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RESEARCH ARTICLE

Dietary variations and their comparison with natural diet on the biological parameters of greater wax moth

Iddi Nangkar[1], Shimpy Sarkar[1*], Arshdeep Singh[2], Zeeshan Ali Sana[2], Ananya Chakraborty[2] & Iza Fatima[3]

[1]Department of Entomology, School of Agriculture, Lovely Professional University, Phagwara 144 411, India

[2]Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara 144 411, India

[3]College of Agriculture Food and Wine, The University of Adelaide, Urbrrae, South Australia, Australia

*Email: shimpy.23645@lpu.co.in, shimpy610@gmail.com

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Abstract

The research trial conducted at Lovely Professional University's Department of Entomology, School of Agriculture examined the efficacy of two distinct diets and one natural diet in the mass rearing of the greater wax moth. The influence of recommended meals on several biological features of the eggs, larvae, pupae and adults of greater wax moths was examined in a laboratory setting. The weight and size of the larvae and pupae were compared with the effects of feeding on natural foods, such as honeybee wax. The goal of the current study was to examine how this feeding strategy affected the biological characteristics of *Galleria mellonella* eggs, larvae, pupae and adults. The study concluded that the D2 diet-which included wheat flour, milk powder, yeast powder, honey, wheat bran and maize flour-was the most successful for the greater wax moths. It was discovered that this diet greatly shortened the larval length while improving the weight (27±0.7) and survival rate (90%) of the larvae, as well as raising adult emergence. The natural diet improved the fecundity of female moths, resulting in an average of 571.5 eggs laid per female and a shorter larval lifespan, among other benefits. The third-most effective diet was D1 feeding which contains 350 g wheat flour, 200 g maize flour, 130 g milk powder, 70 g yeast powder, 100 mL honey and 150 g sorbitol.

Keywords

artificial diets; biological parameters; fecundity of female moths; greater wax moth

Introduction

The historical technique of raising honeybees for commercial gain is known as beekeeping. Honeybees were first found in Africa and Spain circa 7000 BC. An important social and ecological insect is the honeybee (1). They are referred to as social insects since they reside in colonies. Since they produce honey, they are also known as beneficial insects. Bees produce royal jelly, propolis, wax and honey, making beekeeping one of the most lucrative pursuits. Aside from honey products, it is one of the main agricultural operations that provides good opportunities for villagers living below the poverty line (2). Therefore, beekeeping is a viable revenue stream for marginal and landless farmers. India currently produces and exports more honey than any other country 74413 MT in 2021-2022.

Beekeepers across the country are currently coping with challenging issues caused by the wax moth. Wax moths are categorized as belonging to the family Pyralidae of the order Lepidoptera and the subfamily Galleriinae. The lesser wax moth (*Achroia grisella*) and greater wax moth (*Galleria mellonella*) are two species of the order Lepidoptera frequently found in India. These two species of wax moth are either native worldwide or have been introduced by humans and are found nearly everywhere apiculture is practiced. The greater wax moth is the most dangerous to honeybee combs (3). In tropical and subtropical regions, warm temperatures are ideal for promoting their rapid growth. Although greater wax moth infestations occur year-round, the peak larval infection period is in August, September and October.

Since *G. mellonella* is a member of the Lepidoptera order, complete metamorphosis frequently occurs. The larval stage is the most damaging, consisting of seven instars. After hatching, adult females enter the hive at night to lay their eggs in cracks and holes on the bottom board (4). As a result, newly hatched larvae begin eating the honeycomb and digging tunnels to pupate. They will consume one another even if there isn't enough food for them. The majority of yearly financial losses in apiaries are caused by the greater wax moth is the primary cause of annual financial losses in apiariess and is the most destructive insect affecting them. They damage not only the comb but also the hive's wooden components (5).

Within the comb, the larvae form galleries and consume pollen, residual pupal skins, beeswax and brood. The wax moth typically attacks weak colonies. In India, honeybee disappearance from hives due to wax moth infestations is a prevalent and troublesome issue. In addition to larvae, adult moths also cause harm by spreading diseases like foulbrood when moving between colonies (6). Beehive damage is mostly caused by wax moth larvae. It is often referred to as the "micro-pest" by beekeepers because, particularly in weak hives, it can severely damage stored combs and beekeeping equipment.

The greater wax moth, (*G. mellonella* L.) is a significant pest of honeybees. However, due to its value as a surrogate host for different biological control agents, it is currently being artificially raised in lab settings (7). Many biological control agents, such as entomopathogenic nematodes, which are parasites on only target insect pests, are produced in large numbers by wax moth larvae (8). Wax moth larvae are necessary for the raising of some insect natural enemies, such as *Microplitis* spp., *Archytas* spp., *Apanteles* spp. and *Bracon hebetor* L.

Insect physiology, biochemistry and toxicological studies frequently employ wax moth larvae. Moreover, a variety of features of insect physiology, geonomics and proteomics were studied using wax moth larvae as model organisms.

It's critical to assess the adjusted artificial meal composition's effects on a range of biological parameters, including larval weight, duration, pupal weight, duration, fertility, survival and overall developmental period, before to putting it into practice. Before the modified diet is approved for usage, it must undergo a thorough assessment (9). The study focused on feeding wax moth larvae on several artificial diets while they were being raised in a lab. Based on the aforementioned analysis, we investigated the optimal diet to guarantee the production of a large number of healthy larvae while avoiding any negative effects on the target insect model.

Materials and Methods

Research location and source

The trials were carried out at Lovely Professional University (LPU), Phagwara, Punjab, in the Department of Entomology, School of Agriculture. Larvae of *G. mellonella* were collected from combs housed in the greenhouse's apiary at Lovely Professional University's School of Agriculture. Following their collection, the larvae were fed an artificial diet, housed in a plastic jar and monitored until they reached adulthood. After emerging, the adults were moved to another plastic jar for oviposition and mating and to promote egg laying, white-coloured paper was affixed to the jar wall. Following that, a homogeneous population was employed in additional research.

Rearing of G. mellonella under laboratory condition

The greater wax moth larvae have been raised in a proper, supervised laboratory environment at an appropriate temperature of 29.2 °C, using two artificial feeds and one natural diet. The first and second diets recommendation were followed during the experiment (10). A list of the diet's ingredients was presented in Table 1. A few recommended diet items for *G. mellonella* L. mass rearing in a lab environment is listed below (29.2 °C, 61.5 % RH and 24 hr of darkness). Wheat flour (350 g), maize flour (200 g), milk powder (130 g), yeast powder (70 g), honey (100 mL) and sorbitol (150 g) were used to produce the initial diet. The following materials were used to produce the second diet including maize flour (200 g), dried yeast (50 g), honey (175 mL), wheat flour (100 g), wheat bran (100 g) and milk powder (100 g) (11). The preparation of the diet is quite simple; we only need to measure each ingredient. Next, all

Table 1. Ingredients for different artificial diets

S. No.	Ingredients	Quantity of D1	Quantity of D2	Control (old bee wax)
1.	Wheat flour	350 g	100 g	-
2.	Corn flour	200 g	-	-
3.	Milk Powder	130 g	100 g	-
4.	Yeast powder	70 g	50 g	-
5.	Honey	100 ml	175 ml	-
6.	Sorbitol	150 ml	-	-
7.	Wheat bran	-	100 g	-
8.	Maize flour	-	200 g	-
9.	Old bee wax	-	-	50 g

D1 = Diet 1, D2 = 2 and control is old honeybee wax which is natural diet for greater wax moth the ingredients should be placed in a container and mixed well with gloved hands until no lumps remain. To ensure complete mixing, the liquid ingredients-honey and sorbitol-were measured and combined precisely in a plastic beaker. Following that, the liquid ingredients were added to the dry ingredients and thoroughly mixed. The greater wax moth larvae were raised on these diets. The honeybee wax comb (*Apis mellifera*), especially a portion (5×5 cm) and weighing roughly (10 g) for each larva, was fed to the control group when they were one year old. The biological characteristics of *G. mellonella* were recorded and the different developmental stages were examined. Two groups of ten neonates each (D1 and D2) were given test diets with ten replicates of each diet (Table 1).

Rearing procedure

During the experimental period, the three different diets were replicated 10 times for the rearing of larvae. The hatched larvae were reared on three tested diets and they progressed into the pupation stage. The pupae were placed in a pupal jar and kept for adult emergence. Upon emergence, the adults were separated into male and female and placed into a mating jar. A 40 % honey solution was placed inside the jar for feeding the adults. The laid eggs were collected from the laying jar and placed on a wax sheet for observation. The hatching of the eggs was observed regularly and the larvae were transferred to a separate container for feeding on a different artificial diet. The containers were placed inside a BOD incubator and observations were made regularly to record the duration of larval instars. A fresh artificial diet was provided twice a week until the 5th instar, after which the diet was not changed to avoid disturbing the larvae as they were allowed to pupate. Observations were made from the pupation stage to emergence. After emergence, the males and females were separated and placed into a new jar for further experimentation. The experiments were conducted under laboratory conditions, with the temperature controlled at 29.2 °C and the relative humidity (RH) maintained at 61.5 %. The study involved testing three different diets for feeding *G. mellonella* larvae. A control group was fed a honeybee wax comb, while the test diets are described in (Table 1). Each diet was tested with ten replicates of one neonate and their biological parameters were recorded at different stages of development. The honeybee wax comb used as the control weighed approximately 50 g and was one year old. The results were analyzed and compared to determine the effectiveness of the different diets (Fig. 1-3).

Biology of greater wax moth

The adult greater wax moth was collected from a homogenous culture, then moved to a separate plastic container for egg-laying and given a honey-based solution as a food source. The eggs were then separated and transferred to 20 small plastic containers that had been cleaned and sterilized. Each container held only one egg, which was kept separately for observation of the hatching percentage. The duration of the incubation period, which refers to the time required for eggs to hatch, was observed and documented. Additionally, the percentage of successful hatchings was recorded (12). The newly hatched neonates were transferred to a new container filled with freshly prepared food using a brush. To prevent the larvae from escaping, the top of the containers was covered with muslin cloth and fastened with rubber bands. The study recorded various observations related to the life cycle of the greater wax moth. This included fecundity, the developmental duration of each instar, the total number of instars, the total larval development duration, percent larval survival, pre-pupal and pupal periods, sex ratio and adult longevity (13). The formulas used to calculate the hatching and larval survival percentages that were used by various authors.

Hatching percentage

$$\text{Hatching \%} = \frac{\text{Number of egg hatched}}{\text{total number of total egg}} \times 100$$

Larval survival percentage

$$\text{Larval survival \%} = \frac{\text{Number of larva survived}}{\text{Total number of total larva}} \times 100$$

A plastic container was used to house a male and a female adult moth. To sustain them until death, a cotton swab soaked in a 40 % honey solution was provided as food. Daily observations were made to record the number of eggs laid by the female moth each day until her death. After emergence of male and female moths were distinguished based on their mouthparts to calculate the sex ratio.

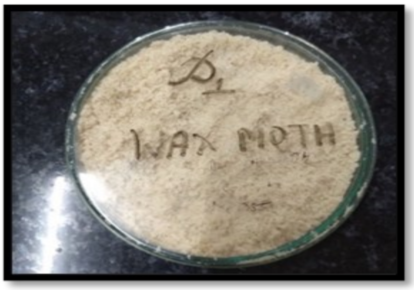

Fig. 1. Diet 1.

Fig. 2. Diet 2.

Fig. 3. Natural diet.

Results and Discussion

The experiments described here were conducted in controlled laboratory environments at the Department of Entomology, School of Agriculture, Lovely Professional University, Punjab. The findings of the experiments are presented in an organized manner, with appropriate headings and subheadings as well as tables and figures to support the results. The present study also includes a detailed investigation of the biology of insects.

Number of eggs

Following mating, female *G. mellonella* will begin egg-laying within a few hours. The rate of egg development can be manipulated by controlling the temperature in an oviposition chamber. Higher temperatures (29°C-35°C) accelerate development, while lower temperatures slow down egg development, taking up to 30 days or more. The eggs are small, elliptical and pear-white in color (14). The number of eggs in each cluster varies depending on the diet of the moths. For the first diet, the cluster contained an average of 329±39 eggs; for the second diet, the average was 610±30.5; and for the natural diet, it was 571.5±41.3 eggs per female. Trials conducted under laboratory conditions yielded similar observations (15). Upon exposure to air, the eggs turned light yellow and had a rough texture due to the presence of wavy lines on the eggshell. The eggs turned black the day before hatching and pre-emerged larvae were visible due to the presence of a prominent black head just before hatching (Fig. 4-7). These observations were consistent with those reported in previous studies (16). The data are presented in Table 2a & 2b.

According to observations, the hatching of *G. mellonella* eggs predominantly takes place in the morning hours, particularly from 8 A.M. to 10:30 A.M. Most eggs hatch within the first 2 to 3 days. Researchers found that the fecundity of the greater wax moth ranged from 650 to 1120 eggs per female (17). Similar results were reported, with mean fecundity values of 790.90 ± 169.78 and 864.55 ± 98.6 eggs per female, respectively (18). The differences in the results of these studies may be attributed to various factors, such as the type of food provided to the insects, the conditions under which they were reared, the season in which the study was conducted and the environmental variables specific to each region (19).

Fig. 4. Egg laying.

Fig. 5. Before hatching.

Fig. 6. Freshly laid egg.

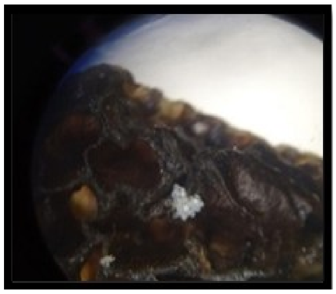

Fig. 7. After hatching.

Table 2a. Different biological parameters of greater wax mot

S. No.	Different biological parameters	Diet 1		Diet 2		Control	
		Av. ±SE	Percentage (%)	Av. ±SE	Percentage (%)	Av. ±SE	Percentage (%)
1.	No. of egg laid by per female	329±39	-	610±30.5		571.5±41.3	-
2.	Oviposition period (days)	6.8±0.3	-	6±0.1		6±0.1	
3.	Incubation period (days)	7.0±0.3	-	5.6±0.4		6±0.3	
4.	Egg hatching %	-	91.50		95		87.50
5.	Female longevity (days)	6.9±0.4	-	7.3±0.3		7.3±0.3	
6.	Male longevity (days)	15.0±0.5	-	10.4±0.8		12.4±0.8	
7.	Larval survival %	-	85	-	90	-	83
8.	Adult emergence %	-	90	-	95	-	82
9.	Total life cycle of male (days)	73.49	-	55.4	-	66.3	-
10.	Total life cycle of female (days)	65.8	-	53.1	-	61.2	-
11.	Suitable temperature (°C)	29.2°C	-	29.2°C	-	29.2°C	-
12.	Relative humidity %	-	61.5	-	61.5	-	61.5
13.	Weight of mature larva (g)	26.4±0.7	-	27±0.7		20.1±0.4	-

Table 2b. Different biological parameters of greater wax moth

Diets	*Growth parameters*						
	Pre-pupation	*Pupation period*	*Fecundity*	*Incubation period*	*Male longevity*	*Female longevity*	*Weight of fully grown Larval*
			Descriptive statistics				
Minimum	2	5	130	3	7	5	18
Maximum	6	12	759	9	21	9	29
Overall average	3.733	10.2	503.633	6.2	12.6	7.16	24.5
			Post hoc Anova (one way)				
Control	3.9^{a}	10.4^{ab}	571.5^{a}	6^{a}	12.4^{a}	7.3^{a}	20.1^{b}
Diet1	4.1^{a}	11.8^{a}	329.2^{b}	7^{b}	15^{c}	6.9^{b}	26.4^{a}
Diet2	3.2^{a}	8.4^{b}	610.2^{a}	5.6^{a}	10.4^{b}	7.3^{a}	27^{a}
MSE	1.237	6.088	13975.76	1.4	10.104	1.670	5.011
Df	27	27	27	27	27	27	27
CV	29.791	24.191	23.473	19.235	25.227	18.034	9.136
LSD	1.020	2.264	-	-	-	-	2.0541

Incubation period of eggs

In a controlled laboratory condition, it was observed that the length of the incubation period for moths varied depending on their diet. Specifically, moths fed the first diet exhibited an average incubation period (7 ± 0.3 days). The larvae fed the second diet had a smaller range of incubation periods, averaging at 5.6 ± 0.4 days. Finally, moths fed a natural diet had an average incubation period of 6 ± 0.3 days. The findings of this study were largely consistent with previous reports. Others reported a similar range of 3 to 6 days, observed a range of 3 to 10 days (20). An example of a similar study is the incubation period of greater wax moth eggs. When the larvae were raised on honeybee wax combs of different species namely *A. cerana, A. dorsata, A. mellifera* and *A. florae* and the mean incubation period ranged from 8.6 to 9.2 days. However, it was found that the incubation period was significantly influenced by the rearing temperature, with mean incubation periods of 13.4, 8.4 and 6.8 days recorded at different temperatures of 22 °C, 27 °C and 32 °C, respectively (21).

Hatching percentage of eggs

During the laboratory experiment, the observed hatching percentage of the greater wax moth on different diets varied. For the first diet hatching percentages was 91.50 %, for the second diet, it was 95 % and for the natural diet, it was 87.50 %. Therefore, the present results are consistent with those of previous studies (22). The variation in results could be attributed to the use of different diets to rear *G. mellonela*.

Larval stage

The larvae went through a total of seven instars, each marked by distinct changes. When they first hatched, the larvae were wedge-shaped, with a pale white hue and a noticeable swiftness in their movements. As they grew and molted, their appearance transformed into a spindle-like shape and their color darkened to a murky gray tone (Fig. 8-16). During their development, insects go through multiple stages known as instars. In this species, the initial instar larvae exhibit slow feeding behavior, while the later instar larvae become more voracious feeders. As the seventh instar larva reached maturity, it began to exhibit restlessness and actively sought out a suitable location for pupation (23).

The larval duration for greater wax moth larvae was recorded for three different diets, including D1, D2 and a control diet of beewax comb. The results reveal that the larvae reared on D1, D2 and the control diet had a larval durations of 3.3 ± 0.3, 4.7 ± 0.3, 5 ± 0.4, 6.2 ± 0.2, 7.1 ± 0.3, 7.7 ± 0.3, 8.5 ± 0.4 days; 2.5 ± 0.2, 3.1 ± 0.2, 3.5 ± 0.3, 5.5 ± 0.3, 6.3 ± 0.3, 6.6 ± 0.4, 6.7 ± 0.3 days and 2.8 ± 0.2, 3.8 ± 0.2, 4.6 ± 0.2, 5.7 ± 0.3, 7.5 ± 0.4, 8.1 ± 0.3 days, respectively. The mean duration of the larval stage was significantly shorter for a second diet compared to the other groups. The outcomes of this study are consistent with the findings of previous researchers who stated that the duration of the larval stage ranged from 23.4 to 40 days, depending on biotic or abiotic factors affecting rearing (24). The reason for the shorter duration of the larval stage in greater wax moth larvae could be linked to the presence of extra ingredients in their diets, as observed in D2 (2). Studies had reported the duration between 23.6 to 29.5 days, when wax moth larvae were reared on wax comb, it took around 39.6 days (25). Greater wax moth larvae had a longer larval duration period when they were raised on honeybee combs of different *Apis* spp, with durations ranging from 49.3 to 110.65 days. In contrast, others reported that the developmental period was not significantly affected by the use of different formulations of artificial feed (25).

Table 3a. Length (mm) of different larval instars

	Length (mm)	Diet 1		Diet 2		Control	
		Av.±SE	Range (mm)	Av.±SE	Range (mm)	Av.±SE	Range (mm)
1.	1st instar	1.5±0.1	1-2	2.4±0.2	1-3	1.7±0.2	1-3
2.	2nd instar	3.1±0.2	2-4	5±0.2	4-6	3.3±0.2	2-4
3.	3rd instar	6.8±0.3	5-9	7.5±0.2	6-9	6.5±0.3	5-8
4.	4th instar	10.1±0.2	9-11	12.8±0.6	10-16	11.3±0.6	8-15
5.	5th instar	16.9±0.4	15-19	17.1±0.3	15-18	15.9±0.3	14-17
6.	6th instar	20±0.3	18-22	21±0.3	20-23	19±0.3	18-21
7.	Male pupa	13.4±0.6	11-16	14.9±0.5	12-18	12.8±0.6	10-16
8.	Female pupa	20±0.2	18-21	21.2±0.7	18-25	19.4±0.4	17-21

Table 3b. Length (mm) of different larval instars

Diets	*Growth parameters*							
	Lmm1st	*Lmm2nd*	*Lmm3rd*	*Lmm4th*	*Lmm5th*	*Lmm6th*	*Male pupa*	*Female pupa*
	Descriptive statistics							
Minimum	1	2	5	8	14	18	10	17
Maximum	3	6	9	16	19	23	18	25
Average	1.866	3.8	6.933	11.4	16.633	20	13.7	20.2
	Post hoc anova (one way)							
Control	1.7[b]	3.3[b]	6.5[b]	11.3ab	15.9[b]	19[b]	12.8[b]	19.4[b]
Diet1	1.5[b]	3.1[b]	6.8[ab]	10.1[b]	16.9[ab]	20[ab]	13.4[ab]	20[ab]
Diet2	2.4[a]	5[a]	7.5[a]	12.8[a]	17.1a	21[a]	14.9[a]	21.2[a]
MSE	0.407	0.555	1.059	3.133	1.285	1.333	3.588	2.740
Df	27	27	27	27	27	27	27	27
CV	34.193	19.614	14.844	15.527	6.815	5.773	13.828	8.195
LSD	0.585	0.683	0.944	1.624	1.040	1.059	1.738	1.519

Fig. 8. 1st instar larva.

Fig. 9. 2nd instar larva.

Fig. 10. 3rd instar larva.

Fig. 11. 4th instar.

Fig. 12. 5th instar.

Fig. 13. 6th instar.

Fig. 14. 6th-7th instar.

Fig. 15. Head capsules.

Fig. 16. Exuviae.

Weight of the larva

The finding of this study revealed a difference in larval weight among the three of diets studies. The average weight of a mature larva reared on D1 and D2 showed almost similar results (26.4 ± 0.7 g and 27 ± 0.7 g). However, larvae that were raised on a natural diet had a significantly lower average weight (20.1 ± 0.4 g). As there are no earlier reports on the weight of mature *G. mellonella* larvae, it is not possible to compare the results (26).

Length (mm) of different larval instars

The larval length of different instars (1st to 6th) that were given diets D1, D2 and a control group that received bee wax was found to be 1.5 ± 0.1 mm, 3.1 ± 0.2 mm, 6.8 ± 0.3 mm, 10.1 ± 0.2 mm, 16.9 ± 0.4 mm, 20 ± 0.3 mm; 2.4 ± 0.2 mm, 5.0 ± 0.2 mm, 7.5 ± 0.2 mm, 12.8 ± 0.6 mm, 17.1 ± 0.3 mm, 21 ± 0.3 mm and 1.7 ± 0.2 mm, 3.3 ± 0.2 mm, 6.5 ± 0.3 mm, 11.3 ± 0.6 mm, 15.9 ± 0.3 mm, 19 ± 0.3 mm, respectively. Larvae fed on diet 2 showed the longest length of larvae from 1st instar to 6th instar compared to larvae fed on diet 1 and the natural diet. The average length of the 1st instar larva was 1-3 mm and the fully grown larva was 12-20 mm according to the data presented in Table 3a & 3b.

Larval survival

The survival rates of wax moth larvae from the first instar to maturity, with 85 %, 90 % and 83 % larvae recorded for those fed on diets D1, D2 and bee wax (control), respectively, indicating that the highest survival rate was observed in the group fed on diet D2 (Table 3a & 3b). In general, the results indicate that the group fed on diet D1 and bee wax had the lowest survival rate, while the group fed on diet D2 had the highest survival rate. Larval survival rates from the first instar to maturity ranged from 28.7 % to 94.2 %, depending on the different types of feeding (27).

Pre-pupation stage

The larva constructed a cocoon, either within its food source or attached upside down to the perforated cap of a plastic vial. The pre-pupal stage of the greater wax moth involves the larva shrinking in size and creating a cocoon using silk filaments and its own excreta. The larva remains inactive inside the cocoon for a few hours before undergoing metamorphosis into a pupa. Interestingly, the larva is still capable of movement and will search for a suitable spot for pupation if disturbed during this stage (Fig. 17). The cocoon formed by *G. mellonella* larva is characterized by its white, oval-shaped, hard and leathery texture. Once the pre-pupal stage is complete and the larva transforms into a pupa, it will undergo further development and eventually emerge as an adult moth (28). The larva created an opening at the front section of the cocoon that served as a wide exit hole for the adult to emerge from. The mean pre-pupal period of greater wax moths that were fed on different diets, including D1, D2 and a control diet of honeybee wax. The results indicate that the mean pre-pupal period for the moth fed on D1 was 4.1 ± 0.4 days, while it was 3.2 ± 0.2 days for those fed on D2 and 3.9 ± 0.3 days for the control group that was fed on honeybee wax. The analysis of variance revealed that the mean pre pupation period of the moth fed on diet D2 was significantly shorter compared to the other diets shown in Table 4a & 4b (29).

Pupation stage

The pupa underwent an object type of metamorphosis, during which all its extremities were firmly attached to its body by a secretion released during the molting process. At the beginning of pupation, the pupa exhibited a yellowish-white hue, later transitioned to brown and eventually dark brown, as it matured and underwent further development (Fig. 18-22). One observation was that the female pupae tended to have a greater length than the male pupae. The greater wax moth pupae's duration period varied depending on the type of diet they were fed during their larval stage, including diets D1, D2 and honeybee wax comb. The range of pupal duration was between 8 to 15 days for diet D1, 5 to 12 days for diet D2 and 8 to 15 days for honeybee wax comb. On average, the pupal duration period was 11.8 ± 0.8 days for diet D1, 8.4 ± 0.6 days for diet D2 and 10.4 ± 0.8 days for honeybee wax comb (30). The duration of the pupal stage was reported to range from 6.8 to 9.4 days. It was found that the pupal duration period was influenced by the rearing temperature, with longer durations observed at lower temperatures. Specifically, the pupal duration periods recorded were 18, 15 and 12.2 days at 22, 27 and 32°C, respectively (31).

Length of male and female pupa

The average length of the male and female pupa varied depending on the type of diet they were fed. On average, the length of male pupa was 13.4 ± 0.6 mm for D1, 14.9 ± 0.5 mm for D2 and 12.8 ± 0.6 mm for honeybee wax comb. The length of female pupa was 20 ± 0.2 mm for D1, 21.2 ± 0.7 mm for D2 and 19.4 ± 0.4 for natural diet. It was observed that the female pupae were generally longer in length compared to the male pupae align with the findings of (32).

Female longevity

The study indicated that female greater wax moths had varying average lifespans based on the type of diet they consumed during their larval stage. The results revealed that moths fed on diets D1, D2 and honeybee wax had mean longevities of 6.9 ± 0.4, 7.3 ± 0.3 and 7.3 ± 0.3 days, respectively. Notably, D2 and a natural diet showed almost the same results. However, it is possible that the disparity in results could be attributed to variations in the nutritional value of the food provided during the upbringing process. The female moths displayed varying lifespans based on their respective diets (33). The lifespan for those fed with D1 was 65.8 days, while those on D2 had a lifespan of 53.1 days. The female moths on the control feed exhibited a lifespan of 61.2 days (Fig. 23)

Male longevity

The findings of the study showed that the average lifespan of male greater wax moths varied depending on the type of diet they were fed during their larval stage (Fig. 24). Specifically, moths that had been reared on diets D1, D2 and control exhibited mean longevities of 15.0 ± 0.5, 10.4 ± 0.8 and 12.4 ± 0.8 days. As a result of the study, it was found that male greater wax moths had a longer lifespan than their female counterparts. The results showed that diet D2 had the shortest significant male longevity when compared to the other diets. A study on the biology of *G. mellonella* and reported that the adult male had an average lifespan of 16.4 ± 2.69 days (25). The male greater

Table 4a. Larval growth parameters of greater wax moth

S. No	Larval instar	Diet 1		Diet 2		Control	
		Av. ±SE	Range (days)	Av. ±SE	Range (days)	Av. ±SE	Range (days)
1.	1st instar	3.3±0.3	2-5	2.5±0.22	2-4	2.8±0.2	2-4
2.	2nd instar	4.7±0.3	4-6	3.1±0.2	2-4	3.8±0.2	2-5
3.	3rd instar	5±0.4	3-7	3.5±0.3	2-5	4.6±0.2	3-7
4.	4th instar	6.2±0.2	5-7	5.5±0.3	4-7	5.7±0.3	4-8
5.	5th instar	7.1±0.3	6-8	6.3±0.3	5-8	7.5±0.4	5-9
6.	6th instar	7.7±0.3	6-9	6.6±0.4	5-8	7.1±0.4	5-9
7.	7th instar	8.5±0.4	7-11	6.7±0.3	4-8	8.1±0.3	6-10
8.	Pre-pupation period	4.1±0.4	2-6	3.2±0.2	2-5	3.9±0.3	2-5
9.	Pupation period	11.8±0.8	8-15	8.4±0.6	5-12	10.4±0.8	8-15
10.	Total development period	58.4		45.8		53.9	

Table 4b. Larval growth parameters of greater wax moth

Diets	*Growth parameters*						
	LS1st instar	*LS2nd instar*	*LS3rd instar*	*LS4th instar*	*LS5th instar*	*LS6th instar*	*LS7th instar*
				Descriptive statistics			
Minimum	2	2	2	4	5	5	6
Maximum	5	6	7	8	9	9	11
Average	2.866	3.866	4.366	5.8	6.966	7.133	7.766
				Post hoc anova (one way)			
Control	2.8^{ab}	3.8^{a}	4.6^{a}	5.7^{a}	7.5^{a}	7.1^{a}	8.1a
Diet1	3.3^{a}	4.7^{b}	5^{a}	6.2^{a}	7.1^{ab}	7.7^{a}	8.5a
Diet2	2.5$^{\mathbf{b}}$	3.1^{b}	3.5^{b}	5.5^{a}	6.3^{b}	6.6^{a}	6.7b
MSE	0.6	0.688	1.144	1.044	1.092	1.459	1.537
Df	27	27	27	27	27	27	27
CV	27.021	21.465	24.498	17.620	15.003	16.934	15.962
LSD	0.710	0.761	0.981	0.937	0.959	1.108	1.137

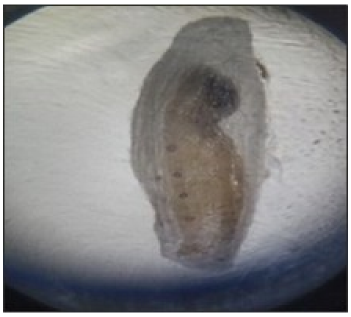

Fig. 17. Pre pupal stage.

Fig. 18. Newly formed pupa.

Fig. 19. Mature pupa.

Fig. 20. Male and female pupa.

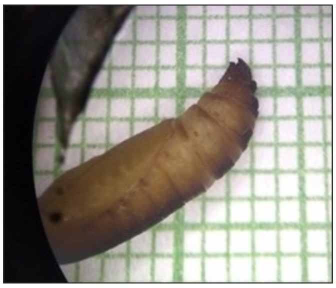

Fig. 21. Female pupa.

Fig. 22. Dark colored pupa.

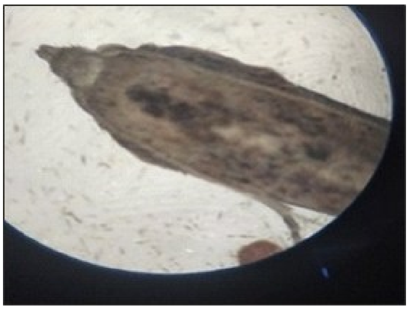

Fig. 23. Female adult.

Fig. 24. Male adult.

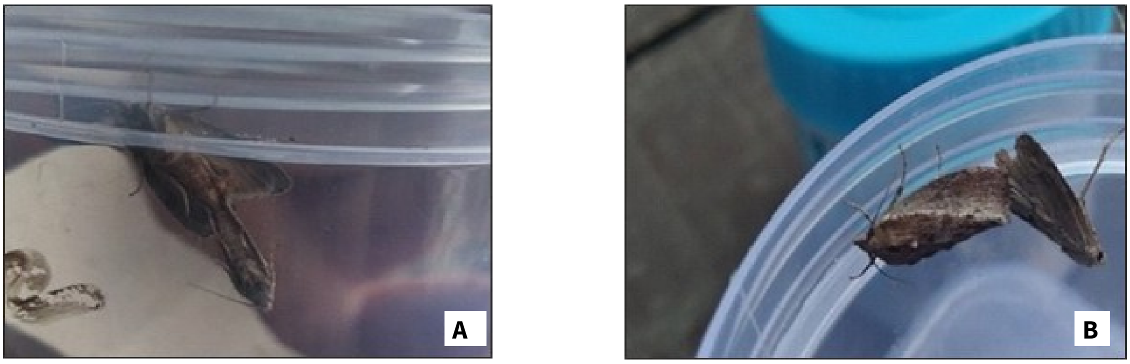

Fig. 25. Mating of male and female moth (a, b).

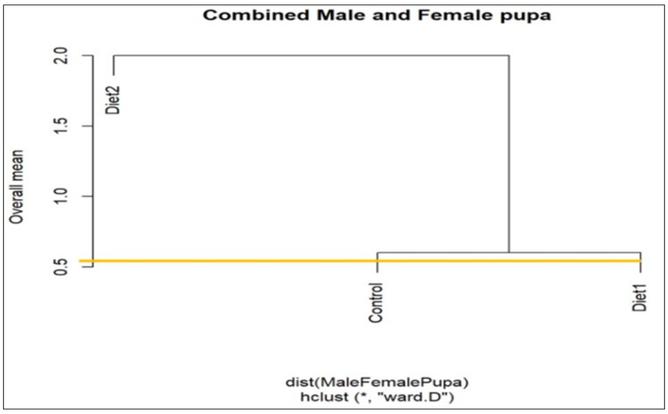

Fig. 26. Four homogeneous (2) and intermediary (1) groups based on male and female pupa mean values (statistical software R was used to get the graph).

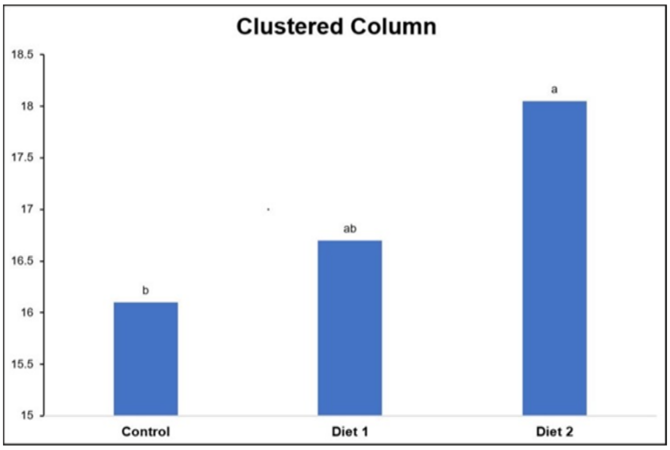

Fig. 27. Growth of wax moth on different diets (statistical software R was used to get the graph).

wax moth had a lifespan of 11.2 days (30). The total lifespan of male moths was different for different diets. It took 73.4 days for D1, 55.4 days for D2 and 66.3 days for the control feed (34).

Adult greater wax moth

The moths that recently appeared had a creamy white hue initially, but eventually, their color transformed into ash gray. This can be expressed as: "initially, the newly hatched moths displayed a creamy white appearance which later evolved into an ash grey colour". In both male and female pupae, the forewings exhibited varying degrees of pigmentation. In contrast to the rear one-third, which was made up of a variety of stripes of darker and lighter pigmentation, the anterior two-thirds of the wings were covered in scales, resulting in a uniformly darker coloration (Fig. 25a & 25b). The antennae of the greater wax moth were long and filiform, extending beyond the length of the head and thorax (35). Female moths were observed to be larger than males and the males were noticeably smaller and lighter in colour than the females. Another distinguishing characteristic between the sexes was the shape of the distal forewing margin, which was almost straight in females but scalloped (notched) in males. Additionally, the female moths had a labial palp that projected forward, giving their mouthparts a beak-like appearance, while in males, the labial palp was sharply curved upward and inward, resulting in a snub-nosed appearance. Among other investigations, similar morphological descriptions were reported (36). In the experiment, adult emergence percentages were different for different diets. For D1 and D2, it was 90 % and 95 % and for the control feed, it was 82 % (Fig. 26 , 27).

Conclusion

Diet 2 has been found to be the most appropriate and optimal artificial diet for *G. mellonella* when compared to the other two compositions. After studying, it was found that the larvae that were fed on diet 2 completed their life cycle in fewer days. Moreover, after rearing the wax moth larvae on the second diet, indicates its effectiveness (Tables 2 - 4). Thus, it can be concluded that the second diet is the most suitable and efficient diet for *G. mellonella,* as demonstrated by its sustained use over multiple generations from the best diet D2. But D1 was also effective for rearing of greater wax moth. Those larvae fed on D1 food took a little more time to complete their life cycle, their growth was as good as D2. Natural diet took less time to complete the life cycle of wax moth larvae, but the growth and survival rate were not as good as in D1 or D2.

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Authors' contributions

IN, ZAS and AC conducted the fieldwork. SS wrote the paper, AS performed the analysis and IF carried out the revisions. All authors have read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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