

Effect of *Apis mellifera* Comb Age on Biology of *Galleria mellonella* in Morena of Madhya Pradesh, India

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ABSTRACT: A lab experiments was carried out to evaluate the effect of *Apis mellifera* comb age on the biology of greater wax moth (*Galleria mellonella* L.). The maximum egg duration (10 days) was found in fresh comb (0-06 month) but maximum egg length and egg width (0.43mm and 0.27mm) was recorded in 72-84 month old comb. Similarly, the maximum larval duration (119.67 days) was observed in fresh (0-06 month) comb but maximum larval length (26.54mm), larval width (5.83mm) and larval weight (297.67mg) was found in 72-84 month old comb age of *A. mellifera*. However, the maximum prepupal and pupal period (5.42 and 11.43 days, respectively) was found in fresh (0-06 month) comb but, maximum cocoon and pupal length (23.35 and 15.23mm), width (8.77 and 5.01mm) and weight (235.52 and 241.24mg), respectively was observed in (72-84 month old) comb. Similarly the maximum male and female longevity (12.58 days and 7.94 days, respectively) was noticed in 72-84 month old comb. Whereas, maximum male and female wing expense of adult was observed 28.30mm and 30.83mm in older (72-84 month old) comb. The pre-oviposition and post-oviposition period was maximum (2.47 and 2.17 days, respectively) in fresh (0-06 month) comb but maximum oviposition period was observed 7.03 days in older (72-84 month) comb. Similarly, the maximum adult female fecundity was resulted 929.43 eggs/F (127.81 eggs/F/Day) in older (72-84 month old) comb age of *A. mellifera*. Unlike other invertebrates, *G. mellonella* larvae can be incubated and can be dosed relatively precisely at 30-35°C temperature as well as 50-60% RH, whereas the environmental condition in Chambal region is extremely warm in summer and also unfavorable in winter. The study was observed on laboratory condition.

Keywords: *Apis mellifera*, *Galleria mellonella*, combs age, biological parameters, growth and development.

INTRODUCTION

Honey bees are an important part of the natural environment and influence the forest as well as the agricultural environment. Nearly one-third of the human diet comes from insect-pollinated plants and 80% of pollinated bees (Anonymous, 2014). The renovations and application of improved beekeeping technologies not only benefits beekeepers but also farmers and the general public in pollinating their crops, maintaining plant biodiversity, and the ecology at large (Mulatu & Gebissa 2021).

Two species of wax moths viz., greater wax moth (*G. mellonella* L.) and lesser wax moth (*Achroia grisella* F.) which occur either naturally or being introduced by human being, cause a considerable loss in bee industry at different regions of the world (Mohamed and Amro 2022). The incubation period varied from 6 to 10 days

(Av. 7.36 days) and the larvae passed through seven successive instars (Desai *et al.*, 2019). *G. mellonella* (Pylalidae; Lepidoptera) is an insect universally distributed all over the world where honeybees are present. This pest causes major harm especially in the tropical and sub-tropical regions, in the honeybee colonies of European honeybee (Kumar *et al.*, 2021). The pest inflicts heavy damage on beekeepers every year by feeding on beewax combs inside the hives as well as stored combs (Rahimi and Parichehreh 2021). The larval moths feed on wax combs, cast honey bee larval skins, pollen, some honey and leaving behind a destroyed comb containing moth silk and frass (Muhammad *et al.*, 2020).

The *A. mellifera* comb demonstrated lesser fecundity and prolonged larval and pupal stages could be attributed to the presence of high content of propolis in

the combs compared to other species of bee combs (Hanumanthaswamy *et al.*, 2009). For larval development, temperature of 40°C was found unsuitable and Haydak diet was proved more suitable as compared to 2 years old *A. mellifera* comb pieces (Kumar and Kaushik 2010).

MATERIALS AND METHODS

Studies on the biology of *G. mellonella* was carried out on the different combs age of *A. mellifera* under laboratory condition at RVSKVV ZARS-KVK Morena (M.P.) during 2020-22. The combs were classified into eight groups based on the time after the comb construction. The combs was grouped into 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old. Totally 80 eggs from the laboratory culture of the wax moth were kept in separate containers and were observed at 24 hrs interval. Newly laid eggs were collected from the stock culture and were observed at 24 hrs interval for hatching. The small piece of different old comb was provided separately for newly hatched larvae. Additional quantity of comb was provided as and when the larvae consumed the food. Observations were made every day for moulting, the number of moults and instars passed during the larval development. Grown up larvae was transferred into the plastic containers. Larval duration, weight, length and width was recorded before construction of the silken webs. Prepupal and pupal period, weight, length and width was determined. After adult emergence longevity of male and female, wing expanse, pre-ovipositional, ovipositional and post-ovipositional periods including number of eggs laid by female was recorded. The data was statistically analyzed in a factorial CRD (Completely Randomized Design) and tabulated. Linear correlation was also employed to verify the association between age of the comb and all the above biological parameters (Fig. 4) (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

A. Effect of comb age on the growth of Greater wax moth

Effect on eggs growth. Egg duration was found to be non significant with age of the comb. The mean egg duration was 10.00, 9.50, 9.53, 8.90, 8.70, 8.37, 8.13 and 8.00 days on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old combs, respectively. On older combs the mean duration was shorter than those reared on other groups of combs. Eggs length measured 0.43 mm when reared on 0-06 months aged comb and were shorter than the eggs obtained when reared on 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old combs, where they measured 0.44, 0.44, 0.45, 0.45, 0.45, 0.45 and 0.46 mm which was found to be significant with age of the comb. Similarly, egg width was significant with age of the comb. Eggs obtained from 72-84 months old comb were 0.32 mm broad and were broader than the eggs obtained from 60-72, 48-60, 36-48, 24-36, 12-24, 06-12

and 0-06 months old combs which measured 0.31, 0.30, 0.30, 0.29, 0.29, 0.28 and 0.27 mm, respectively (Table 1 & Fig. 5). Egg period was found non significant and negatively correlated with age of the comb ($r = -0.97$). Similarly, egg width was also found non significant ($r = 0.97$) and positively correlated with age of the comb. However, egg length was found to be significant and positively correlated with age of the comb ($r = 0.82$) (Table 3 & Fig. 1).

Effect on larval growth. The Larval mean duration was observed 119.67, 108.40, 94.33, 72.87, 64.07, 55.95, 50.35 and 48.88 days on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old combs, respectively (Table 1 & Fig. 5). The mean length and width of the larvae were 17.77 and 4.12 mm on 0-06 months old comb, 19.80 and 4.37 mm on 06-12 months old comb, 21.10 and 4.54 mm on 12-24 months old comb, 22.44 and 4.81 mm on 24-36 months old comb, 23.80 and 5.35 mm on 36-48 months old comb, 24.77 and 5.47 mm on 48-60 months old comb, 25.80 and 5.62 mm on 60-72 months old comb and 26.54 and 5.83 mm on 72-84 months old comb. The mean weight of the larvae was 85.00, 95.35, 121.50, 166.01, 200.33, 225.35, 276.01 and 297.67 mg on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old combs, respectively. Larvae reared on the eight groups of old age combs were found to be non significant with respect to duration, length, width and weight. Combs of less than 2 months old were also tested in this experiment, but the larvae were died without pupation. It was observed that the age of the comb exerted significant influences on the larval duration, negative correlation ($r = -0.96$) was observed between these two parameters. However, larval length, larval width and larval weight were found non significant ($r = 0.98$, $r = 0.98$ and $r = 0.99$, respectively) and positively correlated with age of the comb (Table 3 & Fig. 1). Similar observations were observed by Mohamed and Amro (2022), Desai *et al.*, (2019); Muhammad *et al.*, (2020) observed the average larval period was 50.84 ± 4.90 days and larval length, width and weight was higher in older combs. Swamy (2008) found that the mean egg length and width was longer, and egg period was shorter on older combs than those reared on other groups of combs. Hosamani *et al.*, (2017) found egg length and width as 0.44 and 0.30mm, respectively and larval growth was more or less similar to present findings.

B. Effect of comb age on the development of Greater wax moth

Effect on pre-pupal/cocoon development. The mean duration of the prepupa was recorded 5.42, 4.92, 3.93, 3.17, 2.68, 2.40, 2.25 and 2.00 days on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old combs, respectively. The mean length and width of the cocoons were 16.08 and 4.36 mm on 0-06 months old comb, 17.68 and 4.46 mm on 06-12 months old comb, 17.71 and 5.36 mm on 12-24 months old

comb, 18.45 and 6.00 mm on 24-36 months old comb, 19.74 and 7.07 mm on 36-48 months old comb, 20.32 and 7.73 mm on 48-60 months old comb, 21.27 and 8.33 mm on 60-72 months old comb, where as the length (23.35 mm) and width (8.77 mm) of the cocoons was longer on 72-84 months old comb (Table 1 & Fig. 5). The mean weight of the cocoons was found 166.83, 186.17, 200.33, 208.83, 212.33, 215.00, 223.02 and 235.52 mg when reared on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old combs, respectively. The differences in the duration of prepupa, length, width and weight of the eight sets of the cocoons were statistically non significant. It was observed that the age of the comb exerted significant influences on the prepupal period, negative correlation ($r = -0.95$) was observed between these two parameters. Similarly, Cocoon weight was found significant ($r = 0.94$) and positively correlated with age of the comb. However, cocoon length and cocoon width were found non significant and positively correlated with age of the comb ($r = 0.98$ and $r = 0.99$) (Table 3 & Fig. 2).

Effect on pupal development. The mean pupal duration was noticed 11.43, 11.37, 11.15, 10.27, 10.10, 9.70 and 9.03 days when reared on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60 and 60-72 months old combs, respectively (Table 1 & Fig. 5). On 72-84 months old combs the mean pupal duration was observed 8.83 days and was found to be significantly shorter than those reared on other combs of different ages. The mean pupal length was observed 8.20, 9.60, 9.64, 10.41, 12.41, 12.96, 14.22 and 15.23 mm on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old combs, respectively. The mean width of the pupae was 3.03, 3.11, 3.67, 4.05, 4.27, 4.72, 4.92 and 5.01 mm on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old comb, respectively. The mean weight of the pupae when reared on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old comb was 112.37 mg, 123.67 mg, 142.92 mg, 190.86 mg, 212.71 mg, 224.38 mg, 236.67 mg and 241.24 mg, respectively. Pupae reared on the eight different age old combs were found to be non significant with respect to length, width and weight. Thus the pupae reared on 72-84 months old combs were found to be larger than those reared on other old combs. It was observed that the age of the comb exerted significant influences on the pupal weight, positive correlation ($r = 0.96$) was observed between these two parameters. However, pupal period was found non significant and negatively correlated ($r = -0.99$) with age of the comb. Similarly, pupal length and pupal width were found non significant and positively correlated with age of the comb ($r = 0.99$ and $r = 0.98$) (Table 3 & Fig. 3).

More or less similar result was obtained by Rahimi and Parichehreh (2021); Kwadha *et al.* (2017); Hosamani *et al.*, (2017); Ellis *et al.* (2012). Swamy (2008) observed cocoon and pupal period as 258.50 and 192.00 mg, respectively. Mandal and Vishwakarma (2016); Desai

et al. (2019) found that the cocoon and pupal length and width were longer, and durations were shorter on older combs than those reared on other groups of combs.

C. Effect of combs age on the biological parameters of Greater wax moth

Effect on adult longevity. Males reared on 0-06 months old combs lived for 6.20 days while, those reared on 72-84 months old combs lived for 19.30 days. This difference was found to be highly significant. The males reared on other different age old combs lived for 7.27, 8.93, 10.53, 13.23, 16.90 and 18.23 days on 6-12, 12-24, 24-36, 36-48, 48-60 and 60-72 months old combs, respectively. Females also exhibited similar pattern and lived longer when reared on 72-84 months old combs (10.23 days) followed by 60-72 (9.87 days), 48-60 (9.17 days), 36-48 (8.67 days), 24-36 (7.67 days), 12-24 (6.40 days), 06-12 (6.03 days) and 0-06 (5.47 days) months old combs (Table 2).

Effect on wing expense. The mean wing expense of males reared on 0-06 months old combs was 19.90 mm while, those reared on 72-84 months old combs the wing expense was 28.30 mm. This difference was found to be highly significant. The wing expense of males reared on other different age old combs was 21.00, 22.07, 23.47, 24.20, 24.87 and 27.07 mm on 6-12, 12-24, 24-36, 36-48, 48-60 and 60-72 months old combs, respectively. Females also exhibited similar pattern and wing expense was broader when reared on 72-84 months old combs (30.83 mm) followed by 60-72 (29.17 mm), 48-60 (27.53 mm), 36-48 (25.63 mm), 24-36 (24.20 mm), 12-24 (22.70 mm), 06-12 (21.87 mm) and 0-06 (21.10 mm) months old combs (Table 2 & Fig. 6).

Male and female longevity were found non significant and positively correlated ($r = 0.99$ and $r = 0.98$, respectively) with age of the comb. Similarly, Male and female wing expense were found non significant and positively correlated ($r = 0.99$ and $r = 0.98$, respectively) with age of the comb (Table 3).

Similar findings were obtained by Mohamed *et al.* (2014) and Swamy *et al.* (2008) as wing expense of male and female was long in older combs then other age group of comb. Kumar *et al.*, (2021) and Mohamed *et al.* (2014) also found the average longevity of *G. mellonella* male and female as 18 and 10 days, respectively which was similar to the present findings.

Effect on biological parameters of females. Pre-oviposition, oviposition and post oviposition periods for female moths reared on different age old combs were found to differ significantly. The mean pre oviposition period was 2.47, 2.33, 2.27, 2.23, 2.13, 1.27, 1.13 and 1.00 days for females reared on 0-6 (fresh), 6-12, 12-24, 24-36, 36-48, 48-60, 60-72 and 72-84 months old combs, respectively. The corresponding means for oviposition and post oviposition periods were 1.07 and 2.17 days, respectively on 0-06 months old combs, 1.53 and 2.03 days, respectively on 06-12 months old combs, 2.80 and 1.97 days, respectively on 12-24

months old combs, 3.47 and 1.83 days, respectively on 24-36 months old combs, 4.13 and 1.33 days, respectively on 36-48 months old combs, 5.03 and 1.17 days, respectively on 48-60 months old combs, 6.43 and 1.17 days, respectively on 60-72 months old combs and 7.03 and 1.03 days, respectively on 72-84 months old combs (Table 2 and Fig. 6).

Fecundity was found to differ significantly between the females reared on different old combs. Females reared on 0-06 months old combs laid an average of 40.37 eggs/female with 26.16 eggs/day, on 06-12 months old combs they laid an average of 75.73 eggs/female with 38.13 eggs/day, on 12-24 months old combs they laid an average of 214.37 eggs/female with 60.72 eggs/day, on 24-36 months old combs they laid an average of 318.87 eggs/female with 74.78 eggs/day, on 36-48 months old combs they laid an average of 502.53 eggs/female with 87.81 eggs/day, on 48-60 months old combs they laid an average of 687.00 eggs/female with 102.05 eggs/day, on 60-72 months old combs they laid an average of 869.10 eggs/female with 117.55 eggs/day, whereas on 72-84 months old combs they laid an average of 929.43 eggs/female in their life time

distributing at a rate of 127.81 eggs/day (Table 2 & Fig 6).

It was observed that the age of the comb exerted significant influences on the pre-ovipositional period, negative correlation ($r = -0.94$) was observed between these two parameters. However, ovipositional period was found non significant and positively correlated ($r = 0.99$) with age of the comb. Similarly, post-ovipositional period was found non significant and negatively correlated ($r = -0.97$) with age of the comb. Whereas, eggs per female and eggs per day of adult were found non significant and positively correlated ($r = 0.99$ and $r = 0.99$, respectively) with age of the comb (Table 3 & Fig. 3).

More or less similar result was found by Ramesh *et al.* (2021); Kwadha *et al.* (2017); Rahman *et al.* (2017) as mean pre and post oviposition period was 1.55 and 1.30 days, respectively, whereas average oviposition period was 6.70 days. Desai *et al.* (2019); Hosamani *et al.* (2017); Swamy (2008) observed the fecundity of adult female moth which was higher in oldest comb and consumable for newly born larvae.

Table 1: Effect of different comb age of *Apis mellifera* on growth and development of Greater wax moth (*Galleria mellonella*).

Comb age (months)	Eggs			Larvae				Prepupal stage				Pupal stage			
	Egg duration (days)	Egg length (mm)	Egg width (mm)	Larval duration (days)	Larval length (mm)	Larval width (mm)	Larval weight (mg)	Period (days)	Cocoon Length (mm)	Cocoon Width (mm)	Cocoon Weight (mg)	Pupal Period (days)	Pupal Length (mm)	Pupal Width (mm)	Pupal Weight (mg)
0-06	10.00	0.43	0.27	119.67	17.77	4.12	85.00	5.42	16.08	4.36	166.83	11.43	8.20	3.03	112.37
06-12	9.50	0.44	0.28	108.40	19.80	4.37	95.35	4.92	17.68	4.46	186.17	11.37	9.60	3.11	123.67
12-24	9.53	0.44	0.29	94.33	21.10	4.54	121.50	3.93	17.71	5.36	200.33	11.15	9.64	3.67	142.92
24-36	8.90	0.45	0.29	72.87	22.44	4.81	166.01	3.17	18.45	6.00	208.83	10.27	10.41	4.05	190.86
36-48	8.70	0.45	0.30	64.07	23.80	5.35	200.33	2.68	19.74	7.07	212.33	10.10	12.41	4.27	212.71
48-60	8.37	0.45	0.30	55.95	24.77	5.47	225.35	2.40	20.32	7.73	215.00	9.70	12.96	4.72	224.38
60-72	8.13	0.45	0.31	50.35	25.80	5.62	276.01	2.25	21.27	8.33	223.02	9.03	14.22	4.92	236.67
72-84	8.00	0.46	0.32	48.88	26.54	5.83	297.67	2.00	23.35	8.77	235.52	8.83	15.23	5.01	241.24
Mean	8.89	0.45	0.30	76.81	22.75	5.01	183.40	3.35	19.32	6.51	206.00	10.24	11.58	4.10	185.60
Sem	0.098	0.002	0.003	3.81	0.39	0.103	6.82	0.17	0.41	0.18	6.36	0.09	0.20	0.11	2.05
CD (5%)	0.294	0.007	0.008	11.42	1.17	0.310	20.45	0.50	1.22	0.54	19.08	0.27	0.60	0.32	6.15

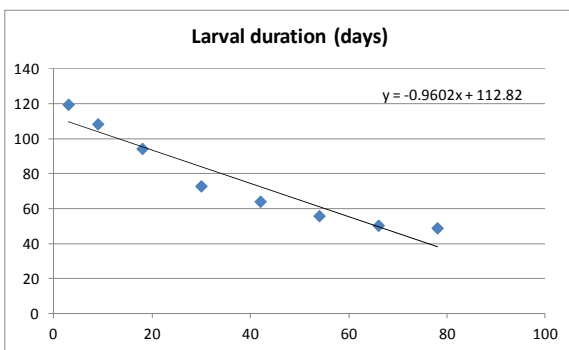
Table 2: Effect of different comb age of *Apis mellifera* on biological parameters of Greater wax moth (*Galleria mellonella*).

Comb age (month)	Longevity (days)		Wing expense (mm)		Oviposition			Fecundity	
	Male	Female	Male	Female	Pre (days)	Ovi. (Days)	Post (Days)	Eggs/F	Eggs/F/Day
0-06	6.20	5.47	19.90	21.10	2.47	1.07	2.17	40.37	26.16
06-12	7.27	6.03	21.00	21.87	2.33	1.53	2.03	75.73	38.13
12-24	8.93	6.40	22.07	22.70	2.27	2.80	1.97	214.37	60.72
24-36	10.53	7.67	23.47	24.20	2.23	3.47	1.83	318.87	74.78
36-48	13.23	8.67	24.20	25.63	2.13	4.13	1.33	502.53	87.81
48-60	16.90	9.17	24.87	27.53	1.27	5.03	1.17	687.00	102.05
60-72	18.23	9.87	27.07	29.17	1.13	6.43	1.17	869.10	117.55
72-84	19.30	10.23	28.30	30.83	1.00	7.03	1.03	929.43	127.81
Mean	12.58	7.94	23.86	25.38	1.85	3.94	1.59	454.68	79.37
Sem	0.49	0.32	0.52	0.39	0.047	0.31	0.057	26.68	2.66
CD (5%)	1.46	0.96	1.56	1.17	0.141	0.93	0.169	79.99	7.99

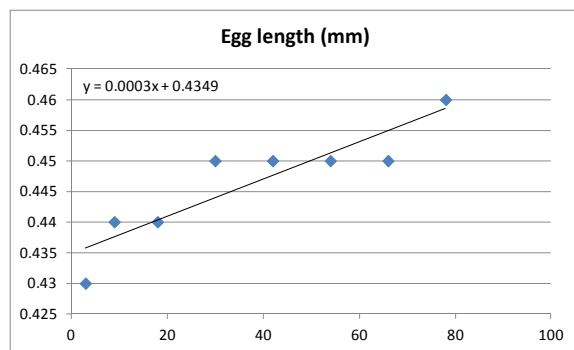
Table 3: Relationship between age of the combs and biological parameters of Greater wax moth.

Parameters (Age of the Comb vs)	r	R ²	Regression equation	P
Egg period	-0.97	0.95	-	2.35
Egg length	0.82*	0.79	$y = 0.0003x + 0.4349$	0.002
Egg width	0.97	0.94	-	5.22
Larval period	-0.96**	0.91	$y = -0.9602x + 112.82$	0.002
Larval length	0.98	0.96	-	2.38
Larval width	0.98	0.97	-	9.33
Larval weight	0.99	0.99	-	3.96
Prepupal period	-0.95**	0.89	$y = -0.0445x + 5.0161$	0.003
Cocoon length	0.98	0.96	-	1.93
Cocoon width	0.99	0.98	-	6.80
Cocoon weight	0.94**	0.89	$y = 0.7453x + 178.06$	0.005
Pupal period	-0.99	0.98	-	2.61
Pupal length	0.99	0.98	-	2.75
Pupal width	0.98	0.96	-	1.86
Pupal weight	0.96**	0.93	$y = 1.8432x + 116.48$	0.001
Male longevity	0.99	0.98	-	1.15
Female longevity	0.98	0.97	-	3.32
Male wing expense	0.99	0.98	-	1.48
Female wing expense	0.99	0.99	-	1.02
Pre oviposition period	-0.94**	0.89	$y = -0.0211x + 2.6449$	0.004
Oviposition period	0.99	0.98	-	4.35
Post oviposition period	-0.97	0.94	-	6.98
Eggs/female	0.99	0.99	-	2.18
Eggs/female/day	0.99	0.98	-	2.40

* significant at 0.05%; ** significant at 0.01%; P: P value; r: Correlation; R²: coefficient of determination.



(A)



(B)

Fig. 1 (A & B). Regression of different combs age of *A. mellifera* with Egg length and Larval duration of *G. mellonella*.

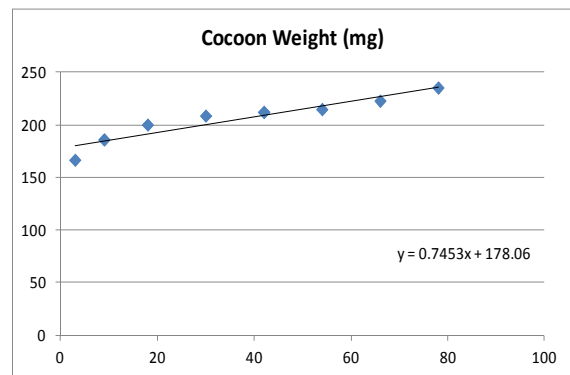
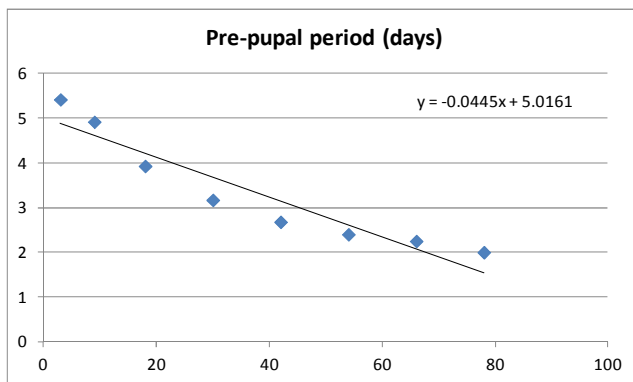


Fig. 2 (A & B). Regression of different combs age of *A. mellifera* with Pre-pupal period and Cocoon weight of *G. mellonella*.

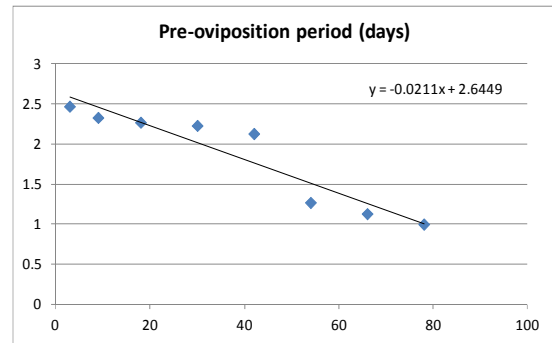
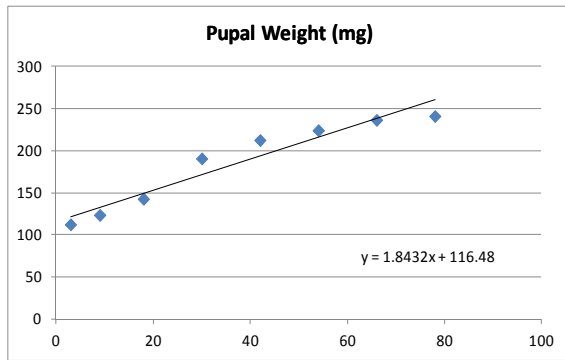


Fig. 3 (A & B). Regression of different combs age of *A. mellifera* with Pupal weight and Pre-oviposition period of *G. mellonella*.

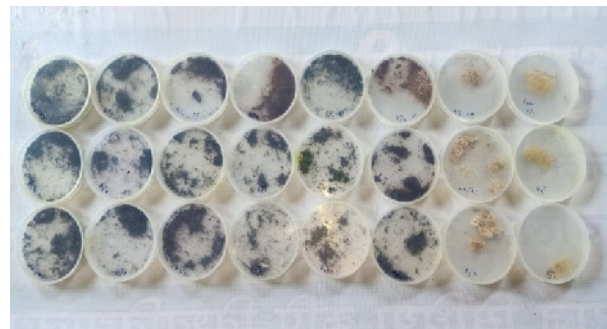
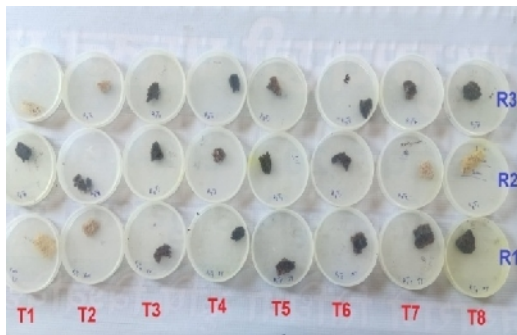


Fig. 4 (A & B). Experiment details of *G. mellonella* on different age combs of *A. mellifera*.

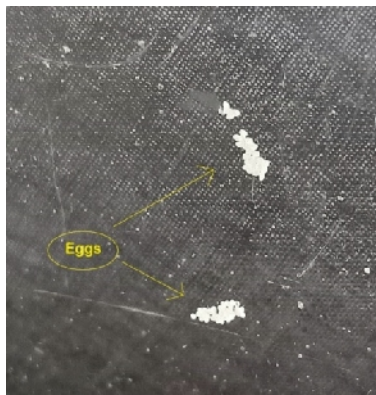


Fig. 5. Growth and Development (Eggs, Larva, Pupa and Adult) of *G. mellonella* on *A. mellifera* colonies.



Fig. 6. (A) Adult Female, (B) Adult Male, (C) Mating and Oviposition of adult *G. mellonella* (Male and Female).

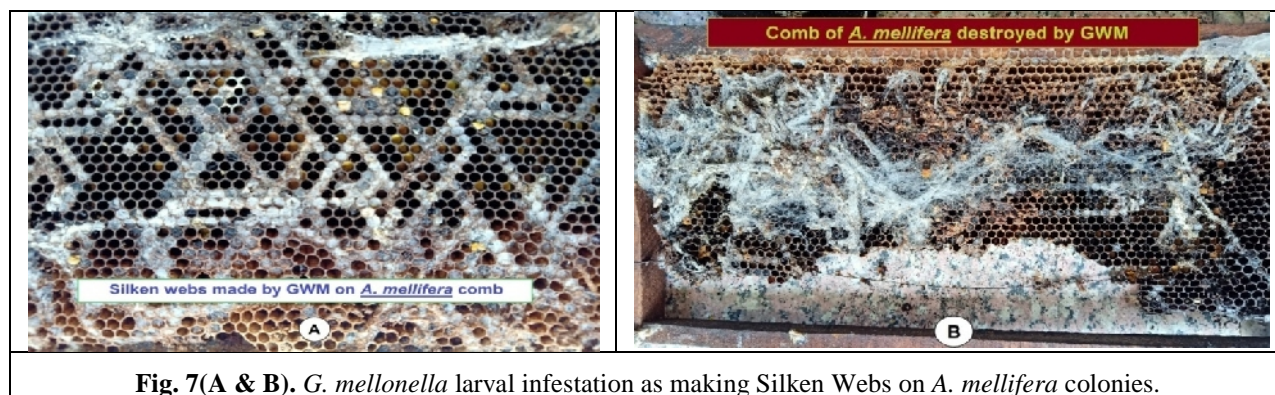


Fig. 7(A & B). *G. mellonella* larval infestation as making Silken Webs on *A. mellifera* colonies.

CONCLUSION

Comb age of *A. mellifera* influenced the growth, development and biology of *G. mellonella*. Egg, larval and pupal duration was shorter when comb age was increased, whereas length and width of egg, larva and pupae were longest in old combs. Similarly, on older combs the longevity of male and female moth of *G. mellonella* was found highest and also the wing development was observed faster in old comb. Fecundity of adult female *G. mellonella* was highest in older comb than other age group of *A. mellifera* combs.

FUTURE SCOPE

Age of comb affected the biology of greater wax moth. So, information regarding the comb age and biology is useful in scientific beekeeping. The greater wax moth biology was conducted under laboratory so the growth and developmental study was not exactly as the natural environment, where the observation may vary depends on different biotic and abiotic factors, but it is similar to the natural condition, so further work is suggested on different natural diet sources for the biological study of greater wax moth.

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Conflict of Interest. None.

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