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Mass rearing of greater wax moth larvae, *Galleria mellonella* for entomopathogenic nematodes studies

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Abstract
Many insects can be reared in artificial diet for experimental purpose under laboratory condition. Composition of the diet, easy availability, cost and feeding behavior of the insect are important consideration for rearing of the insect. The larvae of greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) are pests of honey bee colony and can cause significant damage to stored beekeeping industry. Despite the importance of wax moths to the apicultural industry, they are investigated considerably more as a model organism for studies in biology, behaviour, ecology, molecular biology, physiology, and control. *Galleria mellonella* are good host of many entomopathogens including entomopathogenic nematodes. Due to high susceptibility of the larvae of *Galleria mellonella* to entomopathogenic nematodes (EPNs), they are selected as suitable host for EPNs research. Effective artificial diets are pivotal in *G.mellonella* rearing purposes by decreasing production costs and enhancing the overall quality and fitness of the insect. Research work contributes to make mass rearing of *G.mellonella* less expensive with no adverse effects on quality and quantity of produced larvae as well as their suitability for baiting, rearing or mass multiplication of entomopathogenic nematodes.. This review attempts to compare different studies on composition of diet which has a significant effect on the wax moth larvae as well as production of entomopathogenic nematode and forwarded towards revitalization of research on various aspects of biological science.

Keywords: Greater wax moth larvae, *Galleria mellonella*, Entomopathogenic nematodes (EPNs), rearing of insect, artificial diet

Introduction
Preparation of an artificial diet is essential to the process of laboratory insect rearing. Bogdanov developed the first artificial diet in 1908 to rear blowflies, *Calliphora vomitoria* (L.). Since then, great advances have been made in diet formulation, especially for Lepidoptera, Coleoptera and Diptera by adding essential and non-essential nutrients, conforming to the nutritional requirements of hosts, and thus expanding the pool of insects that could be reared on artificial medium. However, only a few diets have been entirely successful in replacing the natural diet of host insects (Cohen 2004) [16]. Artificial diets can be divided into two categories: the holidic diet and the meridic diet. The holidic diet consists of very few of the ingredients are chemically defined, but the meridic diet the included ingredients are controlled. An artificial diet must be chemically stable, nutritionally complete, palatable to the insect, provide bioavailable nutrients, and support growth, development, and reproduction (Cohen, 2020) [17]. Diet ingredients of the host diets are also easily available and relatively inexpensive, and the life cycles of the hosts are short which will ensure a constant and quick supply of larvae during the production process. It is of extreme importance that diets be optimized to the extent where high-quality insect hosts, in sufficient numbers, are produced using an economically viable diet. Similarly cost of production can be reduced through use of alternative ingredients. The desired outcomes of diet optimization vary depending on the purpose as well as rearing system.

Rearing of Insect: The greater wax moth, *Galleria mellonella*
The greater wax moth, *Galleria mellonella* (order: Lepidoptera) is a member of the Galleriinae subfamily within the family Pyralidae. *G.mellonella* has a wide geographic distribution, particularly in the tropical and sub-tropical regions it lives naturally in beehives of *Apis mellifera* and *Apis cerana*. The moth first enters their hives, whereupon its larvae dig into the cells containing pollen, the bee brood and honey, resulting in severe damage to the hive and the bee population, the greater wax moth is one of the contributing factors to the decline of

domestic and wild honeybee populations (Jindra and Sehnal, 1989; Mohamed *et al*., 2014) [39, 51]. The insect has four developmental stages: egg, caterpillar, pre-pupae/pupa and adult. The eggs are white to light pink in colour and take between 5 to 8 days (24-27^{0}C) to develop and became caterpillars (Cardoso *et al*., 2007) [11]. The caterpillars are creamy white and measures 1-23 mm. This stage takes 6-7 weeks at 28-32^{0}C (Hagstrum and Milliken 1988; Mikolajczyk and Cymborowski 1993; Cymborowski, 2000) [34, 50, 19]. The caterpillars of the insect obtain nutrients naturally from honey, pupal skin, pollen and beewax. During this time, caterpillars undergo 8-10 molting stages and spin silk threads across all stages, but only the last instar spins a cocoon. When the caterpillar ceases feeding and still presents some motility, during the formation of the cocoon, characterizes the intermediate stage of the pre-pupae. Later on the caterpillars became pupae, which are dark reddish brown and immobilized in cocoons. From pupa to moth, takes 1 to 8 weeks. The pupae and adults do not require feeding. Rearing the insects in darkness can increase mating and reproduction (Jorjao *et al*., 2018) [40] because they are active at night (Ellis *et al*.,2013; Kwadha *et al*.,2017) [26, 47]. Moths are a reddish brown and pale cream color, and are able to lay 50-150 eggs .Male moths are slightly smaller and lighter in color than females. Female moths present a bifurcated proboscis and a labial palps projecting forward. The entire life-cycle takes approximately 40 days depending on environmental conditions like temperature and food supply.

Wax moth rearing methods are used in a variety of fields from the simple production of wax moth larvae for reptile, bird food, and fish bait to wax moth control programme and to study as a model insect (Charlis *et al*., 2017; Pereira *et al*.,2020) [15, 53, 54].

The greater wax moth, *Galleria mellonella* (L.), is extensively cultured as a laboratory test animal for basic studies in many disciplines (physiology, biochemistry, toxicology, pathology, etc.). Additionally its egg, larval, and pupal stages are often used as hosts or prey for rearing parasitic and predaceous insects for both laboratory studies and field releases.

They are investigated considerably more as a model organism. Therefore, mass rearing of wax moth had received greater attention in the recent year. The larvae offer many advantages as a model host. The main advantages of this model include the low cost of maintenance, the fast life cycle, the speed and ease of test execution and interpretation, the ability to incubate caterpillars at temperatures between 25^{0}C and 37^{0}C, the possibility of using a large number of caterpillars and the innate immune system, and the evolutionary conservation relative to mammals. It is extensively cultured as a laboratory test animal for basic studies in many disciplines *viz*., physiology, biochemistry, toxicology, and pathology (Champion *et al*., 2018; Ce *et al*., 2020; Pereira and Rossi,2020) [13, 53, 54]. Ellis *et al*., (2013) [26] used it as model organism for studies in insect physiology, genomics and proteomic. Wu *et al*., (2010) [69] used it for study of insect physiology and biochemistry, insecticide screening and insect toxicology.

The greater wax moth *Galleria mellonella* is an increasingly popular infection model to assess microbial virulence and the effectiveness of antimicrobial compounds and can be used to provide faster and cheaper data than traditional test systems (Junqueira 2012; Jacobsen, 2014; Champion *et al*., 2016; Adeline *et al*., 2018; Andrea *et al*.,2019; Cutuli *et al*.,2019; Kazek *et al*.,2019) [41, 38, 14, 1, 2, 18, 44]. Earlier *Galleria mellonella* (wax moth) larvae was widely used as a non-mammalian infection model,now it has been accepted by the scientific community as an experimental model for infection with different bacterial, fungal as well as entomopathogenic nematode species. *G.mellonella* is known to be susceptible to twenty nine species of fungi, seven viruses, one species of parasite and sixteen numbers of biological toxins (Champion *et al*., 2016; Asai *et al*.,2019; Firacative *et al*.,2020) [14, 3, 19]. Additionally its egg, larval, and pupal stages are often used as hosts or prey for rearing parasitic and predaceous insects such as *Microplitis* spp., *Archytas* spp., *Apanteles* spp. and *Bracon hepetor* L. for both laboratory studies and field releases (Kandel *et al*., 2020) [42]. Another advantage of the *G.mellonella* model is the multiple options available for the easy inoculation of the pathogen. Most studies introduce the infection by injecting standardized microbial inoculums or via oral delivery or topical application (Dalton *et al*., 2017) [22].

Simple and easy procedure can support researchers starting to rear *G.mellonella*. Many studies have tried to optimize the mass-rearing of *G. mellonella*, taking into account the cost and availability of diet ingredients, as well as the ability of the insect to adapt to diets without seriously affecting the biological parameters like larval weight, larval phase duration, pupal weight and duration, fertility or egg productivity and overall survival (longevity) and developmental period (Finke 2002; Birah *et al*., 2008; Banville *et al*.,2012; Kulkarni *et al*.,2012; Vanzyl and Malan, 2015; Torres *et al*.,2020; Hickin *et al*.,2021) [28, 8, 4, 46, 67, 66, 36] .

Galleria Mellonella Rearing Methods

Most rearing methods are very similar and share common components. To begin a rearing programme, the initial moths can be obtained from infested honey bee colonies or purchased commercially. Outlined here is the general rearing method of wax moths.

Natural rearing method

1. Create a bee-free hive with frames of pulled, dark comb (dark comb is comb in which brood has been reared) containing honey and pollen.
2. Introduce three, late instar larval wax moths per frame to ensure wax moth presence.
3. The hive and combs should be covered and under some type of shelter to protect it from rain. Darkness, warmth, and lack of ventilation promote colonization.
4. Unattended (bee-free) hives will be highly attractive to adult wax moths if they are present in the area.
5. Provide additional used honeycomb containing honey and pollen as diet for rearing program as the food supply in the box is exhausted.
6. Moth eggs, larvae, pupae and adults can be collected from the hive with an aspirator, forceps, or a small, soft paintbrush. The latter should be used for the immature wax moth stages since they can be damaged easily.

The higher larval survival could be attributed to the adequate supply of the main nutrients as well as pheromones and hormones. In nature, the larvae develop in bee colonies and feed on pollen, honey, cast larval skins and other debris incorporated into the wax comb (Young, 1961) [72]. However, the production and use of wax comb can be expensive and unsustainable if a large number of wax moths are desired.

In vitro rearing method

Most *in vitro* lab rearing techniques follow a simple series of events:

1. Place wax moth eggs on new diet.
2. Allow resulting larvae to feed on diet. The insects are reared under the optimal growing conditions, are reared in glass chambers at 30˚C, 70% relative humidity and in constant darkness. Air movement is also a critical factor to consider in rearing facilities. Too much air movement can lead to drying out of insect diets, while a lack of air movement can lead to humid and unfavourably low oxygen conditions, which are ideal for unwanted microbial growth .
3. Harvest late instar larvae or pupa and place into a second container.
4. Allow late instar larva to pupate or pupa to emerge as adults.
5. Allow adults to mate and allow females to lay eggs.
6. Place eggs on new diet.

Beck (1960) [5], Dutky (1962) [23], Dadd (1964, 1966) [20, 21] and Marston and Brown (1974) originally formulated diets to culture *G. mellonella* in the laboratory. Eischen and Dietz (1990) [25] found that adding 5% pollen and honey or bee wax significantly increased the survival of *G. mellonella* adults. Another economical artificial diet was developed by mixing rice bran, malt and sucrose instead of wheat bran and honey. Metwally *et al*. (2012) [49] using various ingredients instead of natural honey bee wax and found to be suitable for mass rearing of wax moth larvae without adverse effect on quantity and quality of produced larvae with low cost. Ellis *et al*., (2013) [26] described *G. mellonella* breeding using a diet derived by mixing the following ingredients: seven parts dry dog croquettes, one part water, and two parts honey, followed by content adjustment with vitamin A to produce whitish larvae. Commercially available dog croquettes are used to rear *G. mellonella* and are often composed of different ingredients of various origins (Frank and Alfred, 1990; Jorjao *et al*., 2018) [30, 40].

Due to differences in availability and cost of diet constituents and the ability of *G. mellonella* larvae to adapt to different diets without serious effects on their development, many scientists tried to optimize mass production of *G. mellonella* in different countries (Schneider *et al*., 2018) [60]

The following table.1 shows many variations on a generalized artificial diet.

Table 1: Different ingredients for artificial diet of *Galleria mellonella*

Ingredients	Brighenti *et al*., (2005) [10]	PDBC, (2007) [52]	Birah *et al*., (2008) [8]	Kulkarni *et al*., (2012) [46]	Van Zyl & Malan (2015) [67]	Van Zyl & Malan (2015) [67]	Huang *et al*., (2010) [37]
corn meal/maize flour	250 g	200 g	97.5 g	200g			
yeast extract	150 g	70 g	97.5 g	25g	35 g	88 g	5.4g
soy flour	100 g						
powder milk	100 g	130 g	130g	100g		118 g	10.2 g
Honey/Honey waste	200 g	100 ml	195 ml	225g	190.5 ml	175 ml	16.7 g
Glycerol/Sorbitol	200 g	150 ml/150ml	195 ml	125ml	165 ml	175 ml	24 g
beeswax blocks	✓		26 g			24 g	16.7 g
dog croquettes					345 g		
rolled oats					85 g		
wheat bran			130g	100g	85 g	206 g	
wheat flour		350 g	130g	100mg		118 g	51.9 g

Utilization of *G. Mellonella* for Entomopathogenic Nematodes Studies

Entomopathogenic nematodes under the families Steinernematidae and Heterorhaditidae are used as biological insecticides in pest management programmes (Grewal *et al*.,2005) [33]. Host susceptibility, host availability, ease and cost of rearing the host, and quality of nematodes produced by the host are all parameters to take into consideration when selecting a suitable host for entomopathogenic nematodes (EPNs) research (Poinar, 1975; Blinova and Ivanova, 1987; Hatab *et al*., 1998; Shapiro-Ilan and Gaugler, 2002) [55, 9, 35, 32]. The advantages of using *G. mellonella* larvae in entomopathogenic nematode-related studies include high susceptibility of the larvae to EPN (Fuchs *et al*., 2010; Ramarao *et al*., 2012) their size and short lifecycle, easy rearing on artificial diets, rearing at various temperatures (20-37°C), and high nematode yields (Van Zyl and Malan, 2015; Testa and Shields, 2017; Rahoo *et al*., 2018) [67]. The availability of a constant and reliable source of host insects throughout the year can only be achieved by laboratory rearing. For large-scale commercial production as well as for laboratory experimentation or field testing entomopathogenic nematodes are cultured *in vivo* or *in vitro* (Woodring and Kaya, 1988; Shapiro-Ilan and Gaugler, 2002; Gaugler and Han, 2002; Ehlers and Shapiro-Ilan,2005) [68, 63, 32, 24]. *In vivo* production yields vary greatly among different insect hosts and nematode species. The developmental stages of hosts also play an important role in their susceptibility to nematodes (Simoes and Rosa, 1996; Kaya and Stock, 1997) [64, 43]. In an enclosed rearing facility, environmental conditions can be manipulated to increase the efficacy of host production. Besides cost, it is important to know whether the diet influences the effectiveness of EPN emerging from an infected diet-reared host to kill and multiply in the target pest species (Metwally *et al*., 2012) [49]. Under field conditions, application of EPN in insect host cadavers can reduce the quantity of nematodes required for control per unit area compared with their application in water or other solvents (Shapiro-Ilan *et al*., 2012) [61]. The proportion and/or selection of ingredients in the diets play an important role in the development of larvae, as well as in the fitness and quality of the nematodes obtained from them. *In vivo* nematode production yields nematodes with good virulence potential (Kotchofa and Baimey, 2019) [45]. According to Zhen *et al*., (2018) [73], the quality of the insect host can affect the efficacy or persistence of EPN produced *in vivo*. Bhatnagar and Bareth (2004) [7] and Ramakuwela *et al*., (2014) [57] focused on optimization of insect media and its impact on nematode

production and indicated that insect nutrition has a link to EPNs production. A standard *G. mellonella* rearing protocol is fundamental to minimize external influences on the results, and this simple and easy protocol can support researchers starting to rear *G. mellonella*. Attempts were made to develop suitable semi-synthetic diet for mass rearing of *G. mellonella* to ensure uninterrupted EPN production. Calculating the number of nematodes produced per host would assist in determining, in advance, how many larvae need to be reared for a specific field trial, experiment or formulation. The presence of certain lipids in insect diets has also been shown to promote host susceptibility, in increasing the developmental rate and yield of EPNs (Yang *et al*., 1997; Yoo *et al*., 2000; Shapiro-Ilan *et al*., 2012) [20, 71, 61].

Recovery of entomopathogenic nematodes from soil using greater wax moth larvae

This method was developed by Bedding and Akhurst (1975) [6] and Fan and Hominick (1991) [27] and can be used for screening of soils for the occurrence of entomopathogenic nematodes.

1. Collect soil of interest for use.
2. Place 200-250 cm^3 of soil in a plastic or glass dish (~300 cm^3 in volume).
3. Place five late instar *G. mellonella* larvae (late instar larvae are 250-350 mg) on the soil surface.
4. Seal the dish with a tight lid to limit larvae escape.
5. Incubate the dish at 20 °C.
6. Replace the larvae (alive or dead) every 4-6 days. This should be done until larvae in the dish no longer die
7. Dissect all harvested larvae in saline water.
8. Quantify the number of nematode adults.

Infecting greater wax moth larvae with entomopathogenic nematodes

Fan and Hominick (1991) [27] developed this method that is useful for nematologists who need an effective method for rearing nematode species of interest.

Surface sterilization of wax moth larvae (Reddy *et al*., 1979) [59]

1. Surface sterilizes wax moth larvae with a wash (whole body) or rub (target body part) with 70% ethanol.
2. Manipulate (including dissection) the sterilized individual in sterile insect Ringers solution.
1. Wash sand with distilled water.
2. Autoclave
3. Oven-dry.
4. Filter through a 1.18 mm sieve.
5. Moisten the filtered sand with 1 ml of distilled water for every 25 ml of sand (4% V/V).
6. Place 25 ml of moistened sand in a 30 ml plastic tube.
7. Pipette nematodes diluted in 1 ml of water (per producer's instructions or experimental needs) into the sand in the tube. The nematode/water solution brings the V/V content to 8%. Any desired number of nematodes can be introduced to the soil in this way, though including more infective juveniles in the inoculum typically results in greater infestation with nematodes.
8. Invert (turn upside down) the tube multiple times to disperse the nematodes in the sand.
9. Place a single wax moth larva (surface sterilized) on the sand surface in the tube (late instar larvae are 250-350 mg).
10. Replace the tube lid and invert the tube.
11. Leave the tube inverted for set time periods and temperatures per the needs of the study.
12. Recover the wax moth larva and wash it three times with distilled water.
13. Dissect all harvested larvae in saline or maintain on moistened filter paper at 20°C for a period of time before use.
14. Replace the wax moth larva in the tube with a new individual weekly. This should be done until added larvae no longer die, indicating that no nematodes remain in the soil.

Conclusion

Galleria mellonella has all the necessary host features to be model host especially in the microbiology field. Some of the practical recommendations for larval rearing will help in overcoming limitations of the use of the model insect. Artificial diet optimization for *G. mellonella* is important to develop larger larvae with short development time along with other life-history traits. The standardization of parameters related to artificial diet for rearing of *G. mellonella* can be improved by comparison which will provide cost effective and easy application technique. This improvement may lead towards better reliable experimentation using *G. mellonella* for entomopathogenic nematodes studies.

Reference

1. Adeline LJ, Luciane DO, Liliana S, Livia MA, Marcia CA, Antonnio OC *et al*. From moth to caterpillars: Ideal conditions for *Galleria mellonella* rearing for *in vivo* microbiological studies. Virulence. 2018;9(1):383-389. doi: 10.1080/21505594.2017.1397871.
2. Andrea A, Krogfelt KA, Jenssen H. Methods and challenges of using the greater wax moth (*Galleria mellonella*) as a model organism in antimicrobial compound discovery. Microorganisms. 2019;7(3):85. doi: 10.3390/microorganisms7030085
3. Asai M, Li Y, Khara JS, Robertson BD, Langford PR, Newton SM. *Galleria mellonella*: an infection model for screening compounds against the *Mycobacterium tuberculosis* complex. Frontiers in Microbiology 2019;10:2630.
4. Banville N, Browne N, Kavanagh K. Effect of nutrient deprivation on the susceptibility of *Galleria mellonella* larvae to infection. Virulence. 2012;3:497-503.
5. Beck SD. Growth and development of the greater wax moth, *Galleria mellonella* (L.). Transactions of the Wisconsin Academy of Sciences, Arts, and Letters 1960;49:137-148.
6. Bedding RA, Akhurst RJ. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 1975;21:109-110.
7. Bhatnagar A, Bareth SS. Development of low cost quality diet for greater wax moth *Galleria mellonella* (Linnaeus). Indian Journal of Entomology 2004;66:251-255.
8. Birah A, Chilana P, Shukla UK, Gupta GP. Mass rearing of greater wax moth (*Galleria mellonella* L.) on artificial diet. Indian Journal of Entomolology. 2008;70:389-392.
9. Blinova SL, Ivanova ES. Culturing the nematode-bacterial complex of *Neoaplectana carpocapsae* in insects. In: Helminths of Insects. Amerind Publishing, New Delhi, India 1987, 22-26.

10. Brighenti D, Carvalho C, Carvalho G, Brighenti C. Efficiency of *Bacillus thuringiensis* var. kurstaki (Berliner, 1915) for control of the greater wax moth *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae). Ciencia e Agrotecnologia (Science and Agrotechnology) 2005;29:60-8.
11. Cardoso AC, Prata MC, Furlong J, Prezoto F. Thermal requirements of *Galleria mellonella* L. (Lepidoptera: Pyralidae) immature stages. Neotropical Entomology 2007;36:657-661.
12. Ce R, Silva RC, Trentin DS, Marchi JGB, Paese K, Guterres SS, *et al*. *Galleria mellonella* larvae as an *in vivo* model to evaluate the toxicity of polymeric nanocapsules. Journal of Nanoscience and Nanotechnology. 2020;20:1486-94.
13. Champion OL, Titball RW, Bates S. Standardization of *G. mellonella* larvae to provide reliable and reproducible results in the study of fungal pathogens. Journal of Fungi 2018;4:108.
14. Champion OL, Wagley S, Titball RW. *Galleria mellonella* as a model host for microbiological and toxin research. Virulence 2016;7(7):840-845. https:// doi.org/ 10. 1080/21505594. 2016. 1203486 WOS: 000384026200016. PMID: 27362761
15. Charlis AK, George OO, Paul NN, Suresh KR, Ayuka TF. The biology and control of the greater wax moth *Galleria mellonella*. Insects 2017;8(2):61-77.
16. Cohen AC. Insect Diets: Science and Technology. CRC Press, Boca Raton, FL, U.S.A. 2004.
17. Cohen AC. Design, operation, and control of insect rearing systems. CRC Press, Boca Raton, FL. 2020.
18. Cutuli MA, Petronio G, Vergalito F, Magnifico I, Pietrangelo L, Venditti N, *et al*. *Galleria mellonella* as a consolidated *in vivo* model hosts: new developments in antibacterial strategies and novel drug testing. Virulence 2019;10:527-541 DOI:10.1080/21505594.2019.1621649
19. Cymborowski B. Temperature dependent regulatory mechanism of larval development of the wax moth (*Galleria mellonella*). Acta Biochimica Polonica (Biochemical Journal of Poland) 2000;47:215-221.
20. Dadd RH. A study of carbohydrate and lipid nutrition in the wax moth, *Galleria mellonella* (L.), using partially synthetic diets. Journal of Insect Physiology 1964;10:161-178.
21. Dadd RH. Beeswax in the nutrition of the waxmoth *Galleria mellonella* (L.). Journal of Insect Physiology 1966;12:1479-1492.
22. Dalton JP, Uy B, Swift S, Wiles S. A novel restraint device for injection of *Galleria mellonella* larvae that minimizes the risk of accidental operator needle stick injury. Frontiers in Cellular and Infection Microbiology 2017;7:99.
23. Dutky SR, Thompson JV, Cntwell GE. A technique for mass rearing the wax moth (Lepidoptera: Galleriadae). Proceedings of Entomological Society of Washington 1962;64:56-58.
24. Ehlers RU, Shapiro-Ilan DI. Mass production. In: Nematodes as Biocontrol Agents. CABI Publishing, Wallingford, U.K. 2005, 65-78.
25. Eischen F, Dietz A. Improved culture techniques for mass rearing *Galleria mellonella* (Lepidoptera: Pyralidae). Entomological News. 1990;101:123-128.
26. Ellis JD, Graham JR, Mortensen A. Standard methods for wax moth research. Journal of Apicultural Research 2013;52(1):1-17. DOI: 10.3896/ IBRA.1.52.1.10.
27. Fan X, Hominick WM. Efficacy of the *Galleria*(wax moth) baiting technique for recovering infective stages of entomopathogenic rhabditids (Steinernematidae and Heterorhabditidae) from sand and soil. Revue de Nematologie (Nematology) 1991;14:381-387.
28. Finke MD. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology 2002;21:269-285.
29. Firacative C, Khan A, Duan S, Ferreira-Paim K, Leemon D, Meyer W. Rearing and maintenance of *Galleria mellonella* and its application to study fungal virulence. Journal of Fungi 2020;6:130; doi:10.3390/jof6030130.
30. Frank AE, Alfred D. Improved culture technique for mass rearing *Galleria mellonella* (Lepidoptera: Pyralidae). Entomological News. 1990;10(2):123-128.
31. Fuchs BB, O'Brien E, Khoury JB, Mylonakis E. Methods for using *Galleria mellonella* as a model host to study fungal pathogenesis. Virulence. 2010;1(6):475-82.
32. Gaugler R, Han R. Production technology. In: Entomopathogenic Nematology, CABI, New York, NY, 2002, 289-310.
33. Grewal PS, Ehlers RU, Shapiro-Ilan DI. Nematodes as biological control agents. Wallingford: CABI Publishing. 2005.
34. Hagstrum DW, Milliken GA. Quantitative analysis of temperature, moisture, and diet factors affecting insect development. Annals of the Entomological Society of America. 1988;81:539-546.
35. Hatab AM, Gaugler R, Ehlers RU. Influence of culture method on *Steinernema glaseri* lipids. Journal of Parasitology. 1998;84:215-221.
36. Hickin M, Nadel H, Schal C, Cohen AC. Optimization of a diet for the greater wax moth (Lepidoptera: Pyralidae) using full factorial and mixture design. Journal of Economic Entomology. 2021;114(3):1091-1103. doi: 10.1093/ jee/toab 039.
37. Huang CH, Pan XH, Huang DF, Wang BH, Wei JL. Screening of artificial feed formulae for *Galleria mellonella* L. Journal of Guangxi Agricultural. Science 2010;41(7):1002-1161
38. Jacobsen ID. *Galleria mellonella* as a model host to study virulence of Candida. Virulence 2014;5(2):237-239. https://doi.org/10.4161/viru.27434 WOS: 000331447800001. PMID: 24384470
39. Jindra M, Sehnal F. Larval growth, food consumption, and utilization of dietary protein and energy in *Galleria mellonella* L. Journal of Insect Physiology 1989;35(9):719-724.
40. Jorjao AL, Oliveira LD, Scorzoni L, Figueiredo-Godoi MA, Prata MCA, Jorge AOC *et al*. From moths to caterpillars:ideal conditions for *Galleria mellonella* rearing for *in vivo* microbiological studies.Virulence 2018;9:383-389.
41. Junqueira JC. *Galleria mellonella* as a model host for human pathogens. Virulence. 2012;3(6):474-6. https://doi.org/10.4161/viru.22493 WOS: 000312000300002. PMID: 23211681
42. Kandel MA, Said SM, Abdelaal AAA. Effect of food types of *Galleria mellonella* L. (Lepidoptera: Pyralidae) on biological aspects and life table of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). African Journal of Agricultural Research 2020;16(6):884-891. DOI: 10.5897/AJAR2019.14618
43. Kaya HK, Stock SP. Techniques in insect Nematology. In: Manual of Techniques in Insect Pathology. San Diego, CA, Academic Press. 1997, 281-324.
44. Kazek M, Kaczmarek A, Wronska AK, Bogus MI, Diet influences the bacterial and free fatty acid profiles of the cuticle of *Galleria mellonella* larvae. PLoSOne

2019;14:e0211697.
45. Kotchofa R, Baimey H. *In vivo* production of entomopathogenic nematodes using *Galleria mellonella*: costs and effect of diets on nematode pathogenicity. Journal of Nematology. 2019;51:1-15. DOI: 10.21307/jofnem-2019-066
46. Kulkarni N, Kushwaha DK, Mishra VK, Paunikar S. Effect of economical modification in artificial diet of greater wax moth *Galleria mellonella* L. (Lepidoptera:Pyralidae). Indian Journal of Entomology 2012;74(4):369-374.
47. Kwadha CA, Ong'amo GO, Ndegwa PN, Raina SK, Fombong AT. The biology and control of the greater wax moth, *Galleria mellonella*. Insects. 2017;8(2). ARTN 61 https:// doi.org/ 10.3390/ insects 8020061 WOS: 000404617800028. PMID: 28598383
48. Marston N, Brown B. Constituents in diets for *Galleria mellonella*. Journal of Economic Entomology 1974;67:497-500.
49. Metwally HMS, Hafez GA, Hussein MA, Hussein M, Salem H, Saleh M. Low cost artificial diet for rearing the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a host for entomopathogenic nematodes. Egyptian Journal of Biological Pest Control 2012;22(1):15-17. https:// www. researchgate.net/publication/286657015
50. Mikolajczyk P, Cymborowski B. Lower temperature influences developmental rhythms of the wax moth *Galleria mellonella*: Putative role of ecdysteroids. Comparative Biochemistry and Physiology 1993;105:57-66.
51. Mohamed AA, Ansari MJ, Al-Ghamdi A, Mohamed MO, Kaur M. Effect of larval nutrition on the development and mortality of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Revista Colombiana de Entomología (Colombian Journal of Entomology) 2014;40(1):49-54.
52. PDBC. Traning on Entomopathogenic nematode for insect pest control. Training manual for "Hands-on Training on Entomopathogenic nematode" 20th march to 29th March 2007, Project Directorate of Biological Control, Bangalore, India. 2007.
53. Pereira MF, Rossi CC, da Silva GC, Rosa JN, Bazzolli DMS. *Galleria mellonella* as an infection model: an in-depth look at why it works and practical considerations for successful application. Pathogens and Disease 2020;78(8). ftaa056. doi: 10.1093/femspd/ftaa056.
54. Pereira MF, Rossi CC. · Overview of rearing and testing conditions and a guide for optimizing *Galleria mellonella* breeding and use in the laboratory for scientific purposes APMIS. 2020;128(12):607-620. doi: 10.1111/apm.13082. Epub 2020 Oct 9.
55. Poinar GO. Jr. Entomogenous Nematodes- A manual and host list of insect nematode associations. Brill, Leiden, Netherlands. 1975.
56. Rahoo AM, Mukhtar T, Abro SI, Bughio BA, Rahoo RK. Comparing the productivity of five entomopathogenic nematodes in *Galleria mellonella*. Pakistan Journal of Zoology 2018;50(2):679-84.
57. Ramakuwela T, Hatting J, Laing MD, Hazir S. Cost effective solid-state production of entomopathogenic nematodes (Steinernematidae). Journal of Nematology. 2014;46:225-226.
58. Ramarao N, Nielsen-Leroux C, Lereclus D. The insect *Galleria mellonella* as a powerful infection model to investigate bacterial pathogenesis. Journal of Visualized Experiments 2012;70:e4392. doi: 0.3791/4392.
59. Reddy G, Hwang-Hsu K, Kumaran AK. Factors influencing juvenile hormone esterase activity in the wax moth, *Galleria mellonella*. Journal of Insect Physiology. 1979;25:65-71. http://dx.doi.org/10.1016/0022-1910(79)90038-6
60. Schneider JC, Leppla NC, Chaudhury MF, Castrillo LA, Ng S, Fisher WR *et al*. Educating the next generation of insect rearing professionals:lessons from the international insect rearing workshop, Mississippi State University,2000-2017. American Entomologist 2018;64:102-111.
61. Shapiro-Ilan D, Rojas MG, Morales-Ramos JA, Tedders WL. Optimization of a host diet for *in vivo* production of entomopathogenic nematodes. Journal of Nematology 2012;44:264-273.
62. Shapiro-Ilan D, Rojas MG, Morales-Ramos JA, Lewis EE, Tedders WL. Effects of host nutrition on virulence and fitness of entomopathogenic nematodes: lipid and protein based supplements in *Tenebrio molitor* diets. Journal of Nematology. 2008;40:13-19.
63. Shapiro-Ilan DI, Gaugler R. Production technology for entomopathogenic nematodes and their bacterial symbionts. Journal of Industrial Microbiology and Biotechnology. 2002;28:137-146.
64. Simoes N, Rosa JS. Pathogenicity and host specificity of entomopathogenic nematodes. Biocontrol Science and Technology. 1996;6:403-411.
65. Testa AM, Shields EJ. Low labor '*in vivo*' mass rearing method for entomopathogenic nematodes. Biological Control 2017;106:77-82.
66. Torres M, Pinzon EN, Rey FM, Martinez H, Parra Giraldo CM, Celis Ramirez AM. *Galleria mellonella* as a novelty *in vivo* model of host-pathogen interaction for *Malassezia furfur* CBS 1878 and *Malassezia pachydermatis* CBS 1879. Frontiers in Cellular and Infection Microbiology. 2020;10:199.
67. Van Zyl C, Malan AP. Cost-effective culturing of *Galleria mellonella* and *Tenebrio molitor* and entomopathogenic nematode production in various hosts. African Entomology. 2015;23(2):361-375. https://www. researchgate. net/publication /281973658.
68. Woodring JL, Kaya HK. Steinernematidae and Heterorhabditidae nematodes: a handbook of techniques. Southern Coop. Ser. Bull.331. Alkansas Agri. Exp. Stn, Fayetteville, AR, 1988, 29.
69. Wu YY, Zhou T, Wang Q, Dai PL, Gong XX, Luo QH, *et al*. Optimization of artificial diets for *Galleria mellonella* L. Chinese Bulletin of Entomology 2010;47(2):409-413.
70. Yang HW, Jian H, Zhang SG, Zhang GY. Quality of the entomopathogenic nematode *Steinernema carpocapsae* produced on different media. Biological Control 1997;10:193-198.
71. Yoo SK, Brown I, Gaugler R. Liquid media development for *Heterorhabditis bacteriophora*: lipid source and concentration. Applied Microbiology and Biotechnology 2000;54:759-763.
72. Young RG. The effects of dietary beeswax and wax components on the larvae of the greater waxmoth, *Galleria mellonella* (L.). Annals of the Entomological Society of America. 1961;54:657-659.
73. Zhen S, Li Y, Hou Y, Gu X, Zhang L, Ruan W, Shapiro-Ilan D. Enhanced entomopathogenic nematode yield and fitness via addition of pulverized insect powder to solid media. Journal of Nematology. 2018;50(4):495-506.