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# MODIFIED SEMI-SYNTHETIC DIET FOR MASS REARING OF WAX MOTH *GALLERIA MELLONELLA* L.

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**ABSTRACT**

**A semi-synthetic diet with modified composition was used for mass rearing of wax moth *Galleria mellonella* L. (28±2°C, 65±5%RH, 12:12 scoto-photophase). The ingredients consisted of wheat flour (*Triticum aestivum*) (170g), maize flour (*Zea mays*) (170g), oat (*Avena sativa*) (160g), milk powder (170g), yeast powder (20g), honey (160ml), glycerin (150ml) streptomycin sulphate (0.5g) and two vitamin E capsules (Evion 400mg-2). This diet successfully supported growth and development of *G. mellonella* for three generations with enhanced larval weight. The biological parameters based on three continuous generations showed maximum larval weight of 5th instars (44.1mg), pupal weight (24.0mg), pupation (74%), emergence (83%), survival (63%), fecundity (1252 eggs/female) and fertility (77%) with lesser developmental period (egg to adult) of 62 days. All the components of the diet are easily available and economical too as the cost of 1 kg diet was approximately Rs. 500 only, for rearing 1000 larvae successfully.**

**Keywords:** *Galleria mellonella,* synthetic diet, mass rearing, entomopathogenic nematodes, larval weight, pupal weight, emergence, survival, fecundity, fertility

Entomopathogenic nematodes (EPNs) belonging to genera *Heterorhabditis* and *Steinernema* have been extensively accepted as environmentally safe biological control agents of mainly soil insect pests. They are considered to be one of the noble bioagents for IPM in major commercial crops viz., sugarcane, potato, groundnut, soybean, banana, apple, ginger and turmeric because of their ability to kill over 250 species of insect pests including root feeding grubs. For mass production of EPN in *vivo*, the wax moth, *G. mellonella* is known to be the best laboratory host. The fifth instars larvae of *G. mellonella* are known to be the ideal choice for maximized production of EPN. Large-scale mass production in the laboratory using semi-synthetic diet offers the most dependable and preferred method of obtaining large-scale, uniform and constant supplies of fifth instars caterpillars. Mass multiplication of EPNs may be developed as cottage industry by mass rearing of wax moth. Significant progress had been made in mass rearing methods of wax moth (Mohamed and Coppel, 1983; Frank and Alfred, 1990; Ajanta et al., 2008; Jagpal Singh and Sharad Mohan, 2014; Sumit et al., 2015; Charlis et al., 2017 Adeline et al., 2018). Looking deeply into mass production of *G. mellonella* caterpillars as laboratory host of EPNs was the priority in the laboratory during 2017-2018. In the present study, incorporation of natural wax of honey comb for rearing wax moth caterpillars was explored to develop a novel semi-synthetic diet.

## MATERIALS AND METHODS

The initial culture of wax moth eggs were obtained from the Division of Nematology, Indian Agricultural Research Institute, New Delhi. The neonates emerged within one week from the eggs. These neonates were reared on its natural food- honeycomb (bee wax) -up to 7 days, and subsequently on nutrition-fortified semi-synthetic diet of the following composition:

Wheat flour (*Triticum aestivum*)- 200 g, maize flour (*Zea mays*)- 200 g, oats (*Avena sativa)* 200 g, milk powder (Nestle Everyday)- 200 g, yeast powder (Fisher Scientific India Pvt. Ltd.)- 20 g, honey (local apiarist)- 200 ml, Glycerin (Fisher Scientific India Pvt. Ltd.)- 150 ml, Vitamin E capsule (Evion 400 Merck Limited)- 2 Nos, and streptomycin sulphate (Indian Drugs and Pharmaceuticals' Ltd.)- 0.5 g. Adult moths were fed with 30% honey solution fortified with ABDEC drops, i.e. around 1ml/l of honey solution. The rearing process was done in an environmentally controlled growth chamber (28ºC±2, 65±5% RH and 12:12 hr scoto-photophase).

One hundred neonates in four groups were used

for growth evaluation in each generation for three consecutive generations. Development data on larval, pupal and adult development were recorded. The mean fecundity was calculated on the basis of number of eggs laid by 12 females (N=3 pair) per generation. The transparent rectangular plastic containers (37x 30x 17 cm) having lid with embedded plastic mesh were used for larval and pupal rearing. The adult moths were held in 5 l capacity round transparent plastic containers having perforated lids. A layer of soft tissue paper was placed on the top of the container, to offer the oviposition substrate for female. The tissue paper bearing the eggs was replaced with a new one on alternate days. The eggs on tissue papers were transferred to a plastic container having wax comb. Another set up for rearing the emerged neonates on wax comb was also set up. The neonate caterpillars were fed with natural diet of clean wax comb. After seven days growth, one set of 100 caterpillars were given semi-synthetic diet while parallel set of 100 numbers were reared on wax comb till pupation . The rearing containers were cleaned regularly to remove the web. The pupating fifth instar larvae were transferred to another container (37x 30x 17 cm) for pupation, and adults when emerged provided with food.

The mean fecundity/ female was calculated on the basis of the number of eggs laid over three generations by 12 females per generation. The biological attributes were compared with those of larvae reared on its natural food bee wax. Growth and developmental index were calculated as follows: Larval Growth Index (LGI) = % pupation/ larval period (days), Pupal Growth Index (PGI) = % Adult emergence/ pupal period (days), Developmental index (DI) = % survival/developmental period (days)

## RESULTS AND DISCUSSION

The various growth parameters of *G. mellonella* caterpillars, reared on the semi-synthetic diet and on bee wax over three successive generations is given in Table 1. It was observed that pupae yield was 74% while wax comb rearing could attain yield of 58% pupae only. The average of 30 larva weight of wax-comb reared larvae was 35.5 mg, whereas higher larval weight (44.1mg) was recorded on the improved semi-synthetic diet. The pupation stage on semi synthetic diet reached within the least larval duration of 34.33 days as compared to 41.5 days of larval period, on its natural food, wax comb. The larval period was also found to be lower as compared to 49 days as reported by Adeline et al (2018). The larval weight (44.5g) was seen to be higher in this study in comparison to 37.2 g reported by Ajanta et al., (2008) as well as that recorded (35.5g), when reared on bee wax alone.

Table 1. Growth and development of *G. mellonella* over three generations

| Parameters | Rearing medium[1] | |
|---|---|---|
| | semi-synthetic diet | Wax honeycomb (natural food) |
| Larval weight of 5th instars (mg) | 44.1 | 35.5 |
| Pupal weight (mg) | 24.0 | 16.8 |
| Eggs period (days) | 6.5 | 10.3 |
| Larval period (days) | 34.3 | 41.5 |
| Pupal period (days) | 8.1 | 9.3 |
| Male Adult period (days) | 10.3 | 14.5 |
| Female adult period (days) | 15.5 | 18.5 |
| Pupation (Percentage) | 74 | 58 |
| Emergence( Percentage) | 83.4 | 69 |
| Survival (Percentage) | 63.3 | 40 |
| Larval growth index | 2.20 | 1.40 |
| Pupal Growth Index | 10.4 | 4.32 |
| Developmental index | 1.49 | 0.79 |
| Fecundity (eggs/female)[2] | 1252 | 789 |
| Fertility (neonates/ female)[2]percentage | 76.52 | 77.2 |
| Life period (days)[3] | 61.8 | 77.6 |

[1] Mean of 3 generations and 4 replicates per generation (N= 25 for each replication)

[2] Average of 3 generation (N=12 Adult pairs per generation)

[3] Life period (days) = Eggs period+ larval period+ pupal period+ adult period

The mean growth and development were good on semi-synthetic diet, recorded higher larval growth index (2.2), pupal growth index (10.4) and developmental index (1.49) over that when grown on the natural food, bee wax (1.40, 4.32 and 0.79 respectively). Lower incubation period (6.51 days) and pupal period (8.1 days) were also observed in the larvae reared on artificial diet as compared to bee wax (10.3 days and 9.3 days respectively). The female moths laid an 1252 eggs in their lifetime when grown on improved semi-synthetic diet, whereas 789 eggs were recorded on natural food bee wax. Survival % (eggs to adult emergence) was

higher (63.3%) along with lower of developmental period 62 days (eggs to adult) as compared to 40% survival and 77.6 days developmental period recorded in natural diet; wax honeycomb.

For the last three decades, attempts to develop suitable semi-synthetic diet for mass rearing of *G. mellonella* have been made to ensure uninterrupted EPN production. The semi-synthetic diet should be evaluated for several generations to determine maintenance of vital biological parameters for survival, reproduction and behavior (Cohen, 2001). The present findings suggest that the modified semi-synthetic diet contains all the essential nutrients in the balanced proportions needed for higher larval weight and higher pupation %with reduces developmental period, allowing continuous mass rearing. Furthermore, the cost of 1kg diet is approximately Rs 500, on which 1000 larvae of *G. mellonella* can be reared successfully. The cost-effective and efficient diet formula under report enables EPN production at factory scale.

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