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Research Article

Effect of cold storage on laboratory performance of *Trichogramma cacoeciae* and *T. embryophagum*

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ABSTRACT: *Trichogramma cacoeciae* Marchal and *T. embryophagum* Hartig were cold stored (6°C) in their pupal stages for 75 days. Biological parameters like per cent emergence, parasitism and longevity of F_1 progeny of both the species were studied at an interval of 5 days and compared with untreated control. Negative correlations for per cent emergence (r= -0.93 and -0.94), parasitism (r= -0.88 and r= -0.83) and longevity (r= -0.89 and r= -0.73) were observed both for *T. cacoeciae* and *T. embryophagum* respectively. Overall per cent emergence, parasitism and longevity of the F_1 progeny of *T. cacoeciae* were found better and statistically significant than *T. embryophagum*. Regression model for each parameter has also been established. Life time parasitism of Corcyra eggs by F_1 progeny declined from 113.2 to 5.6 and 97.0 to 2.6 in *T. cacoeciae* and *T. embryophagum* respectively. More than 50 per cent loss in emergence, parasitism and longevity in the F_1 progeny of *T. cacoeciae* and *T. embryophagum* were observed after 55 and 50 days, 15 and 20 days and 5 days of cold storage (6°C) respectively.

KEY WORDS: Cold storage, F_1 progeny, parasitism, per cent emergence, longevity, *Trichogramma cacoeciae, T. embryophagum.*

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INTRODUCTION

The property of cold tolerance in the immature stages of *Trichogramma* spp.*,* under cold storage condition is of immense advantage in bio control programmes. In addition to increasing the shelf life of stored *Trichogramma*, the practice of cold storage also enables stockpiling of live culture in their embryonic stages either for shipment, inundative releases or storage of surplus *Trichogramma* culture when not required for immediate use. Since different species of *Trichogramma* vary differently in their responses to the storage temperature as well as period of cold storage (Voegele *et al*., 1988), laboratory evaluation of individual species, for such treatments, therefore becomes mandatory. Documented works regarding well adapted cold tolerant species like *T. acacioi* Brun, Moraes and Soares and *T. rojasi* Nagaraja and Nagarkatti, but simultaneously cold susceptible *T. atopovirilia* Oatman and Platner and *T. dendrolimi* Matsumara also (Hu *et al*., 2005; Foerster and Foerster, 2009) indicate the significance of laboratory assessment of *Trichogramma* spp., for their cold tolerance. Effects of cold storage at 2- 10°C on important biological aspects of different *Trichogramma* spp. have been observed by a number of workers. Some workers have reported the impact of short term storage (5- 25 days) at 8-10°C, without any deleterious effect (Ayvaz *et al*., 2008; Aydin *et al*., 2009; Rodrigues and Sampaio, 2011), whereas long term cold storage (45-100 days) at 2-6°C have been observed by many workers with negative effects on F_1 progeny (Jalali and Singh, 1992; Ahmad *et al*., 2011; Lessard and Boivin, 2013). Techniques for improvement in long term cold storage involving less detrimental effects on F_1 progeny have also been evolved (Lessard and Boivin, 2013; Gardner *et al*., 2012) which are of much significance in applied field of bio control.

In view of reported potential and field results of *T. cacoeciae* Marchal and *T. embryophagum* Hartig especially against codling moth *Cydia pomonella* (Lep., Tortricidae), (Hassan *et al*., 1988; Hassan, 1989; Pawar *et al*., 1980; Botto and Glaz, 2010) and difficulties involved in maintaining the culture of these *Trichogramma* spp. during the harsh and prolonged winter conditions in the Kashmir valley and in order to ensure their uninterrupted supply for inundative releases, the need of present study was realized. Additionally, as no reports are so far available regarding use of eggs of *Corcyra cephalonica(Stainton)* for long term cold storage of *T. cacoeciae* and *T. embryophagum*, the present work therefore is an original contribution.

MATERIALS AND METHODS

The present study was carried out during 2009-10 in the Bio control laboratory of Entomology Division of the

Sher-e-Kashmir University of Agricultural Sciences and Technology- Kashmir, Srinagar (Jammu and Kashmir), India. Cultures of *Trichogramma cacoeciae* Marchal and *T. embryophagum* Hartig were obtained from the National Bureau of Agricultural Insect Resources (ICAR- NBAIR), Bangalore, India, and cultured on the eggs of factitious host, *C. cephalonica* in BOD (27± 1°C, 65± 5 % Relative Humidity, 16 L : 8D photoperiod). Separate Tricho cards of *T. cacoeciae* and *T. embryophagum* in their pupal stages, were prepared to observe the effects of cold storage. For this, about one cc (approx. 22,000 numbers) of freshly laid and UV sterilized eggs of C. *cephalonica* were sprinkled on paper cards (15 x 15cm.) with diluted thin film of gum Arabica, and dried. Each card was exposed to parasitism separately, by about 500 freshly emerged *T. cacoeciae* and *T. embryophagum*, for 24 hours, inside a sealing plastic envelop (25 x 20 cm.). Diluted honey (50 %) was used as food for the parasitoids. After 24 hours of parasitism, the parental *Trichogramma* were killed and discarded, by exposing them to instant cooling at 6°C for 10 minutes. The envelopes with parasitized eggs were then kept in BOD for four days, till the developing embryos progressed into pupal stage, as indicated by darkening of the parasitized eggs. Such prepared pupal stock of *T. cacoeciae* and *T. embryophagum* were then stored in refrigerator, maintained at 6°C, 65± 5 % relative humidity and under complete darkness, for 75 days for further studies.

In order to investigate the effect of cold storage of F_1 progeny of above mentioned *Trichogramma* spp. five strips (2.0 x 1.5 cm.) of parasitized eggs from the pupal stock kept under cold storage, were taken out every fifth day, i.e. from 5- 75th day, cut with the help of scissor, and each strip, holding about 300 parasitized eggs, was transferred in individual glass test tube (12.5 x 2.0 cm.) plugged with cotton, and kept in BOD (27± 1°C, 65± 5 % relative humidity, 16 L : 8D photoperiod) for the emergence of F_1 progeny.

Percent emergence

The number of emerged F_1 progeny at 27± 1°C in each of the above mentioned five replications was counted under stereoscopic binocular, after killing them under sub zero temperature. Per cent emergence of F_1 progeny was determined by dividing the number of emerged parasitoids from the total number of parasitized black eggs (except shriveled or damaged eggs) of a replication. Mean of all the five rep lications represented mean per cent emergence of F_1 progeny of a *Trichogramma* sp. for a given period of cold storage.

Parasitism

Data on parasitism was based on the observations of twenty five F_1 females. For this, a total of twenty five freshly emerged F_1 females were randomly isolated while recording data on per cent emergence. In order to determine average parasitism, both day wise and life time parasitism, five F_1 females, treated as one replication were transferred in a glass test tube (15 x 2.5 cm), clogged with muslin mounted cotton plug, and supplied about 400 UV sterilized fresh eggs of *C. cephalonica* held on a paper card (4.0 x 1.5cm.), daily for parasitism. The parasitized egg cards were replaced by fresh egg cards after every 24 hours, till the death of all the parasitizing individuals in a replication. A thin streak of pure honey at the back of each card was provided as food to the parasitizing females. The parasitized cards were kept separately in glass test tubes for 4-5 days and counted for the number of eggs parasitized. Mean of day wise parasitism for each replication was separately recorded and finally summed up to determine life time fecundity based on adult progeny produced, under each cold storage treatment, for both the species. The experiment was replicated five times to record mean daily as well as life time fecundity.

Longevity

Data on longevity of F_1 progeny, emerged from each treatment of 5-75 days of cold storage, was based on the average survival ability of twenty five parasitizing females. This was duly recorded while observing the daily parasitism of the two *Trichogramma spp*. Each replication was observed 6- hourly to record the survival ability of the F_1 females.

Experiments for untreated control of both *T. cacoeciae* and *T. embryophagum* were also done in BOD (27± 1°C, 65± 5 % relative humidity, 16 L : 8D photoperiod) for the purpose of comparison of data.

Statistical analysis

Data was analyzed using Minitab version 15. C.D. values of percent emergence were based on arc sin transformation whereas those for parasitism and longevity were based on $\sqrt{n+0.5}$ and $\sqrt{n}$ respectively. Student's t- test was used for the comparison of data of the two species. Data of each parameter was regressed against period of cooling. The values of 'Y' for studied parameter was based on linear model $Y= a+bx$. Where 'a' and 'b' represented constant values for emergence, parasitism and longevity whereas 'x' denoted the period of cooling. One way ANOVA was used to obtain F values as well as to understand the effect of interactions between important parameters.

RESULTS AND DISCUSSION

Our findings showed a gradual decline in the emergence of F_1 progeny of both *Trichogramma cacoeciae* and

T. embryophagum, as a result of 5-75 days of cold storage (6°C) (Table 1). Statistically significant decline in the emergences of both, *T. cacoeciae* (one way ANOVA: $F_{15, 60}$ = 75.24, p= 0.001) and *T. embryophagum* (one way ANOVA: $F_{15, 60}$ = 98.38, p= 0.001) were obtained, as a result of above mentioned period of cold storage. The two species differed significantly in terms of per cent emergence when compared with Student's t- test (t= 2.46*; p=0.015; d.f.=148). Interaction between the *Trichogramma* spp. vs. period of storage was also found significant (one way ANOVA: $F_{15, 60}$ = 2.88, p= 0.002). Data on per cent emergence when regressed with period of cold storage, the models Y= a+bx were obtained best fit, both for *T. cacoeciae* (Y= 110 - 0.929x; R2= 85.6; r= -0.93, d.f. = 78) and *T. embryophagum* (Y=112 – 1.26x; R2= 87.6; r= -0.94, d.f.= 78). Loss of per cent emergence in F_1 progeny of both *T. cacoeciae* and *T. embryophagum* after 25, 50 and 75 days of cold storage was 7.3, 36.3, 68.8 and 10.3, 44.7, 92.9 respectively. More than 50.0 per cent decline in the emergence of F_1 progeny in above mentioned species was recorded when duration of cold storage was beyond 55 and 50 days respectively. *T. cacoeciae* however , indicated 5.5 times more emergence than *T. embryophagum* after 75 days of cold storage at 6°C.

Decline in per cent emergence of F_1 progeny, as a result of cold storage, in different *Trichogramma* spp. has been documented by a number of workers (Jalali and Singh, 1992; Pitcher *et al*., 2002; Ozder, 2004). Our observations on negative correlation for per cent emergence vs. period of cold storage at 6°C, for *T. cacoeciae* (r= -0.93**; d.f= 78) and *T. embryophagum*(r= -0.94**; d.f.= 78) get support from Hany *et al* ,(2010). Decline in per cent emergence of F_1 progeny is mainly attributable to cold induced mortality of stored pupae of *Trichogramma* spp. and also due to adverse physical and physiological changes, both in the eggs of *Corcyra cephalonica* as well as diapaused pupae of *Trichogramma* spp. Further, sudden exposure of pupae of *Trichogramma* to low temperature (6°C), without prior acclimatization of maternal generation to low temperature, in addition to use of non diapausing host eggs, as in present case, might have added increased mortality of stored pupae, which resulted in sharp decline in per cent emergence of F_1 progeny in both the species. Failure of emergence of *T. chilonis* (Ishii) at 6°C beyond 25 days is also reported (Nadeem *et al*., 2010). 30 days of prior acclimation (10°C) of maternal generation of *T.* brassicae (Bezdenko) has been documented as favorable for per cent emergence of F_1 progeny after cold storage (Lessard and Boivin, 2013). Laing and Corrigan, (1995) reported 50% emergence in F_1 progeny of *T.* minutum Riley after long term cold storage (300 days), when diapausing host eggs were used for parasitism. Our findings on more than 50% emergence in F_1 progeny of *T. cacoeciae* and *T. embryophagum*, after 55 and 50 days of cold storage respectively, however indicated, feasibility of cold storage of these species at 6°C for future use.

Parasitism

A general decline in both life time (Table 1) as well daily fecundity, as indicated by mean of first two days' parasitism (Fig. 1), in F_1 progenies of *T. cacoeciae* and *T. embryophagum* (N= 25), developed as a result of cold storage, was observed. Statistically significant differences in parasitism by *T. cacoeciae* (one way ANOVA: $F_{15, 60}$ = 168.26, p= 0.001) and *T. embryophagum* (one way ANOVA: $F_{15, 60}$ = 66.02, p= 0.001) were obtained. Overall rate of parasitism however, by both the species was found statistically similar, when compared through Student's t- test (t= 1.8 ns; d.f.= 157; p= 0.07) as was also obvious by the interactions between species vs. period (one way ANOVA: $F_{15, 120}$ = 1.08, p= 0.0378). However, regression between life time parasitism and period of cold storage yielded comparatively better model Y= a+bx in case of *T. cacoeciae* (Y= 88.7 - 1.37x; R2= 76.7; r= -0.88, d.f= 78) than *T. embryophagum* (Y= 69.7 - 1.099x; R^2= 68.1; r= -0.83, d.f= 78). More than 50.0 per cent loss in parasitizing performance in case of both the species was observed when stored (6°C) beyond 15 days. Average decline in parasitism in *T. cacoeciae* and *T. embryophagum* at 25, 50 and 75 days of cold storage was recorded as 72.9, 86.9, 95.0 and 78.3, 89.4, 97.5 per cent respectively. Per cent parasitism of *T. cacoeciae* however after 75 days of cold storage was a little more than twice that of *T. embryophagum*.

Decline in parasitism in F_1 progeny as a result of cold storage, has been documented by a number of workers for different *Trichogramma* spp. In addition to various factors, varying level of cold tolerance in different *Trichogramma* spp. may be one of the reasons. Jalali and Singh, (1992) observed rate of parasitism of *T. chilonis* better than *T. acheae* and *T. japonicum* Ashmead, when cold stored at 10 °C for 49 days. Foerster and Foerster, 2009 found *T. acacoi* and *T. rojasi* more cold tolerant than *T. atopovirilia*. Combination of low temperature and period of storage is also considered as an important factor affecting fecundity of F_1 progeny in many *Trichogramma* spp. (Colinet and Boivin, 2011). Whereas reports of short term cold storage without any detrimental effect on fecundity in species such as *T. ostriniae* Pang and Chen (4 weeks at 9°C, Pitcher *et al*., 2002), *T. carverae* Oatman and Pinto (2 weeks at 10°C, Rundle *et al*., 2004), *T. evanescens* Westwood (3 weeks at 4°C, Ayvaz *et al*., 2008) and *T. pretiosum* Riley (3 weeks at 5°C, Rodrigues and Sampaio, 2011) are available, long term cold storage on the other hand are reported to affect adversely on

the fecundity of *T. ostriniae* (8 weeks at 9°C, Pitcher *et al*., 2002), *T. cacoeciae* (> 4 weeks at 0°C, Ozder, 2004) and *T. evanescens* (8 weeks at 4°C, Hany *et al*., 2010). Qualitative decline in F_1 progeny in the present case is inferred to have arisen due to a number of physical and physiological reasons. Emergence of physically deformed and dwarfed members in the progeny, coupled with their slow to absolute loss of mobility was one of the reasons which might have accounted quantitative loss in parasitism. Loss of mobility, also in non deformed adults of *T. nerudai* Pintureau and Gerding as a result of > 50 days of cold storage at 4±1°C, has also been reported (Tezze and Botto, 2004). Decline in parasitism in present species, beyond 15 days of cold storage, was clearly indicative of the weakening effect of prolonged cold storage on F_1 progeny at 6°C. This might have been due to cold injuries at immature stages, depletion of fat reserve and also retarded somatic maintenance of the embryo for prolonged period of cold storage (Rivero and West, 2002; Chen *et al*., 2008a; Chen *et al*., 2008b). Many workers (Colinet and Boivin, 2011; Denlinger and Lee, 1998; Boivin, 2010) have also indicated damaging effects of low temperature on reproductive organs and fecundity in *Trichogramma*, because of lack of metabolic maintenance at immature stage, at the cost of fat reserve, during prolonged cold storage. Decline in enzymatic activity and associated irreversible physiological changes at molecular level, at low temperature, are also documented to affect adversely parasitism in *Trichogramma* and other parasitoids (Denlinger and Lee, 1998).

Longevity

In comparison to average longevity of females of *T. cacoeciae* (9.6 days) and *T. embryophagum* (11.45 days) in untreated control condition, significant decline in the longevity of F_1 progeny of both *T. cacoeciae* (one way ANOVA: $F_{15, 60} = 98.09$, p= 0.001) and *T. embryophagum* (one way ANOVA: $F_{15, 60} = 54.49$, p= 0.001) was recorded, as a result of cold storage. Average longevity of *T. cacoeciae* in case of 5- 75 days of cold storage was found comparatively more than that of *T. embryophagum*, the difference being statistically significant when compared with Student's t- test (t= 2.33* p= 0.021; d.f. = 156) as also obvious by the interactions between species vs. period of cold storage (one way ANOVA: $F_{15, 120} = 2.97$, p= 0.001). Regression between longevity and period of cold storage indicated better model Y= a+bx for *T.* cacoeciae (Y= 7.15- 0.096 x: R^2= 80.5; r= -0.89**; d.f.= 78) than *T. embryophagum* (Y= 5.83 - 0.086 x: R^2= 53.3; r= - 0.73**; d.f.= 78). More than 50.0 per cent loss in longevity in F_1 progeny of *T. cacoeciae* and *T. embryophagum* was recorded after 20 and 5 days of cold storage (6°C) respectively. Per cent decline in longevity at 25, 50 and 75 days of cold storage in F_1 progeny of *T. cacoeciae* and *T. embryophagum* was recorded as 66.6, 80.7, 90.6 and 82.5, 89.4, 94.9 respectively. Longevity of *T. cacoeciae* was observed to be 1.5 times greater than that of *T. embryophagum*, after 75 days of cold storage.

Observed decline in longevity in F_1 progeny is attributable to cold induced poor health due to insufficient food reserve during their immature stage, as also indicated by early workers (Boivin, 2010). Negative effects of cold storage (< 10°C) beyond three weeks or longer on the longevity of *T. carverae, T. nr. brassicae* and *T. funiculatum* Carver are also documented (Rundle *et al*., 2004). Decline in longevity in F_1 progeny of different *Trichogramma* spp. as a result of cold storage of immature pupae has also been reported by a number of workers (Ayvaz *et al*., 2008; Jalali and Singh, 1992; Ozder, 2008). Significant drop in longevity in present case, might have been due to susceptibility of immature forms to sudden exposure to low temperature, without prior acclimatization. Lessard and Boivin (2013) observed comparatively reduced mortality in F_1 progeny of *T. brassicae*, as a result of prior exposure of maternal generation to acclimatization of 30 days at 10°C than those without an acclimatization period, when the immature stages were stored directly at 5°C. Many workers ascribed strain variation as an important factor deciding longevity, in different *Trichogramma* spp. (Bigler *et al*., 1993; Dutton and Bigler,1996). Leopold (1998) described chilling injury at temperatures well above freezing as common observation in parasitoids, after cold storage. Nedveˇd *et al*. (1998) correlated severity of chilling injury with the drop in storage temperature or with the increase in length of exposure to cooling. According to Chen *et al*. (2008) indirect chilling injury is caused by prolonged exposure to moderately low temperatures. In our study too, indirect chilling injury might have been one of the reasons, which became progressively more lethal, with the increase in the period of cold storage.

Despite adverse effects of cold storage on studied biological parameters of F_1 progeny of both *T. cacoeciae* and *T. embryophagum*, manifestation of cold tolerance (to 6°C) nevertheless, in the immature stages of these species proved to be an important factor in their amenability to long term cold storage, in the eggs of *C. cephalonica*. The results of the study are thus useful to plan on a strategy for maintaining the culture during prolonged harsh winter conditions in Kashmir, for subsequent use. Although the present study revealed sufficient emergence (> 87.0 per cent) of stored progeny until 30 days, but for field use only 15 days' stored *Trichogramma* is recommended against codling moth, *Cydia pomonella* in Laddakh, in view of sharply declining fecundity of the studied parasitoids, beyond this period. However, for the purpose of maintaining laboratory culture and their mass multiplication during winter, progenies devel

oped even up to 30 days' of cold storage (6°C) can be used.

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Table 1. Effect of cold storage (6°C) on per cent emergence, parasitism and longevity of *Trichogramma cacoeciae* and *Trichogramma embryophagum*

Period of cold storage	*T. cacoeciae*			*T. embryophagum*		
	% Emergence	Life time fecundity	Longevity (in days)	% Emergence	Life time fecundity	Longevity (in days)
Normal	99.3 (85.3)[a]	113.2 (10.66)[a]	9.6 (3.09)[a]	98.32 (82.8)[a]	97.0 (9.85)[a]	11.45 (3.4)[a]
5 day	97.8 (81.5)[a]	102.6 (10.14)[a]	7.4 (2.71)[b]	94.3 (76.9)[a]	77.8 (8.84)[a]	5.2 (2.25)[b]
10 day	95.9 (78.5)[a]	87.4 (9.36)[ab]	7.0 (2.64)[b]	93.4 (75.3)[a]	74.2 (8.52)[a]	4.6 (2.13)[b]
15 day	93.6 (75.5)[ab]	78.0 (8.85)[ab]	5.2 (2.3)[c]	91.8 (73.6)[ab]	58.4 (7.64)[ab]	3.1 (1.76)[c]
20 day	92.6 (74.3)[ab]	50.4 (7.13)[b]	4.2 (2.04)[d]	89.1 (71.1)[ab]	30.6 (5.54)[b]	2.6 (1.6)[c]
25 day	92.0 (73.7)[ab]	30.6 (5.55)[c]	3.2 (1.78)[e]	88.2 (69.9)[ab]	21.6 (4.67)[b]	2.0 (1.41)[cd]
30 day	91.7 (73.3)[ab]	21.4 (4.66)[d]	3.2 (1.78)[e]	87.4 (69.5)[ab]	15.4 (3.95)[bc]	2.0 (1.41)[cd]
35 day	82.4 (65.5)[b]	19.6 (4.47)[d]	3.0 (1.73)[e]	78.4 (62.4)[b]	14.4 (3.83)[bc]	1.76 (1.28)[cd]
40 day	82.1 (66.2)[b]	18.2 (4.29)[d]	2.6 (1.6)[e]	74.1 (59.5)[b]	13.6 (3.74)[bc]	1.6 (1.25)[cd]
45 day	81.1 (64.3)[b]	15.4 (3.87)[de]	2.0 (1.41)[ef]	66.4 (54.6)[b]	11.0 (3.31)[bc]	1.3 (1.13)[cd]
50 day	63.2 (52.7)[c]	14.8 (3.85)[de]	2.05 (1.42)[ef]	54.3 (47.5)[bc]	10.2 (3.15)[bc]	1.2 (1.09)[d]
55 day	60.4 (51.1)[c]	13.6 (3.74)[de]	1.85 (1.35)[ef]	34.6 (35.8)[c]	9.8 (3.19)[bc]	1.1 (1.05)[d]
60 day	47.0 (43.2)[d]	12.4 (3.57)[de]	1.7 (1.3)[ef]	31.0 (33.8)[c]	9.2 (3.1)[bc]	1.01 (0.99)[d]
65 day	44.1 (41.6)[d]	10.8 (3.36)[de]	1.56 (1.2)[ef]	25.1 (30.0)[c]	8.8 (3.04)[bc]	1.0 (0.97)[d]
70 day	40.5 (39.5)[d]	8.4 (2.89)[e]	1.4 (1.2)[ef]	17.5 (23.4)[cd]	8.2 (2.93)[bc]	0.91 (0.95)[d]
75 day	31.0 (33.7)[de]	5.6 (2.46)[e]	1.0 (1.0)[f]	6.9 (14.8)[d]	2.6 (1.6)[c]	0.58 (0.76)[d]
CD (p= 0.01)	(6.44)	(0.71)	(0.21)	(7. 31)	(1.04)	(0.3)
SEM	(1.85)	(0.3)	(0.06)	(2.38)	(0.28)	(0.07)

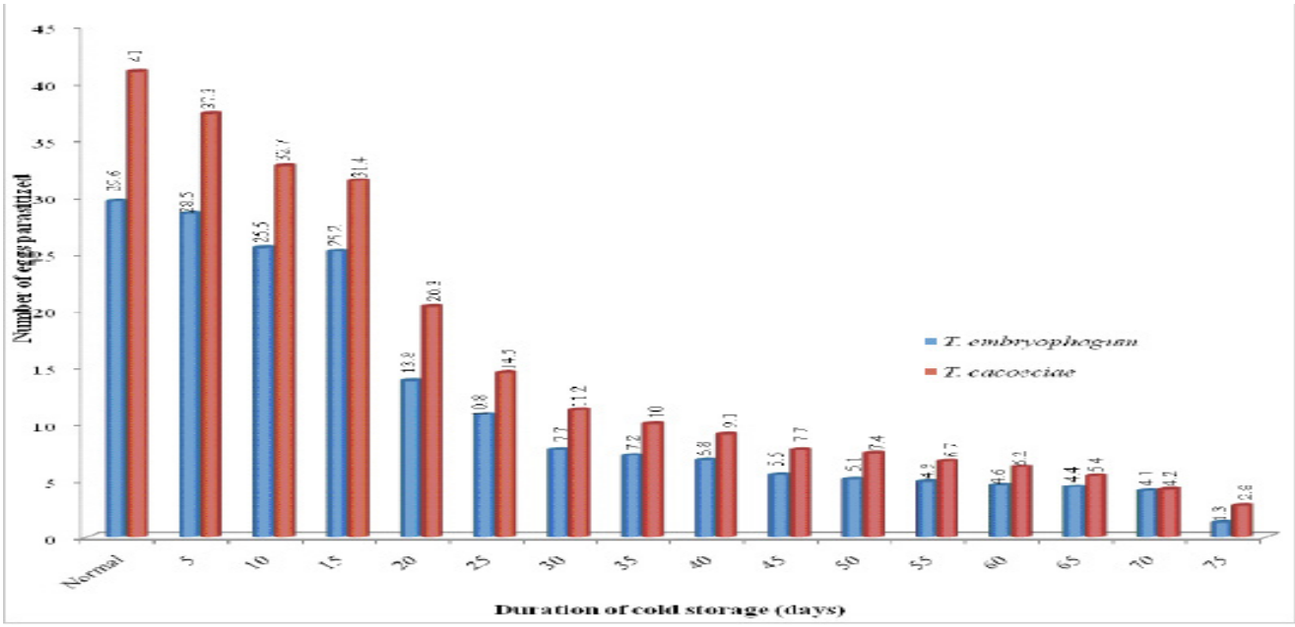

Fig. 1. Effect of Cold Storage on Average fecundity of *T.embryophagum* and *T.cacoeciae* on first two days.

REFERENCES

Ahmad MJ, Ahmed SB, Yaqub M. 2011. Parasitism potential and low temperature response of *Trichogramma kashmirica* Nagaraja, Ahmad and Gupta (Hymenoptera: Trichogrammatidae). *J Biol Cont.* **25**: 143–145.

Aydin ST, Canpolat U, Sumer F. 2009. Suitability of irradiated and cold-stored eggs of *Ephestia kuehniella* (Pyralidae: Lepidoptera) and *Sitotroga cerealella* (Lepidoptera : Gelechiidae). *Biocontrol Sci Technol.* **19**: 127–138.

Ayvaz A, Karasu E, Karabörklü S, Aydln ŞT. 2008. Effects of cold storage, rearing temperature, parasitoid age and irradiation on the performance of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). *J Stor Prod Res*. **44**: 232–244.

Bigler F, Bosshart S, Frei G, Cerutti F. 1993. Product control of *Trichogramma brassicae* in Switzerland. Proceedings of the 7th workshop of the global IOBC working group on the *Quality control of mass reared arthropods*. Rimini, 13-16 September, 1993.

Boivin G. 2010. Reproduction and immature development of egg parasitoids, In: *Egg Parasitoids in Agro ecosystems with Emphasis on Trichogramma* (Consoli FL, Parra JRP and Zucchi RA, eds) (Springer, London, New York, pp. 1–23.

Botto E, Glaz P. 2010. Potential for controlling codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) in Argentina using the sterile insect technique and egg parasitoids. *J App Entomol*. **134**: 251–260.

Chen W, Leopold RA, Harris MO. 2008a. Cold storage effect on maternal and progeny quality of *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). *Biol Cont*. 46: 122– 132.

Chen WL, Leopold RA, Boetel MA. 2008b. Cold storage of adult *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae) and effects on maternal and progeny fitness, *J Econ Entomol*. **101**: 1760–1770.

Colinet H, Boivin G. 2011. Insect parasitoids cold storage: A comprehensive review of factors of variability and consequences. *Biol Cont*. **58**: 83–95.

Denlinger DL, Lee RE Jr. 1998. Physiology of cold sensitivity, In: Hallman GJ and Gardner J, Hoffmann MP, Pitcher SA, Nyrop JP (2012) Recurrent warming to improve cold storage of Trichogrammatids (Hymenoptera: Trichogrammatidae). *Biocontrol Sci Technol.* **22**: 261–270.

Dutton A, Bigler CF. 1996. Quality and environmental factors affecting *Trichogramma brassicae* efficiency under field conditions. *Entomol Exp Applt*. **81**: 71–79.

Foerster MR, Foerster LA. 2009. Effects of temperature on the immature development and emergence of five species of *Trichogramma. Biol Cont*. **54**: 445–450.

Gardner J, Hoffmann MP, Pitcher SA, Nyrop JP. 2012. Recurrent warming to improve cold storage of Trichogrammatids (Hymenoptera: Trichogrammatidae). *Biocontrol Sci Technol*. **22**: 261–270.

Geng Jin Hu, Shen Zuo Rui, Li Zheng Xi, Zhang Fan. 2005. Optimal stage and temperature for cold storage of T*richogramma dendrolimi* reared on *Antheraea pernyi* eggs. *Acta Entomol Sin*. **486**: 903–909.

Hany ASG, Ateef MMS, Ahmed AS. 2010. Impact of cold storage temperature and period on performance of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). *Aus Basic & Appl Sci*. **4**: 2188–2195.

Hassan SA, Kohler E, Rost WM. 1988. Mass production and utilization of Trichogramma: 10. control of the codling moth, *Cydia pomonella* and *Adoxophyes orana* Lepidoptera: Tortricidae), *Entomophaga* **33**: 413–420.

Hassan, SA. 1989. Selection of suitable *Trichogramma* strains to control the codling moth, *Cydia pomonella* and the summer fruit tortrix moth, *Adoxophyes orana, Pandemis heparana* (Lep., Tortricidae). *Entomophaga*. **34**: 19–27.

Jalali SK, Singh SP. 1992. Differential response of four *Trichogramma* species to low temperature for short term storage. *Biocontrol*. **37**: 159–165.

Laing, JE, Corrigan, JE. 1995. Diapause induction and post-diapause emergence in *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae): The role of host species, temperature, and photoperiod. *Can Entomol*. **127**: 103–110.

Leopold RA. 1998. Cold storage of insects for integrated pest management. Pp 235-267. In: Hallman GJ, Denlinger Dl (Eds). *Temperature sensitivity in insects and application in integrated pest management*. West view Press, Boulder, CO.

Lessard E, Boivin G. 2013. Effect of low temperature on emergence, fecundity, longevity and host-feeding by *Trichogramma brassicae*. *BioControl*. **58**: 319–329.

Nadeem S, Ashfaq M, Hamed M, Ahmed S. 2010. Optimization of short and long term storage duration for *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) at low temperatures. *Pak J Zool*. **42**: 63–67.

Nedved O, Lavy D, Verhoef HA. 1998. Modeling the time-temperature relationship in cold injury and effect of high-temperature interruptions on survival in a chill-sensitive collembolan. *Funct Ecol*. **12**: 816–824.

Ozder N. 2008. Effect of cold storage of adult *Trichogramma brassicae, T. cacoeciae* and *T. evanescens* (Hym: Trichogrammatidae). *Arch Phytopath Plant Prot*. **41**: 296–299.

Ozder N. 2004. Effect of different cold storage periods on parasitization performance of *Trichogramma cacoeciae* (Hymenoptera, Trichogrammatidae) on eggs of Ephestia kuehniella (Lepidoptera, Pyralidae). *Biocontrol Sci Technol*. **14**: 441–447.

Pawar AD, Tuhan NC, Balasubramaniam S, Parry M. 1980. Biological control of codling moth in Laddakh. Indian *J Plant Prot*. **8**: 189–191.

Pitcher SA, Hoffmann MP, Gardner J, Wright MG and Kuhar TP. 2002. Cold storage of *Trichogramma ostriniae* reared on *Sitotroga cerealella* eggs. *BioControl* **47**: 525– 535.

Rivero A, West SA. 2002. The physiological costs of being small in a parasitic wasp, *Evol Ecol Res*. **4**: 407–420.

Rodrigues SMM, Sampaio MV. 2011. Cold storage of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). *Biol Sci*. **78**: 45–51.

Rundle JB, Thomson LJ, Hoffmann AA. 2004. Effects of cold storage on field and laboratory performance of *Trichogramma carverae* (Hymenoptera: Trichogrammatidae) and the response of three *Trichogramma* spp. (*T. carverae, T. nr. brassicae, and T. funiculatum*) to cold. *J Econ Ent*. **97**: 213–221.

Tezze AA, Botto EN. 2004. Effect of cold storage on the quality of *Trichogramma nerudai* (Hymenoptera: Trichogrammatidae), *BioControl.* **30**: 11–16.

Voegele J, Pizzol J, Babi A. 1988. The overwintering of some *Trichogramma* species. pp 275-282. In *Trichogramma and other egg parasitoids*. Les Colloques dÍNRA, Paris, San Antonio, USA.