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Mass production of wax moths and economics of parasitoid, *Apanteles galleriae* Wilkinson production

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ABSTRACT: The experiment was conducted in the Department of Entomology, Himachal Pradesh Krishi Vishvavidyalaya, Palampur during 1993-94. The cultures of wax moths namely *Galleria mellonella* (Linnaeus) and *Achroia grisella* (Fabricius) were raised on artificial diet. The larvae developed on artificial diet were utilized for mass rearing of the parasitoid, *Apanteles galleriae* Wilkinson. The maximum number of wax moths larvae were produced when jar size of 20.32x16.24cm was used and when 45 female and 15 male parasitoids were released in the above mentioned jars and 200 larvae were exposed. The economics of parasitoid production was calculated as Rs. 0.66 per 10 adults of *A. galleriae*.

KEY WORDS: *Achroia grisella, Apanteles galleriae, Artificial diet, biocontrol, Galleria mellonella*

The greater wax moth, *Galleria mellonella* (Linnaeus) and lesser wax moth, *Achroia grisella* (Fabricius) are serious pests of honey bees namely *Apis cerana* and *Apis mellifera* (Akratanakul, 1987; *Verma et al.*, 1997; *Viraktanath et al.*, 1998; Verma *et al.*, 1998). These pests cause heavy losses to *Apis cerana* F. and *Apis mellifera* L. colonies. The larvae of the wax moths, besides producing silken tunnels in combs, feed upon the bees wax and also damage the stored combs. A severe infestation by wax moths at times lead to absconding of the bees from their hives.

Various remedial measures have been suggested which include the use of sulphur, besides observing strict cleanliness in the beehives (*Sattigi et al.*, 1993). In case of bee hives or stored comb it is unsafe to use chemicals and it will be appropriate to use biotic agents. *Apanteles galleriae* has been

reported as parasitoid of wax moths (*Ahmad et al.*, 1983; Shimamori, 1987). *Apanteles galleriae* was recorded on lesser wax moth (*Achroia grisella*) caterpillars in the mid hill sub humid zone in 1983 (Anon., 1984). It is desirable to produce the parasitoid in large scale in the laboratory and then utilize for the control of wax moths larvae.

MATERIALS AND METHODS

The experiment was conducted in the department of Entomology, Himachal Pradesh Krishi Vishvavidayalaya, Palampur. Mass production of the parasitoid involves mass production of the host larvae (*Galleria mellonella* and *Achroia grisella*). For rearing the wax moths in the laboratory the artificial diet was prepared according to the method of *Ahmad et al.* (1983). The diet contained maize flour, wheat flour, wheat bran, dry yeast, milk powder, honey and glycerine (4:2:2:1:1:4:4 by weight). In addition, 5g each of terramycine (Pfizer) and multivitamin (Supradyn) were added to one kg diet ingredients to produce disease free and vigorous host larvae for multiplication of the parasitoid. The diet was placed in glass jars for rearing the host larvae and parasitoid. To this diet freshly emerged adults of both the species of wax moths (25 pairs of each species) were released in the glass jars separately for egg laying. A cotton swab containing 30 per cent honey solution was also kept in the glass jars as a feed for adults.

Mass rearing of *A. galleriae*

Mass rearing of parasitoid in individual vials is time consuming and laborious. Hence, an attempt was made to multiply *A. galleriae* in groups. Wax moths larvae were reared on artificial diet as and when these larvae were second instar in case of *G. mellonella* and third instar in case of *A. grisella*. Two hundred larvae of wax moths were exposed to variable number of pairs of *A. galleriae* for 3-4h and then artificial diet was provided. Three replications were maintained. Observations on the emergence of adults were recorded. Honey solution (30%) was given as food supplement to parasitoid.

Cost of Production of *A. galleriae*

The cost of parasitoid production was worked out on the basis of rearing the host insect, *G. mellonella* as well as the production of the parasitoid, *A. galleriae* under laboratory conditions at 23.9±2°C. The experiment was carried out in a 3x3m room. The room was provided with one tube light and one thermometer. The services of the labourer were taken for ½ day per day to prepare the artificial diet and inoculate the diet for the adult wax moths and *A. gallariae*, cleaning, transferring to fresh jars and handling other items in the laboratory for smooth running of the unit. The labour charges were calculated on the basis of daily wage @ Rs.24 per day for skilled labour. The experiment was conducted during July and August, 1994. But the actual period of economics of mass rearing was calculated from the day when egg of wax moth started hatching.

The cost of permanent articles / equipment was calculated at depreciation

rate of 15 per cent per annum and interest on the capital @ 12 per cent per annum and calculated for the experimental period of one month. The life of the incubator was considered as 10 years. Since the economics was calculated only for 15 jars (20.32x15.24cm). Three jars, each containing one kg diet, were used for larval production of wax moth. Further 12 jars containing artificial diet @200 g per jar and each jar containing 200 larvae of wax moth inoculated with 45 female and 20 male parasitoids were used for development of the parasitoid. The depreciation on the permanent equipment, interest on the capital investment, room rent, labour charges and electrical charges were calculated for 15 jars, assuming that the room size mentioned above had the capacity to hold 90 jars (20.32x15.24cm) for maintaining host parasitoid culture. The cost was placed under the head of fixed cost and variable cost. The fixed cost includes the depreciation on equipment and interest over the capital. The variable cost includes consumable articles, electricity and labour charges. The economics of mass production of the parasitoid was calculated by taking into consideration of various aspects elucidated by Puri and Sagwan (1972) and Raj and Ramakrishnan (1978a) under similar studies with other parasitoid.

RESULTS AND DISCUSSION

It is evident from the Table 1 that the number of larvae and moths emerged from each jar varied in respect of size of jars and quantity of artificial diet provided to wax moths. The maximum average number of moths of *G. mellonella* (964.33) and *A. grisella* (953) emerged from the jars measuring 20.32x15.24cm, containing one kg artificial diet and inoculated with 25 pairs of wax moths. Whereas on an average 1218.33 larvae and 953.66 adults of *G. mellonella* and 1267.33 larvae and 921.66 adults of *A. grisella* were obtained from jars measuring 20.32x15.24cm containing 500g of artificial diet inaculated with 25

Table 1. Wax moth emergence in relation to size of rearing jars and quantity of diet

Species of wax moth	Size of Jar (cm)	Quantity of diet (g)	No. of larvae emerged	No. of moths emerged	Sex ratio (F:M)
G. mellonella	20.32 x 5.08	200	320.00	230.33	1: 0.78
	20.32 x 10.16	200	781.66	196.66	1: 0.61
	20.32 x 10.16	500	1034.66	698.33	1: 0.80
	20.32 x 15.24	500	1218.33	953.66	1: 0.77
	20.32 x 15.24	1000	1032.66	964.33	1: 0.77
A. grisella	20.32 x 5.08	200	307.33	218.33	1: 0.78
	20.32 x 10.16	200	836.00	256.00	1: 0.56
	20.32 x 10.16	500	1118.33	743.33	1: 0.84
	20.32 x 15.24	500	1267.33	921.66	1: 0.78
	20.32 x 15.24	1000	1040.00	953.00	1: 0.83

F= Female; M= Male CD (P=0.05) for treatment for *G. mellonella* = 6.41
CD (P=0.05) for treatment for *A. grisella* = 63.33

pairs of each species of wax moth separately. The best combination has been found as the jar measuring 20.32x15.24 cm, containing one kg artificial diet and inoculated with 25 pairs of wax moths. The present findings are in conformity with Marston *et al.* (1975) who reported that optimum density for *G. mellonella* larvae in rearing pans was 1000 / kg diet and that of adults in oviposition cages was 898-1345 pairs/m^2 when reared at room temperature of 30±1°C.

Sex ratio of the wax moths

The population of the females was slightly higher than the males. The sex ratio (female: male) varied between 1:0.56 and 1:0.84 in respect of size of jars and artificial diet added to these jars (Table 1). Space stress resulted in lesser production of larvae of *G. mellonella* without altering sex ratio. However, in case of *A. grisella* the availability of space exhibited influence on larval production as also on sex ratio. Raj and Ramakrishnan (1978b) observed that percentage of female moths of *Corcyra cephalonica* was 65.46 and 69.35, respectively for 2700 and 1350 eggs per kg diet. Studies on influence of population density on size of emerged moths, sex ratio, egg production and total mortality of *Plodia interpunctella* have been conducted by Snyman (1949) who concluded that the adverse effect of density begins when the larval population density exceeds one larva per 0.40 g of food.

Mass rearing of *A. galleriae*

From the results presented in Table 2, it is clear that treatments showed significant difference in average number of adults produced except P3 and P4. The volume of rearing jars also had a significant effect on the average number of *A. galleriae* adults produced on *G. mellonella* as well as on *A. grisella* larvae. The number of parasitoid adults released within examined range had no significant effect on the

Table 2. Effect of parssitoid number released and size of jar on the oviposition by *A. galleriae* on *G. mellonella*

Species of wax moth	Size of glass jar (cm)	Number of adult parasitoid released					
		35 : 15	40 : 20	45 : 20	50 : 25	60 : 30	Mean
		P_1	P_2	P_3	P_4	P_5	
G. mellonella	20.32 x 5.08 V1	76.67	82.00	84.00	90.33	102.33	87.07
	20.32 x 10.16 V2	104.33	120.00	132.00	137.33	151.67	129.07
	20.32 x 15.24 V3	118.67	133.33	149.33	152.67	164.00	143.60
Mean		99.89	111.78	121.78	126.78	139.33	
CD (P=0.05) for pairs		= 6.89					
CD (P=0.05) for volume		= 8.90					
CD (P=0.05) for interaction		= 15.45					

average number of parasitoid produced in the jar size V1 (20.32x5.08cm) in case of *G. mellonella* (Table 2) and V1 (20.32x5.08 cm), V2 (20.32x10.16cm) and V3 (20.32x15.24 cm) in case of *A. grisella* (Table 3). Increase in size of jars from V1(20.32x5.08cm) to V2(20.32x10.16cm) and V3 (20.32x15.24cm) resulted in significant increase in the parasitoid production in case of *G. mellonella* with the increase in parasitoid released from 35 females and 15 males to 40 females and 20 males. Further increase in size of jars to V3 and also increase in number of parasitoid from 40 females and 20 males to 45 females and 20 males also resulted in significant increase in the average number of adult parasitoids produced. Increase in the volume of jars from V1 (20.32x5.08cm) to V2 (20.32x10.16cm) resulted in significant increase in the total number of parasitoid produced irrespective of number of adult parasitoids released on the larvae of either species of wax moths. Further, increase in the volume of jars from V2 to V3 showed no significant increase in the total number of parasitoid produced except P3 (45 femaes and 20 males) in case of *G. mellonella* (Table 2) and P3 and P4 (50 females and 25males) in case of *A. grisella* (Table 3).

In the present studies the best combination has been found when V3 size jars (20.32x15.24cm) containing 200 wax moth larvae were inoculated with 45 females and 20 males (P3) of parasitoid. De Bach and White (1960) while studying the effect of densities of *Aphytis lignanensis* Comp. pointed out that with higher ratio of parasitoid inoculum to host density, a point would reach at which parasitoid progeny produced /cm^2 will decrease. The present investigations also exhibited the same trend as described by these workers. The possible explanation for such phenomenon was that the interference of parasitoids with each other when crowded, resulted in decreased oviposition.

Table 3. Effect of number of parasitoid released and size of jar on the oviposition by *A. galleriae* on *A. grisella*

Species of wax moth	Size of glass jar (cm)	Number of adult parasitoid released					
		35 : 15	40 : 20	45 : 20	50 : 25	60 : 30	Mean
		P_1	P_2	P_3	P_4	P_5	
A grisella	20.32 x 5.08 V1	81.00	86.00	88.33	94.00	107.33	91.33
	20.32 x 10.16 V2	110.00	125.00	136.33	141.33	156.33	133.80
	20.32 x 15.24 V3	123.33	138.33	153.33	156.66	170.00	148.33
Mean		104.77	116.44	125.99	130.66	144.55	

CD (P=0.05) for pairs = 8.74
CD (P=0.05) for volume = 6.77
CD (P=0.05) for interaction = 15.14

Economics of mass rearing of *A. galleriae*

The cost of rearing was worked out as Rs.0.66 per 10 adults of *A. galleriae* (Table 4). The unit of production required the services of one man @Rs.24 for ½ day for the smooth running of the unit. For the purpose of calculating the cost of production monthly depreciation was calculated on the basis of 10 years life period. Scopes (1968) considered the life of equipment as 10 years for calculating the cost of production of *Encarsia formosa*, a parasitoid of whitefly, *Trialeurodes vaporariorum*. The interest on capital investment @15 per cent annum was also included. The cost of production of parasitoid will vary from place to place and laboratory to laboratory due to wide range of differences in price of equipment at different places and at different times. Puri and Sangwan (1972) worked out the cost of mass rearing of *Bracon gelechiae* as Rs.1.79 per 1000 eggs and monthly depreciation was calculated on the basis of 5 years life period. The cost of production of 1000 adults of *T. brasiliense* under controlled conditions in the laboratory was worked out to be Rs.1.10 (Raj and Ramakrishnan, 1978a).

Table 4. Economics of mass rearing of *A. galleriae*

	Rs.
A. Fixed cost	
a) Depreciation [x,y] on the total cost incubator @ 15 per cent per annum	15.97
b) Interest [x,y] on the total cost of incubator @ 12 per cent per annum	12.96
c) Repair and maintainance charges [x,y] incubator @ 2 per cent per annum	3.76
d) Interest [x,y] on the total cost of other equipment @ 12 per cent per annum	1.19
B. Variable cost	
a) Interest @ 12 per cent for 1/2 period of growth (15 days)	2.54
C. Miscellaneous charges	
a) Room rent [x,y] (3 x 3 cm)	4.00
b) *Elęctricity charges [x,y]	12.92
c) Labour charges @ 1/2 day per day for month	60.00
Total cost	113.34
Total numbers of *A. galleriae* adults produced in 12 jars	1695
Cost of production of one parasitoid	0.066

[x]= Calculated for 15 jars (20.32 x 15.24 cm)

[y]= Calculated for one month

*= Calculated on the basis of HPSEB (Himachal Pradesh State Electricity Board)

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