STUDY ON INOCULUM DOSES OF *Corcyra cephalonica* (STAINTON) EGGS FOR ITS EFFICIENT MASS PRODUCTION IN LABORATORY

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ABSTRACT

Rice moth, *Corcyra cephalonica* (Stainton) is a well-established factitious host for laboratory rearing of different egg parasitoids and predators. The insect is mass produced in ventilated plywood boxes with 2.5 kg of food medium in Nepal. In order to increase production efficiency of this rearing system, study was conducted to find optimum dose of egg inoculum. Replicated completely randomized experiment was conducted on crushed maize charging with 0.5, 0.33, 0.25 and 0.2 cc of egg inoculums at National Entomology Research Center during December 2022 to April 2023. Days to first adult emergence, adult emergence period, total adult and egg produced per box, body length and weight of moth, fecundity per female and 100 egg weight were recorded and analyzed using GenStat Discovery computer application. Days to first adult emergence did not differ significantly recording 54-55 days. The adult emergence period was found to increase with higher egg inoculum recording 63, 62, 49 and 42 days for 0.5, 0.33, 0.25 and 0.2 cc egg inoculums, respectively. Moth production was significantly higher in 0.33 cc (n = 1385) followed by 0.5 cc (n = 1169), 0.25 cc (n = 877) and 0.2 cc (n = 825) inoculums. Egg production was significantly highest in 0.33 cc (n = 75539) and remaining 0.5 cc (n = 71801), 0.2 cc (n = 72815) and 0.2 cc (n = 71801) were at par. The body length and weight of moths were found to decrease with increase in inoculum size and female moths were recorded bigger than male. The fecundity per female was higher in 0.25 cc (n = 371) and 0.2 cc (n = 363) followed by 0.3 cc (n = 271) and 0.5 cc (n = 260). The 100 egg weight did not differ among treatments recording 35-36 mg. The inoculum dose of 0.2 cc egg is suitable for efficient mass production of *C. cephalonica*.

Key words : *C. cephoalonica*, egg dose, inoculum, mass production.

INTRODUCTION

Indiscriminate use of chemical insecticides has resulted in several problems such as pest resistance, outbreak and resurgence, toxic residues in food, water, air and soil, elimination of natural enemies of pests, disruption of ecosystem and endanger to workers' health. In order to overcome the hazards of insecticides, safe measure of insect pest management including integrated pest management (IPM) could to be practiced. Biological control of insect pest is one of the key component of IPM which could contribute in a safe and ecofriendly pest management practices. Among various means of biological control, insect parasitoids and predators are proven successful in regulating insect pest population. The success of any biological control program through inoculative and inundative releases depends upon laboratory mass culture of parasitoids and predators in appropriate time. The production of parasitoids and predators depends upon efficient production of host insect or suitable alternate host in laboratory.

Rice moth, *Corcyra cephalonica* (Stainton) is a well-established factitious host for laboratory rearing of different egg parasitoids and predators. The insect is a useful factitious host for 75 biological control agents including 60 parasitoids (Manjunath, 2014). Various biocontrol research, developmental and extension laboratories around the world utilize *C. cephalonica* for mass production of number of natural enemies (Rajasekhar et al., 2016). *C. cephalonica* is utilized by majority of laboratories in Asia for mass production of Trichogrammatid egg parasitoids (Ghosh and Ballal, 2017). In Nepal this factitious host is utilized for scientific studies and mass production of *Trichogramma chilonis* by various institutions (NERC, 2023).

Mass rearing of the *C. cephalonica* is performed in the crushed maize grains as source of food in Nepal. Two and half kg of roughly crushed maize grains mixed with 3 g yeast and 0.5 g streptomycin sulphate is kept in a ventilated plywood box with internal dimensions of 45 cm x 30 cm x 15 cm in length, width and height, respectively (NERC, 2023). Each box with rearing medium is charged initially with 0.5 ml of freshly laid *C. cephalonica* eggs which consists more than 8000 eggs. Moth production per rearing box is quite low compared to number of eggs charged initially. Generally, the large amount of eggs is wasted in this level of initial inoculum, which can be saved for production of *T. chilonis* if the optimum level of initial inoculum can be determined. The doses of eggs used for initial infestation of rearing medium has impact on production of adult moths and amount of eggs laid by these moths (Sharma et al., 2016). Considering the facts, in order to increase production efficiency of the rearing system adopted in Nepal, study on optimum dose of egg inoculum was conducted.

MATERIALS AND METHODS

Laboratory experiment was conducted to find optimum doses of initial egg inoculum to food medium for better and efficient production of *C. cephalonica* adults and eggs at National Entomology Research Center during December 2022 to April 2023. The experiment was conducted in Completely Randomized Design (CRD) with three replications in controlled conditions of 27 ± 2 °C temperature and 75 ± 10% relative humidity. Initial egg inoculum levels of 0.5 cc, 0.33 cc, 0.25 cc and 0.2 cc were studied. Twelve cleaned and dried ventilated plywood boxes with internal dimensions of 45 cm x 30 cm x 15 cm in length, width and height were used for study. Each box filled with 2.5 kg of roughly crushed maize grains which was sterilized at 100 °C temperature for one hour in hot air oven (Faithful 101-3AB). The sterilized maize was thoroughly mixed with 3 g yeast extract powder as protein supplement and 0.5 g of streptomycin sulphate to prevent bacterial contamination. These boxes were charged with four levels of egg inoculum and the boxes were kept in laboratory for production of *C. cephalonic* moths. Egg doses were prepared by measuring one cubic centimeter of eggs with measuring cylinder and divided on weight basis maintaining doses of 0.5 cc, 0.33 cc, 0.25 cc and 0.2 cc. One cubic centimeter of freshly laid eggs (less than 24-hour old) produced in NERC laboratory was counted 16,000 and weighted 5.79 g on average.

After first adult emergence, daily observation was recorded on number of moths emerged from each box. The emerged adults from each box were collected separately in ventilated ovipositional cages made up of 10 L plastic bucket with help of vacuum suction device (Fig. 1). The amount of eggs laid by these moth in 24-hour duration were collected and cleaned using sieves of mesh size 30 and 45. The cleaned eggs were counted visually with help of hand lens on colour chart paper. Daily observations in each box and ovipositional cages continued till cessation of moth emergence and egg laying.

Fig. 1. (a) Collection of adult moths with vacuum suction device and (b) Ovipositional cages for *C. cephalonica* moths.

Another experiment was conducted to find the fecundity of individual moth emerged from each treatment. One pair of newly emerged adults moth from each box were kept separately in culture tube of 100 ml volume and 10 replications were maintained. The male and female moths were identified on the basis of labial palp (Fig. 2). Moths were fed with 10% honey solution on cotton swab. A total number of eggs laid in 24-hour interval was counted. A total number of eggs laid during life time of individual moth was calculated to find the fecundity of individual female.

Body length of freshly emerged male and female moths from each treatment was also recorded. Stage and ocular micrometers were used to measure body length of the moths in stereo-microscope (Bestscope, BS 3040T). Similarly weight of individual male and female moth was also recorded with help of digital weighing balance (Sartorius, Entris 2241-1S). Weight of 100 freshly laid eggs (less than 24-hour old) were also recorded using digital balance.

Data recorded were entered in Microsoft excel and ANOVA of CRD was prepared with GenStat Discovery computer application. Mean separation was done with Duncan Multiple Range Test at 5% probability level.

Fig. 2. Male and female moth of *Corcyra cephalonica*; female with conspicuous projected labial palp.

RESULTS AND DISCUSSION

Average time period from charge of egg inoculum to first emergence of adult moths and total adult emergence period in different treatments is given in the Table 1. Time required to first adult emergence in different doses of egg inoculum did not differed significantly. Days to first adult emergence ranged between 53.7 to 55.0 days in different treatments. Days to first adult emergence is directly related to the total developmental period of egg, larva and pupa. Similar total developmental period of 51.40 days reported by Menge and Naik (2017) while studying biology of *C. cephlonica* under controlled conditions at 28 °C temperature and 64% relative humidity. Total developmental period of *C. cephalonica* ranged between 41 to 59 days at 24 to 28 °C temperature and 70% relative humidity in laboratory (Jagadish et al., 2009). However, total adult emergence period in different treatments were statistically different ($p < 0.001$)). The longest total adult emergence period of 62.7 and 62.3 days was recorded from 0.5 cc and 0.33 cc eggs charged boxes, respectively. The shortest total adult emergence period (42.0 days) was recorded with 0.2 cc egg inoculum followed by 0.33 cc (49.0 days). Adult emergence period of 46.3 to 58.4 days was recorded by Sharma et al. (2016).

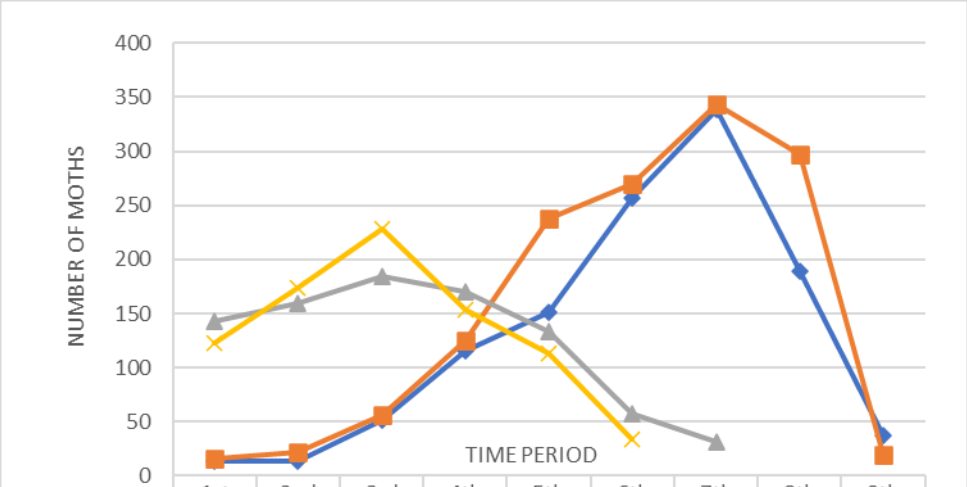
Table 1. Effect of *C. cephalonica* egg inoculum dosages on moth emergence period

Egg inoculum dosage (cc)	Days to first adult emergence	Total adult emergence period (days)
0.50	55.0 ± 0.00	62.7 ± 0.39[a]
0.33	53.7 ± 0.33	62.3 ± 0.33[a]
0.25	54.0 ± 0.00	49.0 ± 0.58[b]
0.20	54.0 ± 0.58	42.0 ± 2.65[c]
F value	3.00	55.33
df	3,8	3,8
P value	0.095	<.001
CV%	1.1	4.4

*Values presented as Mean ± SEM (Standard error of mean); means with different letter are significantly different ($p < 0.05$)

The longer total adult emergence period in boxes with higher volume egg is due to longer developmental period of immature stages of *C. cephalonica*. The longer larval period is responsible for longer developmental period. Such situation of longer larval period with increase in egg volume of initial inoculum was also reported by Sharma et al. (2016). Higher inoculum of eggs makes higher larval population resulting into crowding with more competition for food and space in laboratory multiplication of *C. cephalonica*. Short duration of larval starvation in lepidopteran insects could prolong developmental period, reduce larval growth and survival (Bauerfeind and Fischer, 2009). Such increase in larval developmental period due to increased larval density was also reported by Bhavanam et al. (2012) in Mediterranean flour moth, *Ephestia kuehniella* Zeller. The number of moths emerged over the time period is given in Fig. 3. The maximum adult moths emerged in third week from 0.25 and 0.2 cc eggs charged boxes, whereas, maximum adults emerged in seventh week from 0.5 and 0.33 cc eggs charged boxes in present study. It is clear from graph higher the egg doses initially charged longer the developmental period. The days to first adult emergence from the different level of inoculum did not differed as only few moths emerged in stipulated time though

most of the insect suffered from crowding of larval population. Graph also shows boxes charged with lower inoculum has more uniformity in adult emergence time than higher inoculum.



	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week
0.50 cc	14	14	51	116	151	257	339	189	37
0.33 cc	16	22	56	125	238	270	343	297	19
0.25 cc	143	159	184	170	133	57	31		
0.20 cc	123	174	228	154	113	34			

Fig. 3. Effect of egg inoculum dosages on the production of *C. cephalonica* moths over the time period.

The effect of different doses of egg inoculum on production of total moth and eggs are given in the Table 2. The total number of moths produced significantly differed among the treatments ($p < 0.001$). The highest number of moths (1385) emerged from 0.33 cc inoculum, followed by 0.5 cc inoculum (1169). The lowest number of moths (825) emerged from 0.2 cc and 877 moths emerged from 0.25cc inoculum. The total egg produced per box was significantly high (75539) in 0.33 cc egg inoculum ($p = 0.003$). The rest of the treatments were at par with 72815, 71808 and 71801 total egg production per box from 0.25, 0.2 and 0.5 cc inoculum level. The number of eggs produced in 0.33 cc inoculum was found the highest with highest number of the insect however, egg production in other treatments did not show significant difference although moth population significantly differed. This is due to significant difference in fecundity of female moths emerged from different treatments ($p < 0.001$). The highest fecundity (371) was recorded from 0.25 cc inoculum which was at par with 0.2 cc inoculum recording fecundity of 363 eggs per female. Fecundity of 0.5 cc and 0.33 cc inoculum were at par recording 261 and 271 eggs per female (Table 3). The result showed the higher fecundity of moths emerged from lower egg inoculum. Such finding of higher fecundity rate of *C. cephalonica* with lower egg inoculum was also reported by Lalitha and Ballal (2015). Sharma et al. (2016) also reported increase in fecundity of *C. cephalonica* female is associated with low egg inoculum density. The lower fecundity with higher egg density inoculum is due to crowding during developmental period of the insect. The crowding during larval period might have reduced food availability. Fantinou et al. (2008) reported that the performance of Lepidopteran adults depends upon the food

reserve accumulated during larval stage as adults have few chances for accumulation of additional food resources. In present study the adults emerged from different egg inoculum doses were feed on 10% honey solution however, we find the lower production of eggs with higher inoculum. Manjunath (2014) reveled that overcrowding larvae of *C. cephalonica* had negative impact on its egg production and adult feeding had no effect on it.

Table 2. Effect of *C. cephalonica* egg inoculum dosages on total moth emergence and egg production per box

Egg inoculum dosage (cc)	Total moths emerged	Total eggs produced
0.50	1169 ± 8.2[b]	71801 ± 676.1[b]
0.33	1385 ± 20.1[a]	75539 ± 143.4[a]
0.25	877 ± 9.5[c]	72815 ± 592.9[b]
0.20	825 ± 3.0[d]	71808 ± 628.9[b]
F value	481.80	10.17
df	3,8	3,8
P value	<.001	.003
CV%	1.9	1.3

*Values presented as Mean ± SEM (Standard error of mean); means with different letter are significantly different ($p < 0.05$)

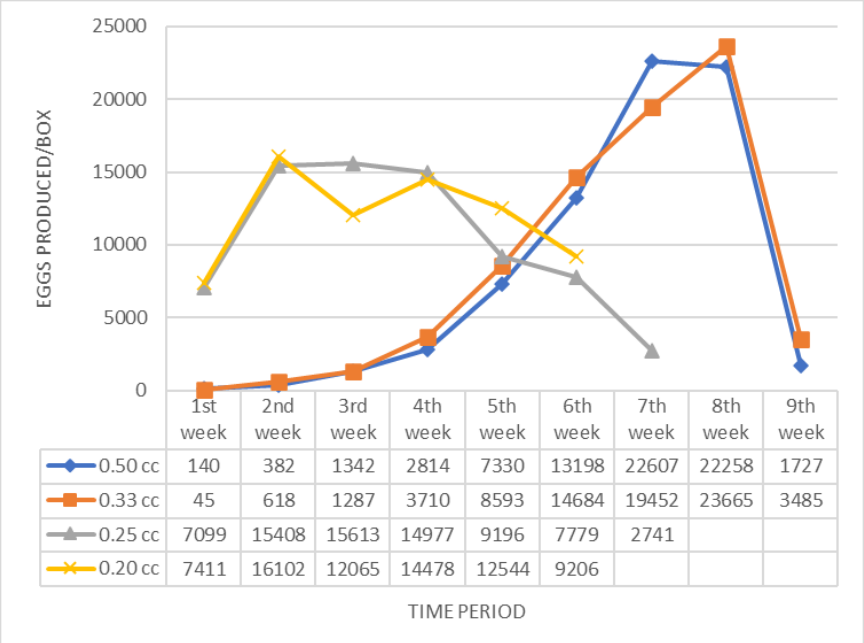
Table 3. Effect of egg inoculum dosages on fecundity and 100 egg weight of *C. cephlonica* moths

Egg inoculum dosage (cc)	Fecundity/ female	100 egg weight (mg)
0.50	261 ± 8.35[b]	3.6 ± 0.15
0.33	271 ± 9.32[b]	3.6 ± 0.10
0.25	371 ± 23.68[a]	3.5 ± 0.08
0.20	363 ± 11.59[a]	3.5 ± 0.09
F value	11.29	0.49
df	3,36	3,36
P value	<.001	0.689
CV%	14.3	9.7

*Values presented as Mean ± SEM (Standard error of mean); means with different letter are significantly different ($p < 0.05$)

The significant effect of egg inoculum on 100 egg weight laid by moths emerged from different treatments was not observed (Table 3). The 100 egg weight ranged between 3.5 to 3.6 mg in all treatments. Comparable 100 egg weight of 3.2 mg was reported by Begum and Qumar (2015) when *C. Cephalonia* was reared in mixed diet of sorghum, maize and millet. They reported the 100 egg weighted 4.2 mg, when the mentioned diet was fortified with 10% groundnut. The egg production per box over the time with different egg inoculum dosages is presented in Fig. 4. The higher egg production among moths emerged from 0.2 and 0.25 cc egg inoculum was recorded during second to

fourth week after first adult emergence, whereas, seven to eight week required in case of adults emerged from 0.5 and 0.33 cc inoculum.



	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week
0.50 cc	140	382	1342	2814	7330	13198	22607	22258	1727
0.33 cc	45	618	1287	3710	8593	14684	19452	23665	3485
0.25 cc	7099	15408	15613	14977	9196	7779	2741		
0.20 cc	7411	16102	12065	14478	12544	9206			

Fig. 4. Effect of egg inoculum dosages on the production of *C. cephalonica* eggs over the time period.

The effect of egg inoculum doses on body length and weight of emerged adult male and female moths are presented in Table 4. The body length of female and male moth differed significantly on different treatments ($p < .001$). The highest female body length (14.6 mm) was reported in 0.25 cc inoculum which was at par with 0.2 cc inoculum with 14.5 mm body length. The body length of female moths was recorded 13.9 mm and 13.1 mm in 0.33 cc and 0.5 cc inoculums, respectively. Similarly, body length of male moths was recorded 12.0 mm and 11.7 mm in 0.2 cc and 0.25 cc inoculum level, which were at par with each other. Males with 0.33 cc and 0.5 cc inoculum were found 11.3 mm and 11.2 mm long. The result clearly indicated that both male and female moths emerged from lower density egg inoculum were bigger in size. The body weight of freshly emerged adult moths from different egg inoculum differed significantly ($p < 0.001$), higher the egg inoculum lower is the body weight. The highest female body weight was recorded in 0.2 cc and 0.25 cc inoculum with 50.6 mg and 49.9 mg, respectively. The lowest female body weight (30.4 mg) of emerged moths recorded in 0.5 cc and 0.33 cc (31.0 mg). Similarly, the male moths were heavier with body weight of 23.3 mg and 21.3 mg in 0.25 cc and 0.2 cc inoculum. The male moths were lighter with17.2 mg and 16.6 mg body weight in 0.33 cc and 0.5 cc inoculum size. Bhardwaj et al. (2017) reported that the weight of male moth ranged between 15.0 to 27.3 mg and weight of female moth ranged between 24.3 to 37.0 mg which is quite similar to our present finding. The result showed lower the inoculum density, heavier and bigger the moths and vice versa. The shortage of food due to competition during larval stage has negative effect on size and weight of adult moths. Moghadaham and Hesami (2022) found the maximum larval weight of the insects produced from minimum amount

of initial egg inoculum in constant amount of diet for laboratory production of Mediterranean flour moth, *Anagasta kuehniella* (Zeller).

Table 4. Effect of egg inoculum dosages on body length and weight of emerged adult *C. cephlonica* moths

Egg inoculum dosage (cc)	Body length (mm)		Body weight (mg)	
	Female	Male	Female	Male
0.50	13.1 ± 0.32^{c}	11.2 ± 0.09^{b}	30.4 ± 0.94^{b}	16.6 ± 0.26^{b}
0.33	13.9 ± 0.17^{b}	11.3 ± 0.13^{b}	31.0 ± 1.67^{b}	17.2 ± 0.81^{b}
0.25	14.6 ± 0.13^{a}	11.7 ± 0.12^{a}	49.9 ± 1.10^{a}	23.3 ± 1.31^{a}
0.20	14.5 ± 0.16^{ab}	12.0 ± 0.09^{a}	50.6 ± 1.14^{a}	21.3 ± 1.07^{a}
F value	10.72	11.45	82.66	11.67
df	3,16	3,16	3,16	3,16
P value	<.001	<.001	<.001	<.001
CV%	3.3	2.1	6.8	10.8

*Values presented as Mean ± SEM (Standard error of mean); means with different letter are significantly different ($p < 0.05$)

Our findings of present study clearly indicated the population density of the *C. cephalonica* affects developmental period of immature stages and size and fecundity of adult moths. The higher density of egg inoculum found to increase developmental period and are less uniform in time of moth emergence. The crowding in immature stage of the insect prolonged the larval development period of the insect (Fantinou et al., 2008). The size and weight of moths were also found to decrease along with reduced fecundity with crowding of immature stage of insect in constant food and space. Generally, fitness of hemimetabolous insects mostly depends on resources gathered in larval stage which affect survival, reproduction and population maintenance of adult (Juliano, Nguyen, Dinh, Than, Tayor and Pontoan, 2019). The weight of egg was not found to be affected by density of the insect.

CONCLUSIONS

On the basis of days to first adult emergence, total adult emergence period, total adult emerged, total eggs produced from emerged adults, fecundity per female, 100 egg weight and body size of emerged adults, 0.2 cc egg inoculum is optimum for efficient production of *C. cephalonica* with 2.5 kg of food medium in 45 cm x 30 cm x 15 cm sized ventilated plywood box at 27 ± 2 °C temperature and 75 ± 10% relative humidity in biocontrol laboratory.

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