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Mass Rearing and Biological Characteristics of *Galleria mellonella* L. (Lepidoptera: Pyralidae) Using an Artificial Diet

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Galleria mellonella L. is an important pest of honeybees. The primary objective of the study is mass-rearing *Galleria mellonella* using a modified artificial diet. 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 duration for the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th instar was 3.2 ± 0.08, 5.2 ± 0.13, 6.1±0.39, 5.6 ± 0.26, 7.4 ± 0.13, 7.6 ± 0.21, 8.5

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± 0.47 days respectively. 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.

1. 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.

2. MATERIALS AND METHODS

2.1 Mother Culture

The first instar larvae of *G. mellonella* were obtained from beehives of Beegarden, Anbil Dharmalingam Agricultural College and Horticultural College & Research Institute at Trichy and reared using artificial diet under the laboratory condition at temperature 27 ± 1°C and relative humidity 65±5%. The adults were fed with 10% sucrose solution.

2.2 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.

2.3 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. Statistical analysis were done by using AGRESS software.

3. 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 was 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 days old larva, with pupal weight at 393.33 ± 6.81 mg. The findings of this study regarding the weight of mature larvae are crucial, as healthy larvae with substantial fatty tissue are essential for effective EPN rearing. Larval weight serves as the most reliable indicator for assessing this (Hussaini, 2003). 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 coinsided 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,

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

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

Mean of three replications

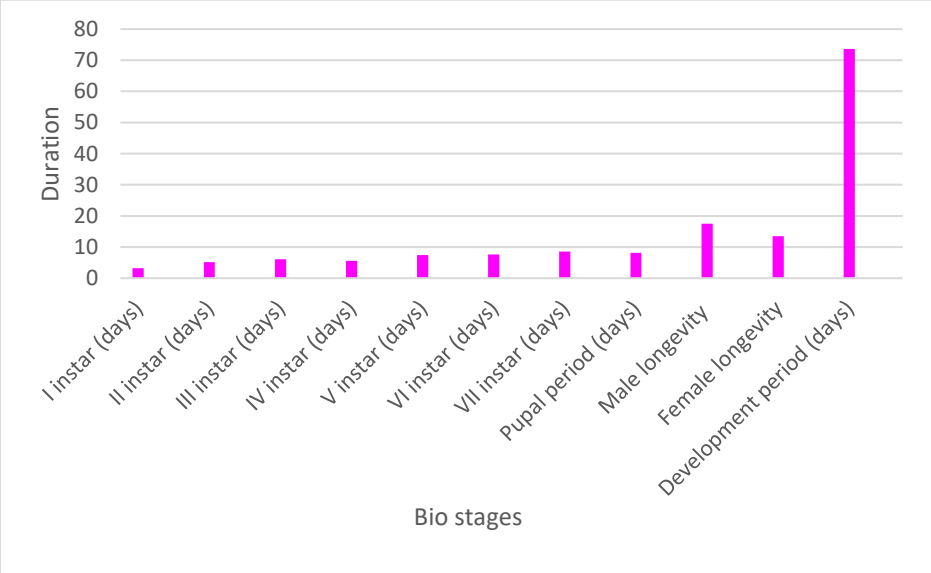

Fig. 1. Biology of greater wax moth, *Galleria mellonella* L. reared using artificial diet

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

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 cylindrosporum* more frequently than *G. mellonella* (Klingen et al. 2002). Hughes et al. (2004) observed a rise in isolations of *Beauveria* and *Metarhizium* when *G. mellonella* and *T. molitor* were used as bait insects, respectively. 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. The *Galleria mellonella* insect model has been widely utilized in various fungal pathogenesis studies, encompassing yeasts like *Candida* spp. (Rajendran et al., 2015) and *Trichosporon* spp.(Marine, 2015); molds such as *Aspergillus* spp. (Maurer, 2015), *Fusarium oxysporum (*Navarro-Velasco, 2011), *Scedosporium aurantiacum* (Kaur, 2015), *Madurella mycetomatis* (Kloezen, 2015), and Mucorales species (Maurer, 2019); as well as dimorphic fungi, including *Histoplasma capsulatum* and *Paracoccidioides lutzii* (Thomaz, 2013). Artificial diet proves to be an effective supplement, achieving a larval survival rate of 82.05%, a pupation rate of 79.48%, an average pupa weight of 76.53 mg, an emergence rate of 95.25%, and an adult fecundity of 121 eggs per female. It can reduce the total developmental cycle to 33 days in *Conogethes punctiferalis* Guenee (Jiang et al*.,* 2021).

4. 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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