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DIETARY VARIATION ON BIOLOGY AND MORPHOMETRIC OBSERVATION OF THE GREATER WAX MOTH, *GALLERIA MELLONELLA* (L.)

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ABSTRACT

Galleria mellonella (L.) is a destructive insect pest of honeybees. *G. mellonella* (L.) is widely used in research, microbiology assays, as pet food and host for biological control agents. Larvae of *G. mellonella* were collected from the main campus, BUAT, Banda, U. P. during 2024 and reared on natural and artificial diet under controlled conditions at 32 ± 2°C and 65 ± 2% R.H. There were 30 observations (3x10) for biological studies and 10 observations for morphometric observation. The mean incubation period was slightly shorter on the natural diet (7.30 days) compared to the artificial diet (7.40 days). Similarly, the total larval duration was reduced on the natural diet (31.55 days) as opposed to the artificial diet (42.89 days). Thus, the overall developmental period of the immature stages was also comparatively shorter on the natural diet (48.91 days) than on the artificial diet (63.45 days). Adult longevity of both sexes was higher on the natural diet (22.83 days) for males and (15.76 days) for females, compared to (16.00 and 12.37), respectively, on the artificial diet. The total life cycle of males and females was shorter on the natural diet (71.75 and 64.67 days) compared to the artificial diet (79.78 and 75.81 days). The survival rate of eggs, larvae and pupa were recorded higher on the natural diet (88.06, 91.67 and 92.91%) respectively compared to artificial diet (84.49, 89.39 and 88.42%). The morphometric observations of different stages of *G. mellonella* were also recorded on natural as well as artificial diet.

Keywords **:** Adult longevity; artificial diet; incubation period; natural diet; survival percentage

Introduction

The greater wax moth, also referred to as the honeycomb moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), is a destructive insect species that poses a serious threat to the apiculture industry globally. It inflicts extensive deterioration on stored honey bee wax combs, commercial apiaries, and frequently results in the collapse of weakened or queen less colonies (Mohamed and Amro, 2022; Amizhthini *et al.,* 2024). The pest's economic significance is primarily attributed to its larval feeding behaviour, where larvae bore through the combs, producing white silken tunnels and contaminating them with frass ultimately leading to structural degradation of the combs (Kwadha *et al.,* 2017; Sohail *et al.,* 2017).

Diet optimization is an important process to increase the efficiency of rearing insects and can be used to develop high-quality insects with specific fitness and life-history traits. *Galleria mellonella* (L.), is widely used in research, microbiology assays, as pet food, and host for biological control agents. Although artificial diets for *G. mellonella* have been researched and optimized for decades, preliminary tests indicated that the predominantly utilized *G. mellonella* diet could be improved to yield larger larvae with a short development time. Despite their pest status, the larvae offer distinct advantages as experimental hosts. In

recent years, the artificial propagation of wax moths has gained considerable momentum due to their emerging role as a model organism. They are extensively employed as biological substrates for the mass cultivation of entomopathogenic fungi and nematodes (Vanzyl and Malan, 2015; Adeline *et al.,* 2018). Additionally, wax moth larvae are used as a host in the mass production of parasitoids and predators, including *Microplitis* spp., *Archytas* spp., *Apanteles* spp., and *Bracon hebetor* L. (Ashfaq *et al.,* 2005; Devi, 2021). Key benefits include minimal maintenance costs, a short developmental cycle, simplified procedures for bioassays, tolerance to a broad range of incubation temperatures (25°C to 37°C). Consequently, *G. mellonella* is extensively used for foundational research in various scientific fields such as insect physiology, metabolic biochemistry, toxicological evaluation, and pathological investigations (Ce *et al.,* 2020; Pereira and Rossi, 2020).

The rearing methodologies for *G. mellonella* larvae have been explored by many researchers. Kwadha *et al.* (2017) reported that the nutritional composition of the larval diet significantly influences growth and development. This study focused on the impact of natural and artificial diet on the biological parameters (viz., eggs, larvae, pre-pupa, pupal duration and male & female longevity), survival percentage (eggs, larvae and pupal) and morphometric observations at different stages of *G. mellonella.* Effective insect diets are pivotal in most rearing operations because they can shape the success of such programs by improving rearing production costs and enhancing the overall quality and fitness of the reared insects. Enhancing the nutritional quality of a diet through diet optimization can improve key life history and biological fitness parameters including growth, flight, fecundity, and longevity. The desired outcomes of diet optimization vary depending on the purpose of the insect and rearing system. (Hickin *et al.,* 2021).

Materials and Methods

Rearing of *G. mellonella* (L.) on Natural and Artificial diet:

The Collection of *G. mellonella* (L.) larvae were done from main Campus of Banda University of Agriculture and Technology (BUAT), Banda, Uttar Pradesh. The collected larvae were reared on old wax combs (natural diet) plate-1. which were also sourced from the same location and on artificial diet. The components for artificial diet were prepared using *viz.*, wheat flour (350gm), wheat bran (200gm), milk powder (130gm), dried yeast powder (70gm), honey (100ml) and bee-wax (20gm). This diet was recommended by Kandel *et al.,* 2020; Iddi *et al.,* 2025 (plate-1). Both the natural and laboratory strains were reared under in the PG Lab, Department of Entomology, BUAT, Banda, at 32 ± 2°C and 65 ± 2% relative humidity. Newly emerged adult males and females were kept in 2 kg glass jars and provided 50% honey solution with vitamin E for feeding in cotton swab, which were covered with muslin cloth and maintained under the above-mentioned controlled conditions. The jars were inspected daily until egg-laying was observed.

For the biological studies, the observation on incubation period, larval duration, pupal duration, pre-oviposition, oviposition, and post-oviposition period, fecundity, adult longevity and total life cycle of adults. Survival percentages of eggs, larvae, pupa and egg to pupa were also recorded in the successive generation of *G. mellonella*. The morphometric measurements were taken using a digital vernier calliper. There were 30 sets for biological studies and 10 sets for morphometric observation.

Statistical analysis

The collected data under each parameter were analysed statistically with suitable software. The formulas used to calculate the eggs hatching, larval survival, adult emergence percentages that were used by various authors.

Plate-1: Showing both diets for rearing *G. mellonella* **(A)** Natural diet; **(B)** Components of artificial diet; **(C)** Prepared artificial diet

Hatching percentage:

Hatching % = Number of eggs hatched/total number of total eggs ×100

Larval survival percentage:

Larval survival % = Number of larvae survived /Total number of total larvae × 100

Adult emergence percentage:

Adult emergence %= Number of adult emerged/Total number of cocoons × 100

Results

Effect of natural and artificial diet on biological studies of *G. mellonella* (L.)

The developmental period of immature stages of *G. mellonella* (L.) on natural and artificial diet are presented in (Table 1 & Fig. 1).

Table 1 : Development period of *G. mellonella* (L.) on Natural & Artificial diet (in days)

Stage	Natural diet		Artificial diet	
	Range	Mean ± SD	Range	Mean ± SD
Egg	7.00-8.00	7.30 ± 0.40	7.00-8.00	7.40 ± 0.41
1st instar	2.00-3.00	2.57 ± 0.45	2.00-3.66	2.90 ±0.45
2nd instar	3.00-4.00	3.40 ± 0.41	4.00-5.66	5.00 ± 0.47
3rd instar	4.00-5.00	4.20 ± 0.36	6.00-7.00	6.27 ± 0.38
4th instar	4.00-5.33	4.56 ± 0.45	5.33-6.00	5.66 ± 0.32
5th instar	4.66-6.00	5.16 ± 0.42	7.00-8.00	7.30 ± 0.43
6th instar	5.00-6.33	5.53 ± 0.48	7.00-8.00	7.37 ± 0.40
7th instar	5.33-7.00	6.13 ± 0.53	8.00-9.00	8.40 ± 0.41
Total larval period	30.32-32.31	31.55 ± 0.61	40.99-44.99	42.89 ± 1.13
Pre-Pupa	2.00-3.00	2.46 ± 0.39	1.33-2.00	1.73 ± 0.26
Pupa	7.00-8.00	7.60 ± 0.44	11.00-12.00	11.43 ± 0.45
Total immature stage	46.98-50.30	48.91 ± 1.32	61.64-64.65	63.45 ± 1.12

The total developmental period was comparatively different between both diets. The incubation period of eggs on natural diet (7.30 ± 0.40) was shorter than the artificial diet (7.40 ± 0.41). The total larval period (31.55 ± 0.61) on natural diet was shorter than the artificial diet (42.89 ± 1.13). The pupal period was also found shorter on natural diet (7.60 ± 0.44) as compare to artificial diet (11.43 ± 0.45) and the total immature stages were also recorded shorter on natural diet (48.91 ± 1.32) compared to artificial diet (63.45 ± 1.12).

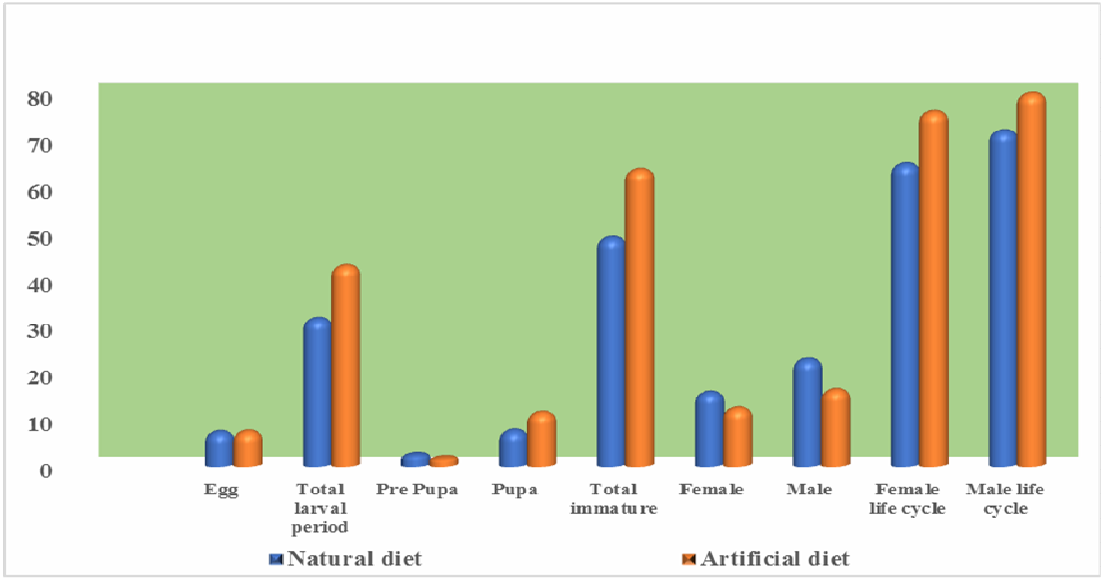

Fig. 1 : Development period of *G. mellonella* (L.) on Natural & Artificial diet

The adult longevity, fecundity and total life cycle were influenced by the provided diet to the larval instars (Table 2)

The pre-oviposition, oviposition and post-oviposition periods of female were higher in natural diet (2.23 ± 0.32, 8.50 ± 0.36 and 4.10 ± 0.28) while it was relatively shorter in artificial diet (1.96 ± 0.29, 6.66 ± 0.35 and 3.20 ± 0.32) respectively. The adult longevity of both males and females was affected by the type of diet. The larvae reared on natural diet showed relatively prolonged adult longevity. Male longevity was recorded (22.83 ± 0.77 days) on natural diet and (16.33 ± 0.35 days) on artificial diet while female longevity was found (15.76 ± 0.39 days) on natural diet and (12.37 ± 0.40 days) on artificial diet. The both provided diet to immature stages had a significant impact on the fecundity of female adults of *G. mellonella*. The fecundity was comparatively higher on natural diet as compared to artificial diet. The fecundity was observed (804.20 ± 46.51 eggs/female) on natural diet and (652.40 ± 44.84 eggs/female) on artificial diet. The total life cycle was also influenced by the type of diet, the total life cycle of adult's male as well as female showed prolonged on artificial diet, as compared to natural diet. The life cycle of male and female were 79.78 ± 1.34 and 75.81 ± 1.41 on artificial diet whereas, it was 71.75 ± 1.61 and 64.67 ± 1.51 as well on natural diet.

Table 2 : Adult longevity & Fecundity of G. mellonella (L.) on Natural and Artificial diet

Adult longevity (days)	Natural diet		Artificial diet	
	Range	Mean ± SD	Range	Mean ± SD
Female				
Pre-oviposition	1.66-2.66	2.23 ± 0.32	1.66-2.66	1.96 ± 0.29
Oviposition	8.00-9.00	8.50 ± 0.36	6.00-7.00	6.66 ± 0.35
Post-oviposition `	3.66-4.33	4.10 ± 0.28	3.00-4.00	3.20 ± 0.32
Total	15.00-16.33	15.76 ± 0.39	12.00-13.00	12.37 ± 0.40
Male	22.00-24.00	22.83 ± 0.77	16.00-17.00	16.33 ± 0.35
Total life cycle				
Male	69.31-73.62	71.75 ± 1.61	77.97-81.63	79.78 ± 1.34
Female	62.32-66.63	64.67 ± 1.51	73.64-77.32	75.81 ± 1.41
Fecundity (Eggs/Female)	725-830	804.20 ± 46.51	585-725	652.40 ± 44.84

The survival percentage of different life stages of *G. mellonella* reared on different food types is presented in (Table 3 & Fig 2).

The result revealed that the egg survival percentage of *G. mellonella* was comparatively greater on natural diet (88.06 ± 1.92) whereas, it was relatively lower on artificial diet (84.49 ± 2.24). The larval and pupal survival percentage also influenced by the different food type. Larval and pupal survival were slightly greater on natural diet (91.67 ± 2.36 & 92.91 ± 1.90) while it was relatively lower on artificial diet (89.39 ± 3.24 & 88.42 ± 3.49) respectively. The overall survival percentage from egg to pupa was significant different between the diets. It was 74.99 ± 74.99 on natural diet and 66.68 ± 4.66 on artificial diet.

Table 3 : Survival percentage of *G. mellonella* (L.) during different development stage on Natural & Artificial diet

Parameter	Natural diet		Artificial diet	
	Range	Mean ± SD	Range	Mean ± SD
Egg survival %	85.09-91.57	88.06 ± 1.92	80.57-88.20	84.49 ± 2.24
Larval survival %	88.34-95.99	91.67 ± 2.36	83.69-93.77	89.39 ± 3.24
Pupa survival %	89.30-94.48	92.91 ± 1.90	81.71-92.06	88.42 ± 3.49
Egg to pupa survival %	71.05-78.43	74.99 ± 74.99	58.72-72.29	66.68 ± 4.66

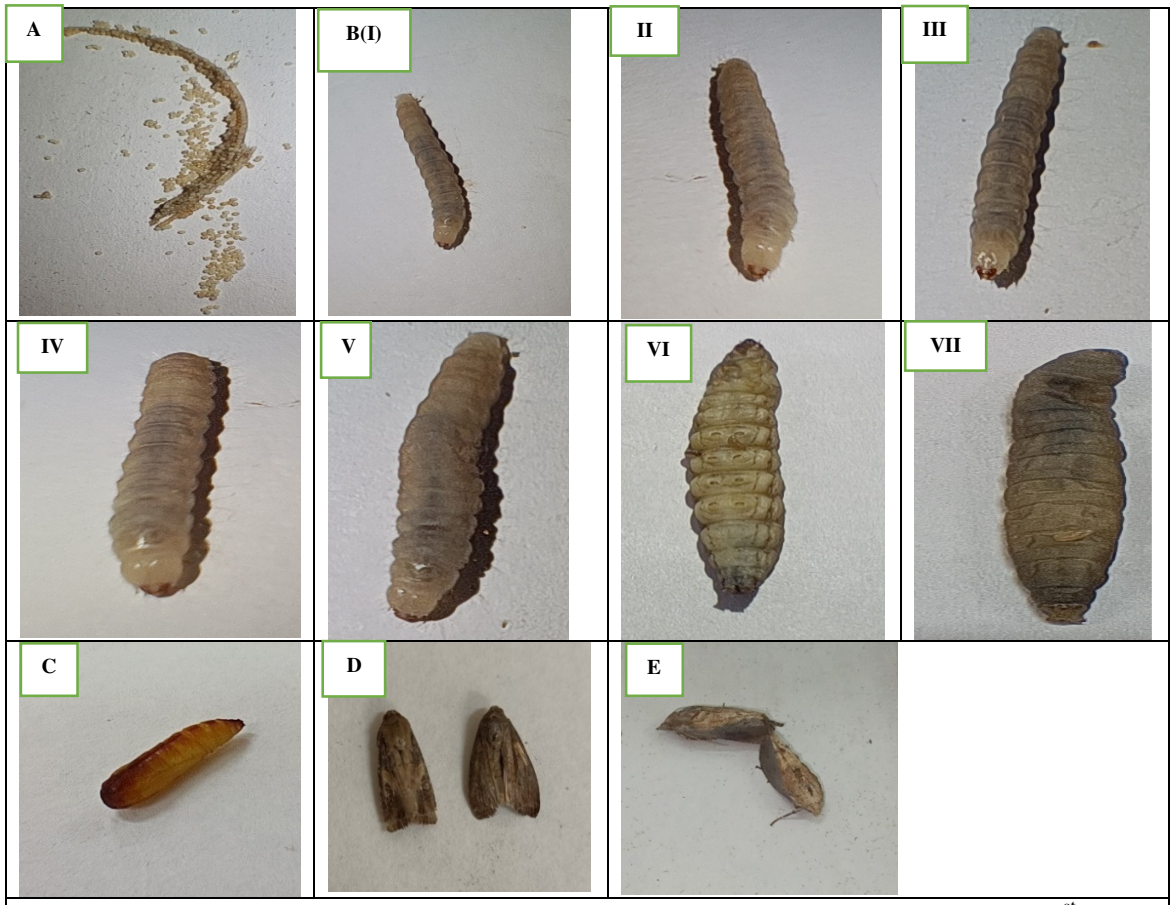

Plate-2 showing the different life stages of *G. mellonella* **(A)** Eggs; **(B)** Larval instarts; **I.** 1st instar; **II.** 2nd instar; **III.** 3rd instar; **IV.** 4th instar; **V.** 5th instar; **VI.** 6th instar; **VII.** 7th instar; **(C)** Pupa; **(D)** Adult (left-male & right-female); **(E)** During mating

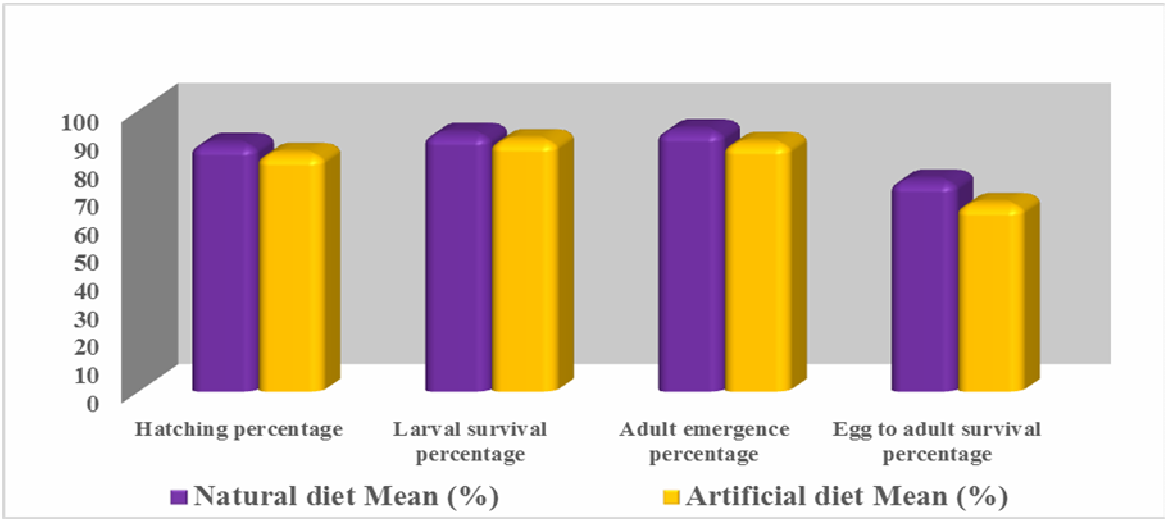

Fig. 2 : Survival percentage of *G. mellonella* (L.) during different development stage on Natural & Artificial diet

Effect of natural and artificial diet on morphometry studies of different life stages of *G. mellonella* (L.)

The morphometric measurement of different life stages of *G. mellonella* are presented in Table 4. The length and width of eggs and all larval instars were significantly influenced by different diet. The eggs of *G. mellonella* were spherical or slightly oblong and smooth. The freshly laid eggs were creamy white in colour. The length and width of eggs were recorded

(0.42 ± 0.07 and 0.51 ± 0.03mm) on natural diet and (0.38 ± 0.02 and 0.30 ± 0.02mm) on artificial diet.

The larvae were eruciform, freshly hatched larvae were white and later turned to dirty grey with brownish head. The 1st, 2nd, 3rd, 4th, 5th, 6th and 7th instar (fully grown) larvae measured (2.11 ± 0.10, 8.16 ± 0.69, 10.68 ± 0.73, 12.41 ± 0.29, 15.23 ± 0.82, 20.89 ± 0.48 and 25.51 ± 0.43mm) in length and (0.35 ± 0.02, 0.97 ± 0.16, 1.27 ± 0.03, 1.51 ± 0.04, 2.44 ± 0.16, 3.24 ± 0.10 and 4.63 ± 0.34mm) in width on natural diet whereas, (1.91 ± 0.11, 7.04 ± 0.17, 9.18 ± 2.84, 11.97 ± 0.10, 14.68 ± 0.36, 20.26 ± 0.37 and 24.23 ± 0.85mm) in length and (0.31 ± 0.01, 0.90 ± 0.03, 1.23 ± 0.02, 1.23 ± 0.02, 2.34 ± 0.04, 2.91 ± 0.06 and 4.01 ± 0.07mm) in width on artificial diet. This study is more or less supported by various authors.

In 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 and width were measured (22.57 ± 0.52 and 4.81 ± 0.32) on natural diet while (21.49 ± 0.31 and 4.25 ± 0.05) respectively, on artificial diet. The pupal period was also significantly influenced by different food, it was found (15.40 ± 0.40) in length and (5.08 ± 0.16) in width on natural diet and the length & width were (14.36 ± 0.30 and 4.82 ± 0.05) respectively, on artificial diet.

The adult male and female obtained from the different food types showed significant difference in their length. The length of female moths was relatively greater than males. 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 length of both male and female was greater on natural diet (17.14 ± 0.26 and 19.15 ± 0.28 mm) as compared to artificial diet (15.21 ± 0.32 and 17.45 ± 0.10 mm). Adult females showed wider wing span than adult males. The wings span of adult male and female was relatively broader on natural diet (25.02 ± 0.36 and 33.76 ± 0.94 mm) as compared to artificial diet (24.49 ± 0.07 and 32.85 ± 0.16 mm). The female moth had an almost straight distal forewing margin while in males the distal border of forewings was notched.

Table 4 : Morphometric study of *Galleria mellonella* (L.) on Natural and Artificial diet

Life stages	Natural diet				Artificial diet			
	Length (mm)		Width (mm)		Length (mm)		Width (mm)	
	Range (mm)	Mean	Range (mm)	Mean ± SD	Range (mm)	Mean ± SD	Range (mm)	Mean ± SD
Egg	0.30-050	0.42 ± 0.07	0.47-0.56	0.51 ± 0.03	0.36-0.41	0.38 ± 0.02	0.28-0.33	0.30 ± 0.02
1st instar	1.93-2.33	2.11 ± 0.10	3.34-0.37	0.35 ± 0.02	1.75-2.10	1.91 ± 0.11	0.29-0.33	0.31 ± 0.01
2nd instar	6.95-8.74	8.16 ± 0.69	0.65-1.10	0.97 ± 0.16	6.75-7.25	7.04 ± 0.17	0.84-0.95	0.90 ± 0.03
3rd instar	9.42-11.56	10.68 ± 0.73	1.22-1.32	1.27 ± 0.03	9.80-10.22	9.18 ± 2.84	1.20-1.26	1.23 ± 0.02
4th instar	11.95-12.74	12.41 ± 0.29	1.44-1.57	1.51 ± 0.04	11.00-12.15	11.97 ± 0.10	1.42-1.52	1.47 ± 0.03
5th instar	14.10-16.65	15.23 ± 0.82	2.10-2.65	2.44 ± 0.16	14.10-15.20	14.68 ± 0.36	2.31-2.39	2.34 ± 0.04
6th instar	20.42-21.40	20.89 ± 0.48	3.10-3.39	3.24 ± 0.10	19.75-20.85	20.26 ± 0.37	2.82-3.00	2.91 ± 0.06
7th instar	24.80-26.10	25.51 ± 0.43	4.25-5.10	4.63 ± 0.34	23.95-24.80	24.23 ± 0.85	3.92-4.12	4.01 ± 0.07
Pre Pupa	21.80-23.30	22.57 ± 0.52	4.10-5.13	4.81 ± 0.32	21.00-21.95	21.49 ± 0.31	4.15-4.33	4.25 ± 0.05
Pupa	14.75-16.00	15.40 ± 0.40	4.80-5.30	5.08 ± 0.16	13.95-14.75	14.36 ± 0.30	4.74-4.90	4.82 ± 0.05
	Body Length (mm)					Body Length (mm)		
Male	16.75-17.50	17.14 ± 0.26				Male	14.80-15.90	15.21 ± 0.32
Female	18.60-19.55	19.15 ± 0.28				Female	17.25-17.56	17.45 ± 0.10
	Wing expanse (mm)					Wing expanse (mm)		
Male	24.55-25.60	25.02 ± 0.36				Male	24.38-24.58	24.49 ± 0.07
Female	33-34.75	33.76 ± 0.94				Female	32.65-33-10	32.85 ± 0.16

Discussion

The total immature stages were recorded shorter on natural diet compared to artificial diet. This study was supported by Kandel *et al.,* 2020, who also reported that the growth of *G. mellonella* immature stages was shorter on natural diet (47.26 ± 2.8) as compared to laboratory diet (57.5 ± 2.3). The pre-oviposition, oviposition and post-oviposition periods of female were higher in natural diet while it was relatively shorter in artificial diet respectively. Findings of Tiwari, 2022 was similar and found the pre-oviposition, oviposition and post-oviposition period was prolonged in natural diet as compared to artificial diet. The larvae reared on natural diet showed relatively prolonged adult (Male & Female) longevity compared to artificial diet. The fecundity was comparatively higher on natural diet as compared to artificial diet. Kandel *et al.,* 2020 reported, that feeding on natural food of honey bee comb is the most favourable food kind to larvae of *G. mellonella* for

reproduction; showing the highest value of net reproductive rate. The total life cycle of adult's male as well as female showed prolonged on artificial diet, as compared to natural diet. This study is supported by Nangkar *et al.,* 2025 they reported female moths displayed varying lifespans based on their respective diets.

The result revealed that the survival percentage of eggs, larval, pupal was comparatively greater on natural diet whereas, it was relatively lower on artificial diet. Desai *et al.,* 2019, reported the hatching percentage of *G. mellonella* eggs varied from 84.72 to 99.06 per cent with an average of 91.54 ± 4.27 per cent under laboratory conditions. Pastagia and Patel (2007) noted 83.33 to 93.54 per cent hatching of eggs. Abdel-Gali *et al.,* 2024 presented the larval survival rates from the first instar to maturity ranged from 28.7 % to 94.2 %, depending on the different types of feeding. Thus, the present findings are more or less similar to past workers the difference in results may be due to different diet used for rearing *G. mellonella.*

In the morphometric studies the result is showing the length and width of all stages *viz.,* eggs, larval, pre-pupal, pupal and adults were greater on natural diet as compare to artificial diet. This study was more or less supported by various authors, Rahman *et al.,* 2017 reported eggs measured 0.32 to 0.47mm (0.39±0.04mm and 0.45 to 0.55mm (0.50±0.02mm) in width on natural diet. Desai 2019 reported, the average length of first to seventh instar larva were 0.81±0.03, 2.10±0.04, 5.86±0.19, 8.78±0.70, 14.24±0.95, 19.58±0.63 and 23.88±1.35mm while, breadth was 0.29±0.01, 0.44±0.02, 1.03±0.03, 1.99±0.08, 2.03±0.95 2.54±0.22 and 3.55±0.20mm, respectively.

Conclusion

This study focused on the impact of natural and artificial diet on the biological parameters (*viz.,* incubation period, larvae, pre-pupal, pupal duration and male & female longevity), survival percentage (eggs, larvae and pupal) and morphometric observations at different stage of *G. mellonella*. The methodology and diet implemented in this study provide a consistent and reliable source of information for maintaining populations of *G. mellonella* for future research as a model organism in biological control studies. This study demonstrate that is a useful and efficient tool for optimizing insect diets. The importance of considering combinations of ingredients and their interactive effects on insect fitness is highlighted. Generally, the positively differences in all biological and morphometric parameters of *G. mellonella* may be related to the host nutrition on natural and artificial diet. These effects include development and longevity of larvae feed on the natural diet. Perhaps the components of the natural diet, its pollen and its high protein contents may have a positive effect on the growth of the developmental stages of wax larvae. This approach to diet optimization is useful to support small lab-based colonies in addition to large-scale production.

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Author's contribution

In this research, AKM conducted the research work, SKS and RP guided throughout research work, SS and PK analysed data and Sapna and AD helps in written this work. All authors have read and approved the final manuscript.

Conflict of interest Authors do not have any conflict of interests to declare.

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