J. Biol. Control, 16 (1): 19-25, 2002

Storage conditions conducive for holding the egg parasitiod, *Trichogrammatoidea bactrae* Nagaraja

P. R. GUPTA and P. C. BHARDWAJ
Department of Entomology and Apiculture
Dr Y. S. Parmar University Horticulture and Forestry
Nauni 173230, Solan, Himachal Pradesh, India
E-mail: eap@yspuhf.hp.nic.in

ABSTRACT: **Studies on *Trichogrammatoidea bactrae* Nagaraja were carried out with the objective of determination of storage conditions conducive for holding the parasitiod. From parasitized eggs of *Corcyra cephalonica* (Stainton), stored after 3-7 days of parasitization for 5 days at 10°C, above 50 per cent adult emergence occurred and eggs could not be stored for more than 7 days. However, at 15°C, storage could be extended up to 18 days but with low emergence (17.2%). In another experiment, exposure of eggs to 10 and 15°C for 5, 10 and 15 days after 3, 5 and 7 days of parasitization, provided satisfactory adult emergence from eggs stored at 15°C for 5 days after 5 and 7 days of parasitization (72.3 and 72.1%) and for 10 days after 5 days of parasitization (69.8%). Occasionally from the eggs stored for more than 5 days at 15°C after 7 days of parasitization adult emergence occurred in storage. In eggs without adult emergence hole, mortality often occurred in the pupal stage (55.9 and 61.6% after 3 and 5 days of parasitization, respectively), followed by larval (21.3 and 6.6%, respectively) and adult stage (22.8 and 31.7%, respectively). Thus storage at 15°C for 10 days after 5 days of parasitization will be the right choice.**

KEY WORDS: *Corcyra cephalonica* , storage temperature, *Trichogrammatoidea bactrae*

The egg parasitoid, *Trichogrammatoidea bactrae* Nagaraja has a wide distribution in the Oriental region, attacks a variety of lepidopteran eggs on paddy, cabbage, colocasia, *Cyperus rotundus* (Linnaeus), sugarcane, maize, etc., and is adapted to terrestrial humid habitats (Nagaraja, 1978). This parasitoid along with *Trichogramma chilonis* Ishii was observed to parasitize over 50 per cent eggs of *Helicoverpa armigera* (Hubner) on cotton in Thailand (Supharngkasen, 1979). Several species of *Trichogramma* can be stored in pre-pupal/pupal stage (*T. minutum* at 12° C and 85% relative humidity up to 25 days; *T. brasiliense* at 10° C up to 50 days and *Trichogrammatoidea eldanae* at 5° C up to 88 days) in the refrigerator for a prolonged period before field use (Singh and Jalali, 1994). Unlike them, *Trichogrammatoidea bactrae* was found to be sensitive to low temperature. After storage of parasitized eggs of *Corcyra cephalonica* (Stainton) under refrigeration, often poor emergence of *T. bactrae* adults was obtained upon returning the tricho-cards to room temperature. In order to find out the suitable storage conditions of tricho-cards containing eggs parasitized by *T. bactrae*, an experiment was conducted.

MATERIALS AND METHODS

The culture of *T. bactrae* obtained from Project Directorate of Biological Control was further multiplied in the laboratory. Present studies were conducted in Department of Entomology and Apiculture of the University during 1998-99. Cards containing glued and utraviolet irradiated eggs of *Corcyra cephalonica* were exposed to *T. bactrae* for 24 hours. From these, small strips were cut at one-day interval still seventh day of parasitization. Two such strips were inserted into individual glass vials and placed in B.O.D. incubator maintained at 10 or 15°C. One glass vial was removed at 5 day interval and shifted to the culture room for adult emergence. Adults emerged from parasitized eggs on such strips in 2-3 days. Once the emergence of adults ceased, counts on number of adults emerged and that of eggs with and without parasitoid exit holes were made. The percentage of eggs with exit holes was worked out. Eggs without exit hole were dissected to know the stage of mortality of the parasitoid. Emerged adults were sexed and the number of males and females was counted. As no adult emergence occurred after 7 and 18 days of storage at 10 and 15°C, respectively, another experiment was performed at these temperatures by exposing 3, 5 and 7 day old parasitized eggs to 10°C for 5 days and to 15°C for 5, 10 and 15 days. For each treatment, three replications were maintained. Observations were recorded as mentioned above. Data on percent adult emergence and mortality were analyzed by randomized block design after arcsine-transformation.

RESULTS AND DISCUSSION

In the preliminary screening test, from eggs stored at 10°C after 1-7 days of parasitization, adult emergence was more than 50 per cent when stored for 5 days after 3-7 days of parasitization and the maximum storage period was 7 days (Fig.1). In the culture room maintained at 26±1°C the emergence was 83.8 per cent. However, from eggs kept at 10°C for 5 days after 7 days of parasitization, it was 74.6 per cent and declined to 69.8, 64.3, 69.1and 58.8 per cent when stored after 6, 5, 4 and 3 days of parasitization, respectively. The emergence was

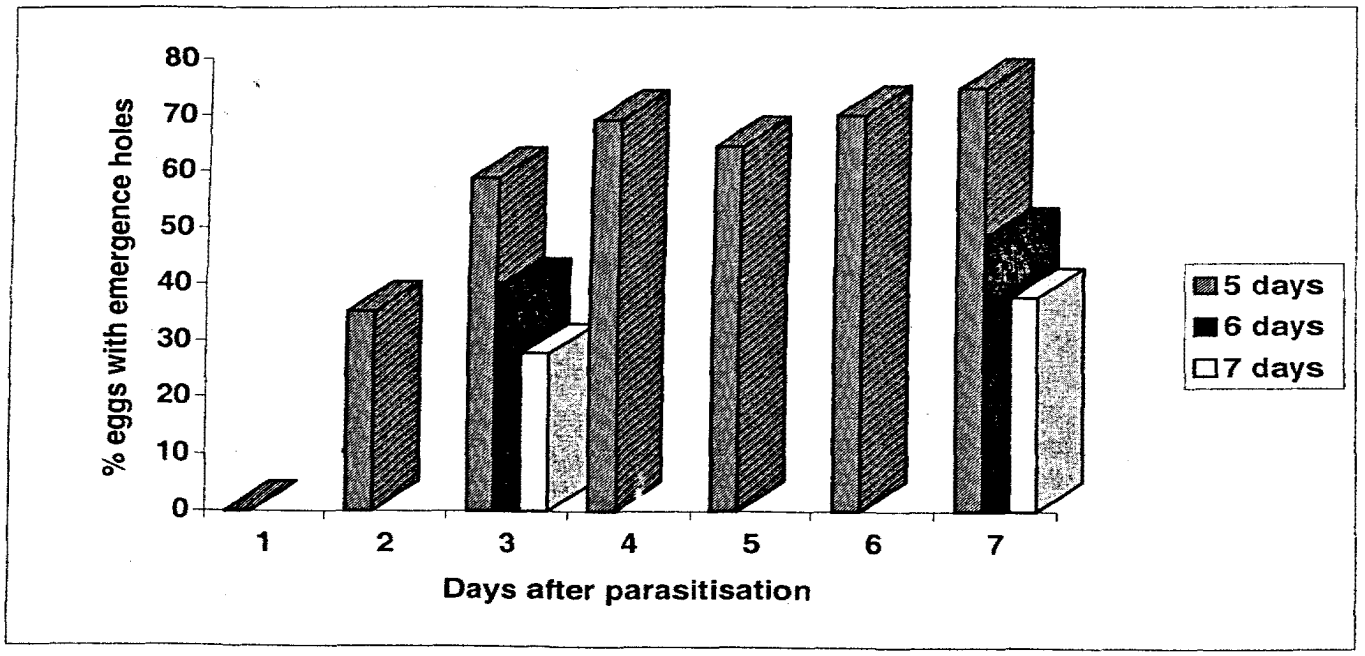

Fig. 1. Emergence of *T. bactrae* from parasitised *C. cephalonica* eggs stored at 10°C for 5, 6 and 7 days after 1-7 days of parasitization

drastically reduced to 35 per cent and zero when these strips were stored after 2 and 1 day of parasitization; in the latter case, there was no blackening of eggs. At 15°C, adult emergence from eggs stored for 10 days after 7 to 1 day of parasitization was comparable with that at 10°C for 5 days and was above 50 percent if stored after 3-7 days of the parasitization. But such eggs stored for 15 days resulted in comparatively low adult emergence, which declined from 55.3 to 33.3 percent when stored after 7 to 1 day of the parasitization

Further, from eggs stored for 20 days at 15°C, no adult emergence took place. Emergence occurred at a low percentage from eggs stored after 3 days of parasitization for 16, 17 and 18 days (34.1, 30.0 and 17.2%, respectively) (Fig. 2). Thus at 15°C eggs can be stored after 2-7 days of parasitization for 10 days and to a maximum of 15 days after 4-7 days of parasitization on basis of criterion of adult emergence from more than 50 per cent eggs. It indicated that suitable stage for storage of eggs is prepupa to pupa, as at 26°C most of eggs held the parasitoid at this stage of development after 3 days of parasitization. Venkataraman and Govil (1952) had reported the storage of *T. chilonis* (quoted as *T. minutum*, but later on corrected as *T. chilonis* by Nagarkatti and Nagaraja, 1968, 1979) in prepupal stage at 12°C for 15 days to be safe and could be protracted to 25 days but at the cost of reduced survival and fecundity of emerged adults. Although lower developmental threshold for *T. bactrae* is 9.54°C (Hutchison *et al.*, 1990), yet in the pupal stage it could tolerate 10°C for 7 days only and at 15°C for a maximum of 18 days. On the other hand

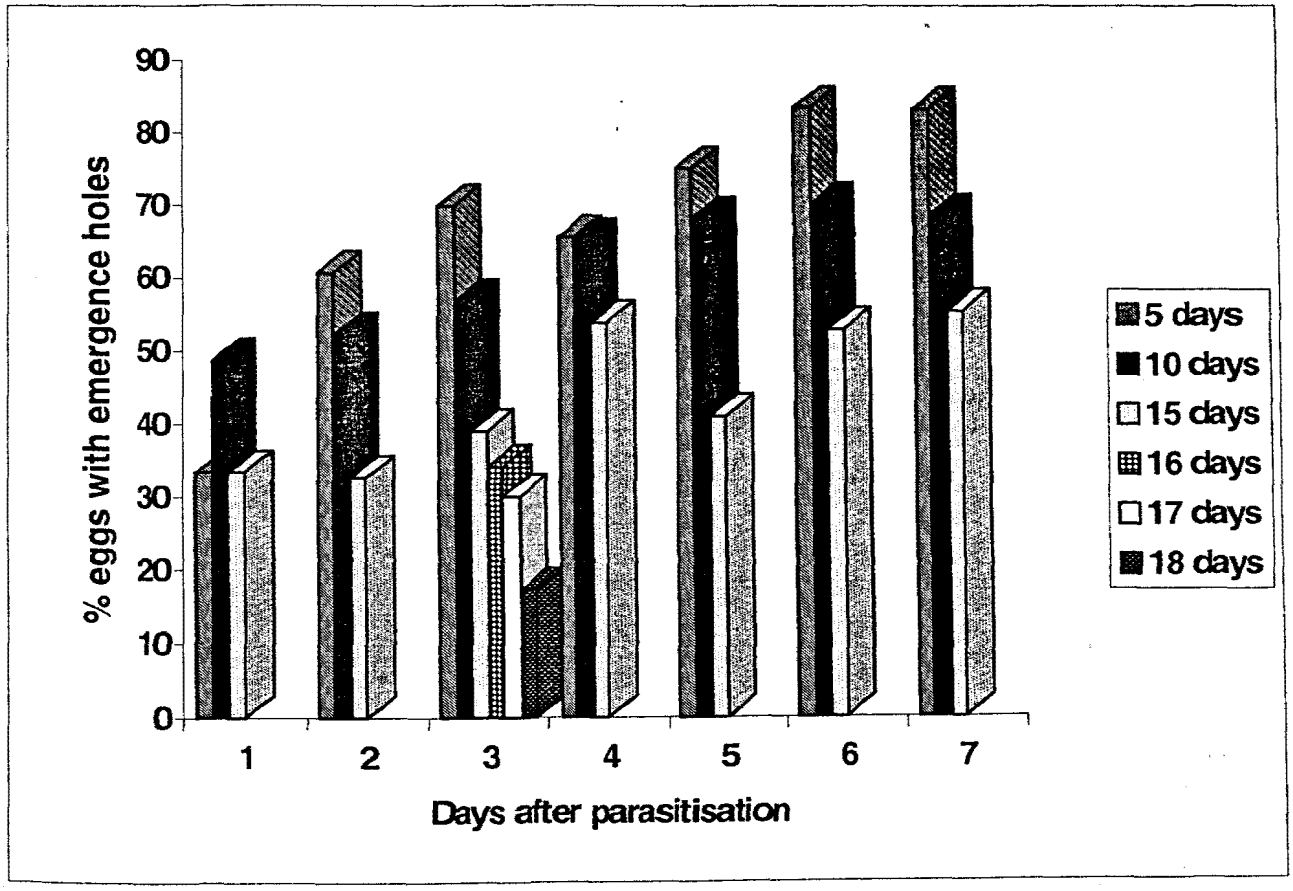

Fig. 2. Emergence of *T. bactrae* from parasitised *C. cephalonica* eggs stored at 15°C for 5, 10, 15, 16, 17 and 18 days of storage after 1–7 days of parasitisation

Trichogrammatoidea eldanae could be stored up to 80 days at 5°C and *Trichogramma brasiliense* up to 50 days at 10°C (Singh and Jalali, 1994).

Results of the second experiment revealed that temperature, duration of cold storage and the period after which parasitized eggs were transferred for cold storage, were significant in F-test and same trend was reflected in their interaction (Table 1). Maximum mean adult emergence (71.3%) occurred after 5 days of storage at 15°C. However, storage of eggs for 10 days at this temperature significantly lowered the percentage of eggs with adult emergence hole to 62.2. This treatment was on par with that of eggs stored at 10°C for 5 days. The least percentage (43.1) was recorded in case of eggs stored at 15°C for 15 days. Irrespective of the storage conditions, eggs stored after 7 and 5 days of parasitization had significantly higher percentage of adult emergence (63.1 and 60.9, respectively) than those stored after 3 days of parasitization (54.2%).

Among the treatment combinations, best treatment proved to be storage of cards at 15°C for 5 days after 5 days of parasitization as in this case highest parasitoid emergence was observed (72.3%). However, this was on par with storage at 15°C for 5 days after 7 days of parasitization (72.1%), at 15°C for 10 days after 5 days of parasitization (69.8%), and at 15°C for 5 days after 3 days of parasitization (69.4%). Occasionally from the eggs stored after 7 days of parasitization, adult emergence occurred during the period of cold storage, usually when storage was for more than 5 days and many such adults were found dead at the time of removal of tricho-cards from the B.O.D. incubator. This gives circumstantial evidence that threshold of development of the parasitoid at the pupal stage is below 15°C. Dead parasitoids were present among some eggs having parasitoid emergence hole that

Table 1. Effect of storage conditions on emergence of *T. bactrae* from parasitized eggs of *C. cephalonica*

Storage temperature and period	Percentage of eggs with adult emergence hole stored after indicated days of parasitization			
	3 days	5 days	7 days	Mean
10°C, 5 days	58.4 (49.84)	61.5 (51.68)	63.0 (52.64)	61.0 (51.38)
15°C, 5 days	69.4 (56.43)	72.3 (58.26)	72.1 (58.37)	71.3 (57.69)
15°C, 10 days	55.1 (47.91)	69.8 (56.55)	61.9 (51.90)	62.2 (52.12)
15°C, 15 days	33.7 (35.43)	40.1 (39.26)	55.5 (48.17)	43.1 (40.94)
Mean percentage	54.2 (47.39)	60.9 (51.44)	63.1 (52.76)	

		CD(P=0.05)
Storage conditions (a)	=	(2.76)
Days after parasitization(b)	=	(2.39)
Interaction (a x b)	=	(4.77)

Figures in parentheses are arcsine-transformed values.

were stored after 3 and 5 days of parasitization under different storage conditions. The percentage of such eggs was low and varied non-significantly from 3.8 to 6.5 for different storage conditions. Storage of eggs after 3 and 5 days of parasitization also did not affect the proportion of eggs with exit hole having dead parasitoid inside, the value being 4.1 and 6.7 per cent, respectively.

Data on stage-specific mortality during post-embryonic development of the parasitoid in parasitized eggs revealed that even among eggs with parasitoid emergence hole, dead larva, pupa or adult parasitoid was present (Table 2). Irrespective of storage conditions, maximum mortality occurred in pupal stage (43.5%), significantly higher than that in adult (17.8%) or larval stage (9.6%). Although data for storage condition and the stage when parasitized eggs were stored did not differ significantly, there is indication of higher mortality in larval stage when eggs were stored after 3 days (16.9%) than after 5 days (2.3%) of parasitization. However, in pupal stage there was no such difference (38.1 and 48.1%, respectively). Relatively higher percentage of adults was dead in eggs with exit hole stored after 5 days (24%) than after 3 days of parasitization (11.7%).

In eggs without exit hole, maximum mortality also occurred in pupal stage (58.8%) followed by adult (27.3%) and larval stage (14.0%), all values being significantly different from one another (Table 3). Significant interaction existed between the stage-specific mortality and days after parasitization before cold storage. There was higher

Table 2. Stage-specific mortality of *T. bactrae* in eggs of *C. cephalonica* having parasitoid emergence hole

Storage temperature and period	Percent mortality during different stages of the parasitoid in eggs of *C. cephalonica* stored after indicated days of parasitization								
	Larva			Pupa			Adult		
	3 days	5 days	Mean	3 days	5 days	Mean	3 days	5 days	Mean
10°C, 5 days	13.3 (13.08)	0.0 (0.00)	6.7 (6.54)	40.0 (33.85)	58.3 (50.00)	49.2 (41.92)	13.3 (13.08)	8.3 (10.00)	10.8 (11.54)
15°C, 5 days	33.3 (35.25)	0.0 (0.00)	16.7 (17.62)	50.00 (45.00)	42.2 (35.18)	46.1 (40.09)	16.7 (19.79)	24.4 (24.83)	20.6 (22.31)
15°C, 10 days	0.0 (0.00)	9.3 (14.53)	4.6 (7.26)	33.3 (30.00)	61.1 (56.29)	47.2 (43.15)	0.0 (0.00)	29.8 (27.74)	14.9 (13.87)
15°C, 15 days	20.8 (21.90)	0.0 (0.00)	10.4 (10.95)	29.2 (27.59)	33.3 (30.00)	31.3 (28.79)	16.7 (15.00)	33.3 (30.00)	25.0 (22.50)
Mean	16.9 (17.56)	2.3 (3.63)		38.1 (34.11)	48.8 (42.87)		11.7 (11.97)	24.0 (23.14)	
Overall mean			9.6 (10.59)			43.5 (38.49)			17.8 (17.55)

CD(P=0.05)

Mortality stage = (13.98)
Rest = NS

larval mortality when eggs were stored after 3 days (21.3%) than after 5 days of parasitization (6.6%). But the trend was reverse for adult mortality, being higher (31.7%) in the treatments stored after 5 days than those stored after 3 days of parasitization (22.8%). However, there was no significant difference in pupal mortality, 55.9 and 61.6 per cent for eggs stored after 3 and 5 days of parasitization, respectively.

The percentage of females emerged from different lots of eggs stored after 3 and 5 days of parasitization at four storage conditions varied from 56.6 to 65.9, the variation being statistically non-significant. The overall percentage of females was 63.3 in this experiment.

On the basis of this experiment storage of parasitized eggs at 15°C for 10 days after 5 days of parasitization would be a right choice. The immature mortality during cold storage was often encountered in pupal stage and this was because of the fact that at the time of storage (after 5 days of parasitization), usually pupal stage was present in such eggs. At 15°C, mortality (in terms of eggs without parasitoid emergence hole) after 10 and 15 days of storage was 38.1 and 45.5 per cent. According to Hutchison *et al.* (1990), mortality in immature stage at 15°C is low (10.6%), but at 25-30°C, it is 28.2-34.6 per cent. Even after storage for 10 days, the mortality was 38.1 percent, which was marginally more than that recorded by Hutchison *et al.* (1990) at 25-30°C. After 15 days of storage of

Table 3. Stage-specific mortality of *T. bactrae* in eggs of *C. cephalonica* without parasitoid emergence hole

Storage conditions (Temperature, period)	Stage of mortality; stored after 3 (a) and 5 (b) days of parasitization								
	Larva			Pupa			Adult		
	(a)	(b)	Mean	(a)	(b)	Mean	(a)	(b)	Mean
10°C, 5 days	25.7 (30.19)	7.0 (15.22)	16.3 (22.71)	56.2 (48.64)	56.9 (49.99)	56.6 (48.81)	18.1 (25.16)	36.1 (36.90)	27.1 (31.03)
15°C, 5 days	19.7 (26.14)	7.5 (13.05)	13.6 (19.59)	54.6 (47.70)	61.2 (51.48)	57.9 (49.59)	25.7 (30.20)	31.3 (33.93)	28.5 (32.06)
15°C, 10 days	20.5 (26.80)	5.1 (7.70)	12.8 (17.25)	55.9 (48.45)	61.6 (51.73)	58.8 (50.09)	23.6 (28.90)	33.2 (35.06)	28.4 (31.98)
15°C, 15 days	19.2 (25.48)	6.9 (1.54)	13.1 (19.01)	57.0 (49.07)	66.7 (54.82)	61.9 (51.95)	23.7 (28.74)	26.3 (30.65)	25.0 (29.69)
Mean	21.3 (27.15)	6.6 (12.13)		55.9 (48.46)	61.6 (51.76)		22.8 (28.25)	31.7 (34.14)	
Overall mean			14.0 (19.64)			58.8 (50.11)			27.3 (31.19)

CD(P=0.05)

Stage of mortality = (3.67)

Interaction between stage and days after parasitization = (5.19)

Non-significant for storage conditions, days after parasitization and other interactions

Figures in parentheses are arcsine-transformed values.

eggs, however, adults failed to emerge from 59.9 per cent eggs. Thus, at the cost of increased mortality parasitized eggs can be stored for 5 more days. On the other hand, *Trichogrammatoidea eldanae* can be stored at 2 and 5°C for less than 14 days and at 10°C for less than 21 days without affecting longevity and fecundity of parasitoid (Jalali and Singh 1992). As compared with *T. eldanae*, *T. bactrae* seems to be more sensitive to temperature (≤ 10°C). Hence, it is concluded that *T. bactrae* in *Corcyra* eggs can be stored up to a maximum of 15 days at 15°C after 5 days of parasitization.

ACKNOWLEDGEMENTS

The authors are grateful to Project Director, Project Directorate of Biological Control, Bangalore, for the supply of the parasitoid.

REFERENCES

Hutchison, W., Moratoria, M. and Martin, J. M. 1990. Morphology and biology of *Trichogrammatoidea bactrae* (Hymenoptera: Trichogrammatidae), imported from Australia as a parasitoid of Pink bollworm (Lepidoptera: Gelechiidae) eggs. *Annals of Entomological society of America*, **83**: 46-54.

Jalali, S. K. and Singh, S. P. 1992. Differential response of four *Trichogramma* species to low temperature for short term storage. *Entomophaga*, **37**: 159-165.

Nagaraja, H. 1978. Studies on *Trichogrammatoidea* (Hymenoptera: Trichogrammatidae). *Oriental Insects*, **12**: 489-523.

Nagarkatti, S. and Nagaraj, H. 1968. Biosystematic studies on *Trichogramma* species: I. Experimental hybridization between *Trichogramma australicum* Girault, *T. evanescens* Westwood and *T. minutum* Riley. *Commonwealth Institute of Biological Control Technical Bulletin* No. 10, C A B, England.

Nagarkatti, S. and Nagaraj, H. 1979. The status of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae). *Oriental Insects*, **13**: 115-118.

Singh, S. P. and Jalali, S. K. 1994. *Trichogrammatids*. Technical Bulletin No. 7, 93 pp. Project Directorate of Biological Control (ICAR), Bangalore.

Supharngkasen, P. 1979. Studies on hymenopterous parasites of *Helicoverpa armigera* (Hb.) eggs in Thailand. *The Journal of Agricultural Science*, **12**: 35-42.

Venkataraman, T. V. and Govil, M. L. 1952. Some effect of low temperature on the biology of *Trichogramma evanescens minutum* Riley, in mass breeding. *Indian Journal of Entomology*, **14**: 215-227.