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Host egg parasitization and development of *Trichogramma brasiliense* Ashmead (Hymenoptera: Trichogrammatidae) in a modified illumination and parasitization chamber

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ABSTRACT: **An illumination chamber and a parasitization chamber was designed and modified for the mass multiplication of the egg parasitoid, *Trichogramma brasiliense* Ashmead on *Corcyra cephalonica* (Stainton) egg cards. The illumination chamber maintained a light intensity of 100.3 lux and a maximum and minimum temperature of 29 and 24°C, respectively, at the centre. The parasitization chamber consisted of a glass box with eight wire mesh supports inside to keep the egg cards. Placing two parasitization chambers inside the illumination chamber, 21 ml of eggs could be exposed for parasitization at a time resulting in uniform parasitization of eggs. No specific pattern in parasitization was perceived between the layers of the chamber at different ratios and exposures. Estimation of egg parasitization at 4 selected locations constituting 0.22 per cent of the total area of the egg card was found ideal for sampling the level of overall parasitization in the egg cards. Exposure of mother to daughter cards in the ratio of 1:7 for 48 h in the parasitization chamber resulted in the optimum parasitization of the eggs by the parasitoid, higher percentage of adult emergence and less number of runts in the resultant progeny.**

KEY WORDS: Egg parasitization, illumination chamber, parasitization chamber, runts, *Trichogramma brasiliense*

The parasitic wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are the most widely used biological control agents in the management of insect pests all over the world (Li, 1995). For field releases, *Trichogramma* are mass multiplied on the egg cards of the *Corcyra cephalonica* (Stainton), in commercial insectaries and other multiplication centres using different types of containers or cages as parasitization chambers. However, maintenance of proper ratio of parasitized eggs cards (as source of adult parasitoids) to fresh egg cards and providing required light intensity inside the parasitization chambers are the two important factors for getting optimum parasitization of the eggs by the parasitoid (Manjunath, 1988). Excess parasitization of the egg cards may result in reduced emergence of healthy parasitoids and development of more number of deformed parasitoids called 'runts'. Less parasitization below desired level makes the biological control programmes ineffective at field levels. The first attempt to develop a parasitization chamber using plywood box for commercial rearing units was made by Morrison *et al.* (1976). Thereafter, several studies were carried out to further improve the

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chamber (Morrison and King, 1977; Morrison, 1985). The uses of transparent polymethyl metacrylate cage for the mass multiplication of *T. australicum* (Liang *et al.*, 1982) and wooden cages for *Trichogramma* spp. (Collazo and Hernandez, 1985; Liang and Eden, 1990) are also reported. In India, transparent bottles or polythene bags are used in mass rearing programmes of the parasitoids, which is labour intensive (Manjunath, 1988). This paper reports the development of an illumination chamber and parasitization chamber for the mass multiplication of *Trichogramma*, a sampling technique for assessment of the parasitization of host eggs and the developmental attributes of *T. brasiliense* in the chambers.

MATERIALS AND METHODS

Illumination chamber

A teak wood box of size 53 x 25 x 41 cm fitted with a door was modified as the illumination chamber (Fig. 1). Four holes of one cm diameter were made at the bottom and the top of the box for sufficient aeration. Two fluorescent lamps of 30 cm length were provided inside the chamber on both sides. The tubes were wrapped with aluminium foil to prevent light from directly falling on the parasitization chamber, kept inside the illumination chamber. A wax paper screen was also kept between the parasitization chamber and the light source to reduce the light intensity. The whole inner side of the illumination chamber was lined with white paper having a uniform reflecting surface.

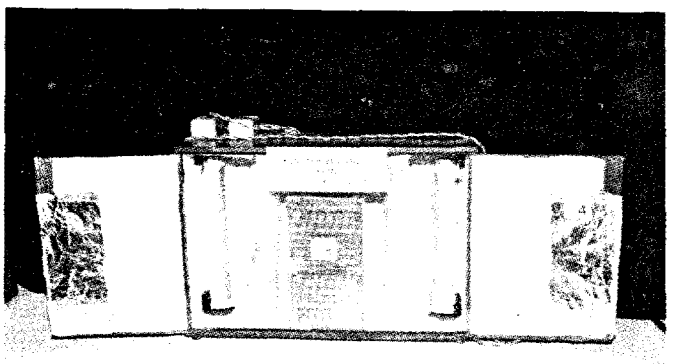

Fig.1. Illumination chamber with parasitization chamber inside

Parasitization chamber

The parasitization chamber was designed and built using glass and thick polyvinyl sheets. Four glass pieces joined together by Araldite® formed the frame of the chamber (Fig.2). Eight struts were provided on the inner sidewall of the chamber at 1.3 cm interval, to support the 8 cut pieces of wiremesh of 20.5 x 12 cm size for keeping the egg cards in eight layers. The two sides of the glass frame were closed by fixing a thick (25μ) polyvinyl sheet with Araldite® on one side and cellophane tape on the other. The polyvinyl sheet jointed with cellophane formed a door for the chamber. Eight holes of 0.5 cm diam, made and sealed with double layered markin cloth on the polyvinyl sheets facilitated the exchange of air and prevented the build up of humidity inside the chamber. Two such parasitization chambers were kept inside the illumination chamber for further studies.

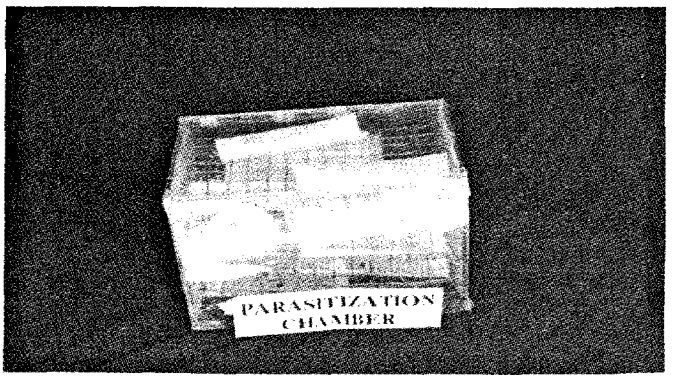

Fig.2. Parasitization chamber will egg cards

Insect culture

Parasitized *Corcyra* egg cards containing ready to emerge *Trichogramma brasiliense* pupae were used as the source of the parasitoid (mother cards) and freshly prepared unparasitized *Corcyra* egg cards (daughter cards) served as the hosts. The sizes of the mother and daughter cards were maintained uniformly at 5 x 9 cm throughout the studies. The mother cards were always kept in the third layer of the parasitization chamber and the daughter cards were kept in other layers @ two cards per layer and the layers were serially

numbered from top to bottom. Fifty per cent honey solution provided as fine streaks on the inner side of the chamber served as the food to the newly emerged parasitoids and the chamber was sealed using cellophane tape.

Sampling technique, parasitization, and emergence of parasitoids

To standardise a sampling technique to study the levels of egg parasitization in the parasitization chamber, the host eggs were exposed to the parasitoid at 1:5 ratio of mother to daughter cards for 48 h. Each daughter card was divided into 28 equal segments. The alternate segments on the card were serially numbered from 1 to 14 and 0.5 x 0.5 cm area of each numbered segment (n = 14) was cut from the centre and the percent parasitization in the samples was calculated. Mean per cent parasitization of the 14 samples of a card constituted the card mean. Ten such cards were sampled. The results were analyzed using chi-square test and the combination of the sample positions in the card that best represented the overall parasitization of the card was selected. The findings of this study was used in the estimation of percent parasitization of eggs on card in the following experiments. Mother to daughter card ratios ranging from 1:4 to 1:8 were exposed at 24, 48 and 96 h interval in the parasitization chamber to study the per cent parasitization of the daughter cards, emergence of parasitoids and runts and per cent parasitization in different layers of the parasitization chamber. The results were statistically analysed using Analysis of variance (ANOVA). All the treatments were replicated 10 times.

RESULTS AND DISCUSSION

The modified illumination chamber provided a light intensity of 100.3 lux and a minimum and maximum temperatures of 24 and 29°C, respectively, inside the parasitization chambers. These conditions were found ideal for optimum parasitization of the eggs by the parasitoids. Results of the sampling scheme are presented in Table 1. Among all the combinations, only combinations at sl. nos. 5, 11, 14, 17, and 21 were found statistically significant ($\chi 2 < 2.08$). Sample combinations at 11, 21 and 17 were rejected as they contained more number of sample segments (n >6). Among the rest, sl. no. 5 with minimum combination of sample segments (n = 4) having a χ^2 value of 1.757 was accepted. Since the positions of the sample segments in this combination were 2, 6, 10 and 14, they well represented the margins, centre and middle positions of the egg card and hence this combination was selected for further studies on egg parasitization. In this method, only 0.22 per cent area of the card is needed to be sampled to get an accurate estimate of the parasitization levels of the egg cards that are mass produced following the specific illumination and card positions of the present studies.

Per cent egg parasitization under different ratios of mother to daughter cards and exposure periods are shown in Table 2. Significant interactions on parasitization of host eggs were observed between exposure periods and mother to daughter cards ratios. A maximum of 96.5 per cent parasitization was recorded in 1:4 ratio in 96 h exposure period. A general enhancement in parasitization was observed with the increase in exposure period and reduction in parasitization with the increase in card ratios. Liang *et al.* (1982) observed 81.1 per cent parasitization in polymethyl metacrylate cage with *T. australicum*.

Mean percent emergence of *T. brasiliense* was not significantly influenced by mother to daughter card ratios and exposure periods, whereas their interactions were significant (Table 3). Mother to daughter card ratios and exposure periods, significantly influenced percent development of runts in the resultant population of *T. brasiliense* (Table 4). A decreasing trend in the percentage of runts in the resultant progeny was exhibited with increase in the ratio of mother to daughter cards. Maximum host egg parasitization (96.5 %) and emergence of highest percentage of runts (12.75) were observed when the eggs were exposed for 96

Table1. Sampling technique for egg parasitization by *T. brasiliense* in the modified parasitization chamber

Sl. No.	No. of samples drawn	Combination of segments	χ^2
1	3	2, 5, 9	5.325
2	3	6, 10, 14	15.165
3	3	3, 6, 10	8.801
4	3	6, 9, 12	8.178
5	4	2, 6, 10, 14	1.757
6	4	3, 6, 9, 12	4.063
7	4	1, 5, 9,13	4.443
8	4	4, 7, 10, 13	3.056
9	4	2, 6, 9, 13	2.345
10	4	3, 6, 10, 14	3.168
11	4	3, 6, 10, 13	1.960
12	4	8, 9, 10,11	2.169
13	5	2, 5, 6, 9, 13	13.901
14	5	3, 6, 8, 9, 10	1.635
15	6	2, 6, 8, 9, 10, 14	5.370
16	6	3, 6, 9, 10, 11, 12	2.085
17	6	4, 7, 8, 9, 10, 13	1.028
18	6	2, 3, 5, 6, 9, 10	5.370
19	6	2, 5, 6, 9, 10, 14	6.842
20	8	1, 2, 5, 6, 9, 10, 13, 14	9.554
21	8	3, 4, 6, 7, 9, 10, 12, 13	0.988

Probability of a larger value of χ^2 (d.f. 9) at 0.99 is 2.08.

h in the mother to daughter card ratio of 1:4. This may be due to the non-availability of sufficient numbers of host eggs to the parasitoids, leading to super parasitization of eggs. Development of more than one larvae of the parasitoid in an egg leads to competition for food and space resulting in the emergence of malformed adults. With the increase in number of daughter cards, level of parasitization decreased with concomitant decrease in percentage of runts in the progeny. Liang and Eden (1990) reported similar reduction in percentage of runts in the population *of T. pretiosum* reared on *Sitotroga cerealella* eggs.

Significant differences in per cent parasitization in the egg cards kept in different layers of the parasitization chamber were noticed in the following combinations of mother to daughter card ratios and exposure times, *viz.* 1:4, 24 and 48 h; 1:6, 24 h; 1:7, 24 h; 1:8, 24, 48 and 96 h out of the 15 such combinations. Of the three exposure periods, 24 h resulted in significant differences in parasitization of the egg cards kept in different layers of the parasitization chamber, irrespective of the ratios used. Significant increases in the per cent emergence of *T. brasiliense* and population of runts in the progeny was observed at 48 h exposure, in 1:8 ratio. It is evident that the movement of the parasitoids inside the chamber was highly irregular, since the differences in the levels of parasitization of the egg cards kept in different layers of the chamber was non-significant in majority of the cases. With the increase in time of exposure, the

Table 2. Parasitization of *Corcyra* eggs by *T. brasiliense* under different ratios of mother to daughter cards and exposure times

Ratio of mother card to daughter cards	Mean per cent parasitsation			Mean of ratios card to daughter cards
	Exposure time (hours)			
	24	48	96	
1:4	93.00	93.92	96.50	94.47
1:5	91.40	81.20	89.90	87.55
1:6	63.80	79.39	80.70	74.64
1:7	73.20	85.66	87.00	81.95
1:8	81.90	68.00	76.30	75.43
Mean of exposure times	80.68	81.63	86.13	

CD for exposure times (P=0.05) = 4.317 = NS
CD for mother to daughter card ratios (P= 0.05)= 3.344 = NS
CD for exposure time x ratio interaction = 7.477
NS = Non-significant

Table 3. Per cent emergence of *T. brasiliense* under different ratios of mother to daughter cards and exposure times

Ratio of mother card to daughter cards	Exposure time (hrs) Mean per cent parasitisation			Mean of ratios card to daughter cards
	24	48	96	
1:4	90.90	82.05	81.37	84.77
1:5	92.15	85.50	85.36	87.67
1:6	88.86	85.74	87.35	87.32
1:7	89.25	87.96	91.46	89.56
1:8	84.80	86.21	89.13	86.60
Mean of exposure times	89.10	85.49	86.93	

CD for exposure times (P=0.05) = NS
CD for mother to daughter card ratios (P= 0.05)= NS
CD for exposure time x ratio interaction = 7.066
NS= Non significant

Table 4. Population of runts of *T. brasiliense* under different ratios of mother to daughter cards and exposure times

Ratio of mother card to daughter cards	Mean per cent parasitisation			Mean of ratios card to daughter cards
	Exposure time (hours)			
	24	48	96	
1:4	7.85	11.67	12.75	10.76
1:5	4.22	4.68	5.40	4.77
1:6	2.80	4.60	4.16	3.85
1:7	3.38	4.14	4.93	4.15
1:8	4.30	1.87	3.60	3.26
Mean of exposure times	4.51	5.39	6.17	

CD for exposure times (P=0.05) = 1.24

CD for mother to daughter card ratios (P= 0.05)= 1.61

CD for exposure time x ratio interaction = NS

differences in the levels of parasitization reduced. The present findings are in agreement with the results of the parasitization study carried out by Liang *et al.* (1982) in a multi-layered cage with *T. australicum.*

In the present study, optimum egg parasitization and emergence of lower proportion of runts were noticed at 1:7 ratio with 48 h exposure. The egg parasitization in different layers of the parasitization chamber was also uniform at this ratio. Hence, we propose the above ratio of mother to daughter cards and exposure time as optimum for mass production of *T. brasiliense.* Placing two parasitization chambers in the modified illumination chamber, 21 ml of eggs could be effectively exposed for parasitization at a time.

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