

Biology of Greater Wax Moth, *Galleria mellonella*, (Galleridae: Lepidoptera) using Artificial Diet

Abstract

Galleria mellonella L. is an important pest of honeybees. Globally, *Galleria* baiting technique with larvae of the greater wax moth, *G. mellonella* L., is the most commonly used method for recovering entomopathogenic fungi and infective-stage juveniles of entomopathogenic nematodes from soil. Therefore *G. mellonella* was mass reared at laboratory condition using modified method of Birah *et al.* (2008) by artificial diet. The mean larval period was 3.2 ± 0.08 for 1st instar, 5.2 ± 0.13 for 2nd instar, 6.1 ± 0.39 for 3rd instar, 5.6 ± 0.26 for 4th instar, 7.4 ± 0.13 for 5th instar, 7.6 ± 0.21 days for 6th instar, 8.5 ± 0.47 days for 7th instar. The mean pupal period was 8.1 ± 0.29 days. The total developmental period was 73.56 ± 1.27 days. Mean fecundity was 680 ± 42.5 numbers, larval weight was 19.98 ± 0.91 mg for 7 days old larva, 376.48 ± 6.52 mg for 14 days old larva, mean pupal weight was 393.33 ± 6.81 mg and adult emergence was 91.15 ± 3.97 per cent.

Keywords: Greater wax moth, *Galleria mellonella*, Biology, Artificial Diet

Introduction

The greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), is a major pest of honeybees and it is a great threat to India's beekeeping industry. It feeds on honey and comb within honeybee nests. In the wild, these moths invade honeybee hives, laying eggs on the comb. After hatching, the larvae burrow into the comb, consuming wax and honey before pupating and transforming into adult moths. Waxworms are rich in fats and proteins, making them an excellent food source for fish, reptiles, amphibians, and invertebrates. Raising waxworms in captivity is more challenging than raising mealworms or superworms, as moths can fly and are harder to contain. When the larvae grow to approximately three-quarters of an inch in length, they spin cocoons, pupate and emerge as adult moths after about two weeks. Adult moths live for only a week, enough time to mate and lay eggs. Providing crumpled balls of waxed paper or chunks of beeswax (such as beeswax candles) in their enclosure encourages egg-laying. The larvae typically take around five weeks to reach full size. Parthasarathy and Rabindra (2002) and Singh (1994) conducted mass rearing of *G. mellonella* on an artificial diet for GmNPY production. Over the past four decades, significant advancements have been made in the mass rearing of *G. mellonella*. However, previous studies have not thoroughly explored various biological characteristics of this insect and there

are differing opinions on several biological parameters. Hence, the current study was conducted to evaluate the feasibility of rearing *G. mellonella* in a meridic diet.

Materials and methods

Mother culture

The first instar larvae of *G. mellonella* were obtained from beehives of Bee garden, Anbil Dharmalingam Agricultural College and Horticultural College & Research Institute at Trichy and reared using artificial diet under the laboratory condition at temperature $27 \pm 1^\circ\text{C}$ and relative humidity $65 \pm 5\%$. The adults were fed with 10% sucrose solution.

Artificial diet preparation

The artificial diet prepared by using milk powder (130g), wheat flour (130g), wheat bran (130g), dried yeast powder (97.5g), maize flour (97.5g) and wax powder (26g). The ingredients were combined with the liquid components like honey (195ml) and glycerine (195ml). Three replications were maintained. Each replication contained 6 larvae.

Observations

Observations on time taken for the first instar to seventh instar, pupal duration, larval and pupal weight, male and female longevity and fecundity for one generation.

Results and Discussion

Galleria baiting method was followed to isolate EPF from soil. Hence, *G. mellonella* was mass cultured at laboratory condition using artificial diet. The larval period ranged from 3.2 ± 0.08 for 1st instar, 5.2 ± 0.13 for 2nd instars, 6.1 ± 0.39 for 3rd instars, 5.6 ± 0.26 for 4th instar, 7.4 ± 0.13 for 5th instar, 7.6 ± 0.21 days for 6th instar, 8.5 ± 0.47 days for 7th instar. The pupal period was 8.1 ± 0.29 days, and adult emergence was 91.25 ± 3.97 per cent. Male longevity averaged 7.2 ± 0.46 days, while females lived longer for 13.5 ± 0.75 days. The total developmental period was 73.56 ± 1.27 days. Fecundity was 680 ± 42.5 eggs (Table 1 & Fig. 1). Weight increased with larval progression, with 19.63 ± 0.91 mg for the 7 days old instar and reached 376.48 ± 6.52 mg during 14 day old larva, with pupal weight at 393.33 ± 6.81 mg. The mean total larval period was 43.6 ± 1.67 days. This is in contrary to Chandel et al. (2003) who noted 23.6 to 29.5 days. and Birah et al. (2008) with 28.6 to 30.6 days. But it was in consistent with Desai et al. (2019) with 44.84 ± 2.98 days. The duration of pupal stage was 6.8 to 9.4 days and it was found to coincide with Chandel et al. (2003). The duration of pupal

stage varied from 9.0 to 10.1 days in active generations (Birah *et al.*, 2008). Fecundity coincided with Desai *et al.*, (2019) who reported *G. mellonella* laid 464 to 964 eggs / female.

Artificial diet developed by Birah *et al.* (2008) was found increase the growth of *G. mellonella* larvae. Gross *et al.* (1996) found that adding torula yeast to the diet of *Galleria mellonella* resulted in increased weight of larvae. Chandel *et al.* (2003) reported that the life cycle of greater wax moth ranged from 23.6 to 29.5 days, which aligned with our findings. The pupal duration ranged from 9.0 to 10.1 days in active generations, with 94.2% of larvae successfully transformed into pupae reached a maximum weight of 394 mg (Birah *et al.*, 2010). In general, it seems that insect species living outside the soil on special substrates, e.g. honey combs, flour, bark or wool are preferably used for baiting entomopathogenic fungi. Starvation and stress conditions in the soil probably increase their susceptibility to pathogens (Zimmerman, 1986). Bait insects have also been used along with either or both of the common bait insects, *G. mellonella*, *Tribolium castaneum*. For example, Vänninen (1996) used *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and *Acanthocinus aedilis* Linnaeus (Coleoptera: Cerambycidae), Klingen *et al.* (2002) employed *Delia floralis* Fallén (Diptera: Anthomyiidae), Goble *et al.* (2010) used *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) and *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), and Rudeen *et al.* (2013) used *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae).

Studies have evaluated the use of several bait insects from different taxa for isolating entomopathogenic fungi. Klingen *et al.* (2002) found that dipteran larvae isolated fungi differently than *G. mellonella*. More specifically, larvae of *Delia floralis* (family Anthomyiidae) isolated *Tolypocladium cylindrosporium* more frequently than *G. mellonella* (Klingen *et al.* 2002). Thus the use of insect baits can also be considered to be a selective isolation method. However, the "*Galleria* bait method" appears to be more sensitive than traditional plating on media (Keller *et al.*, 2003) and is therefore useful for isolation and identification of the spectrum of entomopathogenic fungi indigenously from soils.

Conclusion

The artificial diet formulated in our laboratory met the nutritional needs of the larvae from the neonate stage and promoted their growth and development. The larval period was 3.2 ± 0.08 for 1st instar, 5.2 ± 0.13 for 2nd instars, 6.1 ± 0.39 for 3rd instar, 5.6 ± 0.26 for 4th instar, 7.4 ± 0.13 for 5th instar, 7.6 ± 0.21 days for 6th instar, 8.5 ± 0.47 days for 7th instar. The

pupal period was 8.1 ± 0.29 days. The total developmental period was 73.56 ± 1.27 days. The *Galleria baiting* method remains a reliable and sensitive technique for isolating entomopathogenic fungi from soil, as it effectively supported the survival and development of larvae in artificial diet under controlled conditions. This study refined the methodology of rearing *G. mellonella* and its application in soil pathogen studies and laid the groundwork for further advancements in biological pests management research.

References

- Birah, A., Chilana, P., Shukla, U. K., & Gupta, G. P. (2008). Mass rearing of greater wax moth (*Galleria mellonella* L.) on artificial diet. *Indian Journal of Entomology*, 70(4), 389-392.
- Chandel, Y. S., Sharma S. and Verma K. S. 2003. Comparative biology of the greater waxmoth, *Galleria mellonella* L., and lesser waxmoth, *Achoria grisella*. *Forest Pest Management and Economic Zoology*, 11: 69-74.
- Desai, A. V., Siddhapara, M. R., Patel, P. K., & Prajapati, A. P. (2019). Biology of greater wax moth, *Galleria mellonella* L. on artificial diet.
- Goble, T. A., Dames, J. F., P Hill, M., & Moore, S. D. (2010). The effects of farming system, habitat type and bait type on the isolation of entomopathogenic fungi from citrus soils in the Eastern Cape Province, South Africa. *BioControl*, 55, 399-412.
- Keller, S., Kessler, P. & Schweizer, C. (2003) Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metharhizium anisopliae*. *Biocontrol*, 48, 307-319.
- Klingen, I.; Eilenberg, J.; Meadow, R. Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. *Agric. Ecosyst. Environ.* 2002, 91, 191–198.
- Rudeen, M.L.; Jaronski, S.T.; Petzold-Maxwell, J.L.; Gassmann, A.J. Entomopathogenic fungi in cornfields and their potential to manage larval western corn rootworm *Diabrotica virgifera virgifera*. *J. Invertebr. Pathol.* 2013, 114, 329–332.
- Vänninen, I. Distribution and occurrence of four entomopathogenic fungi in Finland: Effect of geographical location, habitat type and soil type. *Mycol. Res.* 1996, 100, 93–101
- Zimmerman, T. G., Lanier, J., Blanchard, C., Bryson, S., & Harvill, Y. (1986). A hand gesture interface device. *ACM Sigchi Bulletin*, 18(4), 189-192.



Galleria mellonella 1st day of diet



Pupation



Before pupation – inactive stage

Plate 1 : Mass culturing of Greater wax moth, *Galleria mellonella* F. in artificial diet

Table 1. Biology of Greater wax moth (*Galleria mellonella*) reared using modified artificial diet

Bio stagess	Mean \pm SD
I instar (days)	3.2 \pm 0.08 (1.92) ^d
II instar (days)	5.2 \pm 0.13 (2.39) ^d
III instar (days)	6.1 \pm 0.39 (2.57) ^d
IV instar (days)	5.6 \pm 0.26 (2.47) ^d
V instar (days)	7.4 \pm 0.13 (2.81) ^d
VI instar (days)	7.6 \pm 0.21 (2.85) ^d
VII instar (days)	8.5 \pm 0.47 (3.00) ^d
Pupal period (days)	8.1 \pm 0.29 (2.93) ^d
Adult emergence (%)	91.15 \pm 3.97 (72.69) ^c
Male longevity	17.5 \pm 0.46 (4.24) ^d
Female longevity	13.5 \pm 0.75 (3.74) ^d
Development period (days)	73.56 \pm 1.27 (8.61) ^c
Fecundity (No.)	680 \pm 42.5 (26.09) ^a
Larval weight (mg) (7 days old)	19.98 \pm 0.91 (4.53) ^d
Larval weight (mg) (14 days old)	376.48 \pm 6.52 (19.42) ^b
Pupal weight (mg)	393.33 \pm 6.81 (19.85) ^b
SEd	8.93
CD (P=0.05)	18.18

Mean of three replications

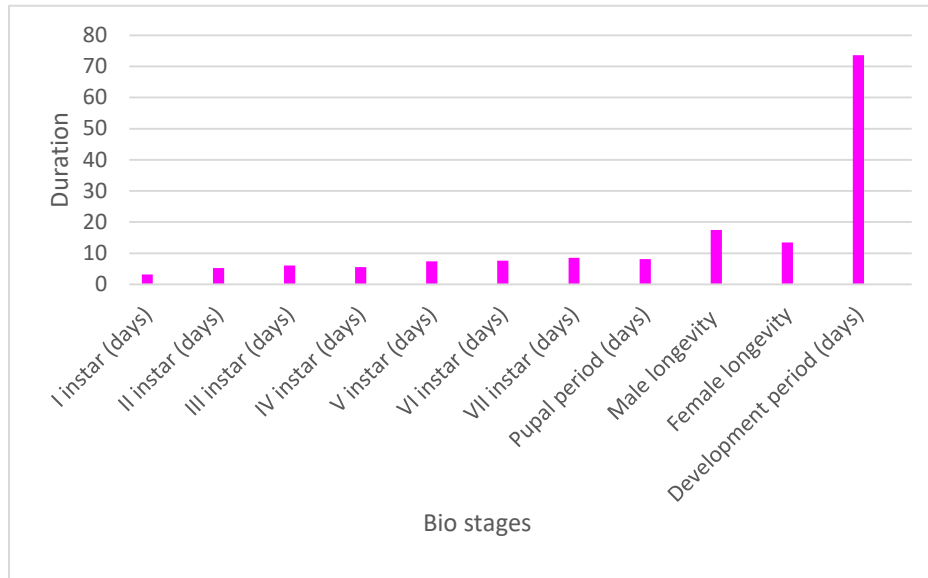


Fig 1. Biology of Greater wax moth, *Galleria mellonella* L. reared using artificial diet.