Research Article

Rearing of *Galleria mellonella* Under Controlled Environments: A Study on Diets, Temperature, and Management Strategies

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Abstract | Honey beekeeping in Pakistan faces significant challenges from various insect pests, with the wax moth (*Galleria mellonella*) as a dangerous, causing substantial losses to the honey bee industry. These trials investigated the development of the *G. mellonella* under laboratory settings, examining the effects of different temperatures and diets, as well as evaluating control strategies targeting various larval instars. At 25 °C, development on old wax took 54 days, which was significantly shorter compared to other diets. At 30 °C, development time decreased, with the moth completing its life cycle in 40 days on old wax, 42 days on a mixture of old and new wax, and 50 days on new wax. At 35 °C, the development period further shortened to 28 days on old wax, 33 days on the wax combination, and 37 days on new wax. Cold treatment (T1 temperature 2°C) caused 100% mortality in 1st instar larvae within 24 hours, and this remained unchanged through day 7. In contrast, the control group (T4 25°C room temperature) showed minimal mortality, reaching only 10% by day 7. For 3rd instar larvae, cold treatment resulted in 20% mortality after one day. By 72 hours, neem (T2) and marigold (T3) treatments showed the highest mortality at 30%. After 7 days, all three treatments (cold, neem, and marigold) resulted in 50% mortality. Neem treatment (T2) caused 20% mortality in 5th instar larvae at both 24 and 48 hours, increasing to 70% by day 7. Cold (T1) and marigold (T3) treatments were less effective against 5th instar larvae, with mortality rates remaining at 20%. The ideal temperature ranges from 30-35°C and old wax are ideal for rearing on the larva. Cold treatment is good control for early stage larvae and neem is good in controlling the wax moth larvae.

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Introduction

Honey beekeeping is practiced in Khyber Pakhtunkhwa on large scale. Two honey bee species are reared for honey production, the Asian honey bee *A. cerana*) and the exotic honey bees *A. mellifera*. Both have good potential for honey production and more 0.5 million people of Khyber

Pakhtunkhwa are involved in this sector. The sector is greatly challenged by climate change, floral resources, agricultural pesticides application, pests and diseases. Among the pests mites and wax moth have great impact on honey beekeeping (Ali *et al.*, 2022).

Wax moths tend to cause more problems in leftover combs and weak colonies. Their larvae typically eat wax combs, pollen, larval exuviae, and honey (Graham, 1992). The presence of wax moth infestation can be detected by silken threads or tunnels within the wax combs (Hepburn and Hepburn, 2005). Heavy larval feeding can break down the combs into piles of debris made up of frass and wax moth webbings, and the larvae may even bore into the midribs (bases) of the combs. They usually prefer dark combs, which are those used for brood rearing, making these combs more vulnerable to damage (Ellis *et al.*, 2013).

The *G. mellonella* consume wax combs, discarded honey bee larval skins, honey and pollen (Graham, 1992). Their occurence within frames is indicated by the formation of silken threads, which often create tunnel-like structures inside the wax. This pest tends to cause more damage in weak or stressed colonies compared to stronger ones (Ellis *et al.*, 2013; Sohail *et al.*, 2017; Hood *et al.*, 2003).

Average temperatures ranges from 25-30°C, to complete the developmental stages the duration is 5 days for the egg hatching, 30-40 days larva, 14 days for pupal stage. Under warmer conditions (33.8 °C), the larvae go through 8 to 9 molts during their development (Charriere and Imdorf, 1999).

Chemical fumigants are commonly used to control greater wax moth (GWM) in beekeeping. These include ethylene bromide, methyl bromide, sulphur, phosphine etc. However, these fumigants are toxic to bees and beneficial insects (Ellis *et al.*, 2013).

The present study was conducted to evaluate the larval development of the greater wax moth (*Galleria mellonella*) on various diets across different temperature conditions, and to assess the effectiveness of multiple control methods for its management under laboratory conditions.

Materials and Methods

Location

The trials were carried out at Entomology Research Programme at Agricultural Research Institute (ARI), Tarnab farm Peshawar during 2019.

Wax moth culture

Greater Wax moth (*Galleria mellonella*) cultures were developed using old, infested frames recovered from bee colonies at ARI Tarnab, Peshawar. These frames provided a consistent and reliable source of wax moth culture for the experiments.

Rearing and testing of development on different cultures

The trials for rearing and treatments were started in first week of April 2019 and ended in June 2019. First instar larvae were selected from the culture. Ten larvae were assigned to each treatment and placed in separate containers enclosed with mesh. The containers were made of plastic and were then placed in an incubator (Infitek-ICB Series, China) set to three temperatures: one group at 25 °C, second at 30 °C, and third at 35 °C to observe the effects of temperature variation. At each temperature, four types of diets were provided as food sources: new wax (new prepare wax sheets), old wax (one year old frame wax), a mixture of old and new wax (50:50 of both), and paper wasp nests (abandoned nests rom trees and old buildings).

Treatments for wax moth control

Different larval instars were separated for experimental purposes, specifically the 1st, 3rd, and 5th instars. Ten larvae from each instar stage were selected for each treatment group. They were placed in individual plastic containers (7x4.75x2.5 inches), which were enclosed with mesh. These containers were then kept in an incubator, where various treatments were applied. Temperature (25±2°C) and humidity at (60+5%) was maintained according to the recommended laboratory standards for wax moth rearing (Ellis *et al.*, 2013). These treatments were applied T1= Cold treatment for three hours, T2= 5% neem spray (extracted from need seeds) on larva, T3= Marigold extraction (flowers extraction) spray and T4= control no treatment was applied. The mortality of wax moth larvae was recorded after 24 hours, 48 hours, 72 hours and 7days.

Statistical analysis

The data were recorded for rearing and treatments trials and means were calculated, the mean were subject to statistical analysis using computer program Statistix-10.

Results

Development under varying feeds and temperatures

The *G. mellonella* development on four diets varied at 25 °C as presented in Figure 1. Significant differences were recorded in development on different feeds. The development was completed in significantly less time on old wax (54 days), combination of old and new wax (58 days) as compared to new wax (62 days) however development on paper nest was was slow (73 days).

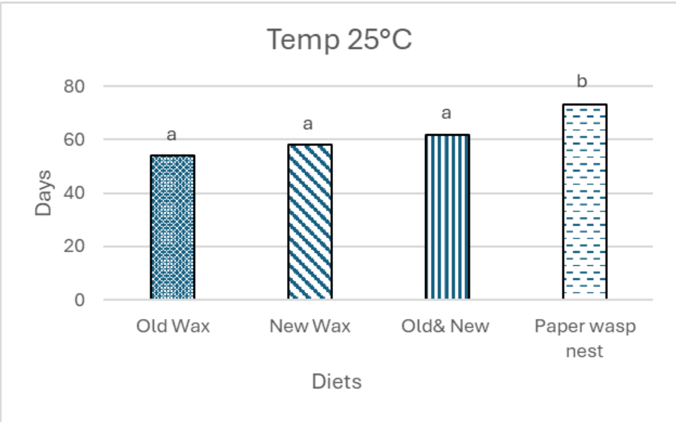

Figure 1: *Wax moth development on different diets at 25°C under laboratory conditions.*

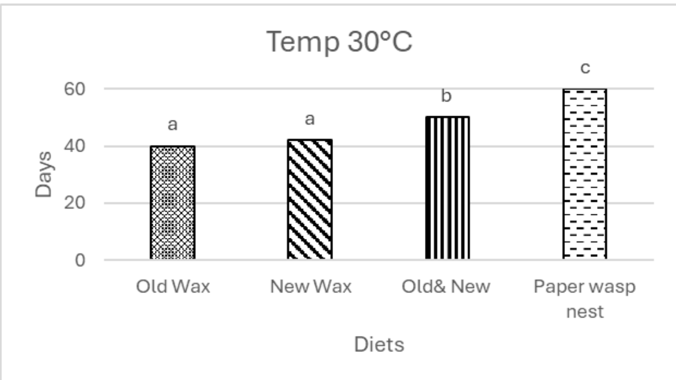

Figure 2: *Wax moth development on different diets at 30°C under laboratory conditions.*

The development of the wax moth on four diets varied at 30 °C as presented in Figure 2. Significant different were recorded in development on different feeds. The development was completed in significantly less time on old wax (40 days), combination of old and new wax (42 days) as compared to new wax (50 days) however development on paper nest was slow (60 days).

The development at 35° showed accelerated development as compared with 25 °C and 30°C. The development of the wax moth on four diets varied at 35 °C as presented in Figure 3. Significant differences were recorded in development on different feeds. The development was completed in significantly less time on old wax (28 days), combination of old and new wax (33 days) as compared to new wax (37 days) however development on paper nest was slow (40 days).

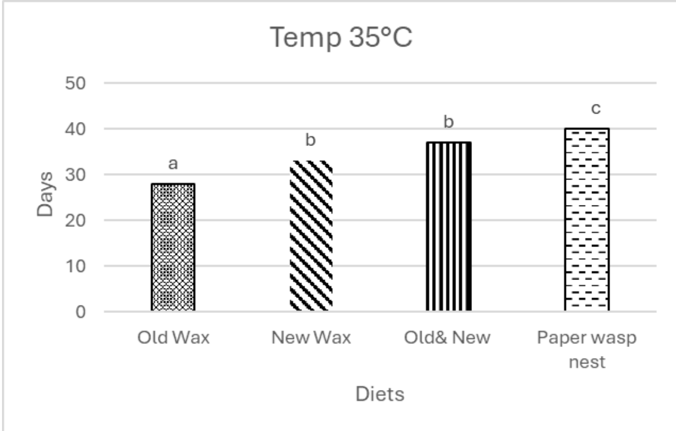

Figure 3: *Wax moth development on different diets at 35°C under laboratory conditions.*

Management of wax using different control strategies

1st instar larvae: Figure 4 illustrates the percentage of dead wax moth larvae (1st instar) under various treatments. After 24 hours, the highest mortality was observed in the cold treatment (T1), with 10 dead larvae, while no mortality was recorded in the control group (T4). At 48 hours, complete mortality (all larvae dead) was again recorded in the cold treatment (T1). In contrast, the lowest mortality was observed in both the marigold treatment (T3) and the control (T4), with only one dead larva in each. After 72 hours, cold treatment (T1) continued to show the highest mortality (5 larvae), whereas the control (T4) had the lowest. By the seventh day, T1 (cold treatment) still exhibited the highest mortality (10 dead larvae), while the control group (T4) had the lowest, with only 1 larva dead.

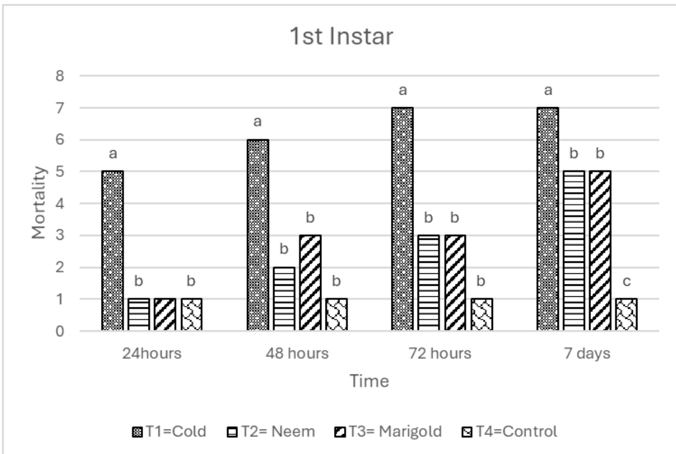

Figure 4: *Efficacy of different treatments against 1st instar larvae.*

3rd instar larvae: The percentage of dead wax moth larvae (3rd instar) due to different treatments is presented in Figure 5. After 24 hours, the highest mortality (2 larvae) was observed in both the cold treatment (T1) and the marigold treatment (T3), while no mortality was recorded in the control group (T4). After 48 hours, the same trend continued, with the highest mortality (3 larvae) again observed in cold (T1) and marigold (T3), and no mortality in the control. After 72 hours, the maximum deaths (3 larvae) reported in the neem (T2) and marigold (T3) treatments. After 7 days, the maximum mortality (5 larvae each) was recorded in cold (T1), neem (T2), and marigold (T3) treatments. The minimum mortality (1 larvae) in the control group (T4).

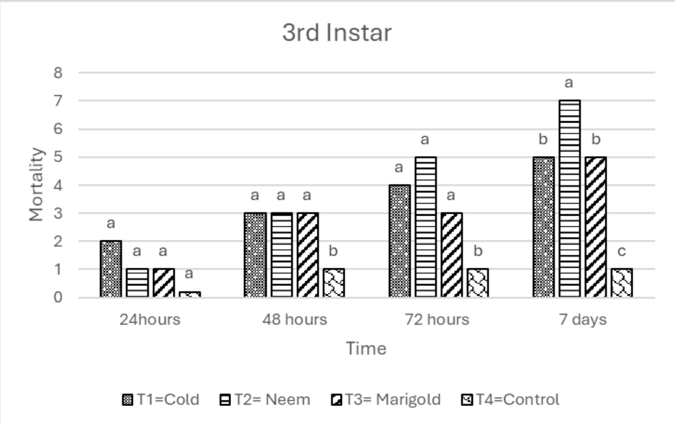

Figure 5: *Efficacy of different treatments against 3rd instar larvae.*

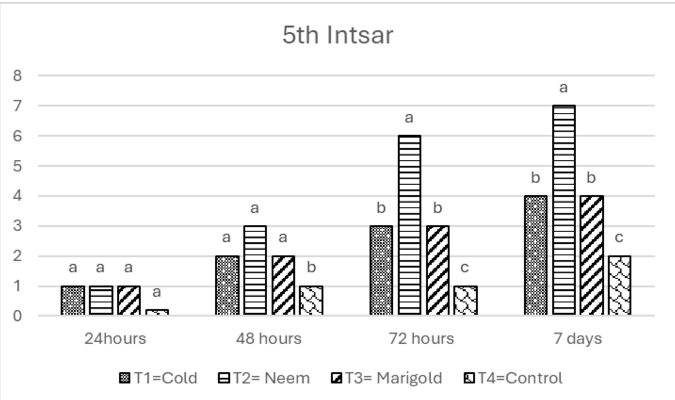

Figure 6: *Efficacy of different treatments against 5th instar larvae.*

5th instar larvae: Figure 6 shows how many wax moth larvae (5th instar) died under different treatments at different times. After 24 and 48 hours, only the neem treatment (T2) caused any deaths, with 2 larvae dying. No larvae died in the other treatments (cold (T1), marigold (T3), and control (T4). After 72 hours, the control group (T4) had only one dead larvae. After 7 days, the highest number of deaths was again in the neem treatment (T2), with 7 larvae dead. The cold (T1) and marigold (T3) treatments had the lowest, with 2 larvae dead in each. Only one dead larvae was recorded in control (T4).

Discussion

The current study reported that temperature has great impact on the life cycle of *G. mellonella*. At 25°C the development took longer time as compared to 30°C and 35°C. Similar results were also has been reported by Hanumanthaswamy *et al.* (2013), who reported that the optimum temperature for wax moth rearing under lab condition ranges from 28°C and 34°C. Kwadha *et al.* (2017) also reported that temperature below than 25°C and above 35°C can affected life cycle of this pest. At this range of temperature, the metabolism remains at peak and is critical for development, growth rate and reproduction. So, the temperature has an important role in larval development, pupation and adult emergence. The humidity during the experiments were kept constant at 60± 5 %. The humidity is also an important factor in rearing of the wax moth under laboratory conditions. Earlier studies of Ranjbar-Aghdam *et al.* (2015) and Borah *et al.* (2024) supported our results that humidity ranges from 50±10% and temperature ranges from 28-35°C are ideal for mass rearing of wax moth under laboratory conditions. Krams *et al.* (2015) reported that diets with varying compositions affect the life cycle of the moth due to differences in their nutritional quality. Some diets promote faster and earlier development compared to others. Temperature also plays a crucial role in the development of the wax moth across different diets.

In the trials, cold treatment, neem extract spray, and marigold extract were applied at different larval stages of the wax moth to assess their effectiveness in causing mortality. Previous findings by Gulati and Kaushik (2004) indicated that early instar larvae of the wax moth are susceptible to cold treatment; however, this method is less effective against later larval stages, which show higher survival rates under cold conditions. Cold treatment remains a simple and effective method for managing early larval stages of moth in wax storage. In the current study, neem extract also demonstrated high effectiveness, resulting in significant mortality across all larval stages. These findings are consistent with the results of Elbehery *et* *al*. (2016), who reported neem's efficacy in controlling wax moth larvae. Neem extract showed promising results both in laboratory and field conditions, with significantly higher larval mortality.

Conclusions and Recommendations

The temperature and diets influenced the life Cyle of *G. mellonella*. Optimal growth was observed on old wax at temperatures of 30°C and 35°C. Early instar larvae in infested frames can be effectively managed using cold treatment for 3–5 hours. However, later instar stages are more resistant to cold, requiring the implementation of proper management techniques for effective control. The beekeeper should used pollen diets in dearth period to improve the health and brood rearing of colonies.

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Novelty Statement

The paper provides insightful information on rearing wax moths on different diets and includes additional methods for their management.

Author's Contribution

Hussain Ali: Conducted the experiments, collected the data and submitted the paper for publication.
Mirzakhmedov Muhammadjon Fazliddin Ogli: Did the analysis.
Saparov Kuanishbay Abdirakhmanovich, Sultanov Esbosin Kosimbetovich and Embergenov Muratbay Amanbaevich: Wrote this first draft.

Generative AI and AI-assisted technology statement
In this paper, AI was used primarily for correcting grammar and improving sentence structure only.

Conflict of interest
The authors have declared no conflict of interests.

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