Ed±		3.56	4.23	3.52	2.84	0.00
CD (0.05)		7.22	8.59	7.15	5.77	0.00

Data based on replications each with 10 insects

Figures within the parentheses are angular transformed values.

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