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Effect of Cold Temperature Durations on the Emergence and Parasitization Efficiency of Laboratory Reared *Trichogramma chilonis* (Ishii)

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A B S T R A C T

The result over fitness life parameters of *Trichogramma* revealed that the percentage emergence of *Trichogramma* recorded after 5 days of storage at different temperature revealed that 10^{0}C gave significantly highest emergence of parasitoid (96.20%) and was similar to control (97.4%) however; other temperatures, 12, 14 and 16^{0}C showed 90.90%, 89.43% and 88.33% emergence of *Trichogramma*, respectively. At 10^{0}C, the highest parasitism percentage (96.00%) was observed and further decreased to 53.66% at 10^{0}C when stored for up to 30 days whereas, the lowest parasitism percentage assessed at 16 (89.83%) and 6^{0}C (90.03%). The maximum adult longevity measured, while it was stored at 6^{0}C (4.50 days), whereas shortest adult longevity noted at 16^{0}C (3.00 days). Further, adult longevity was decreased from 4.26 to 0.00 days when stored at 10°C from 5 to 50 days. At 6^{o}C, *Trichogramma* gave considerable emergence (76.13-20.63%) and parasitization (91.46-42.83%) from 5 to 50 days storage, whereas at 12, 14 and 16^{o}C, development was completed during storage. It was evident that storage at 10^{o}C and 6^{o}C were very conducive for life parameters of *Trichogramma* to get short term and long storage, respectively.

Introduction

Trichogramma species are widely used egg parasitoids for biological control of insect pests of different crops through augmentation and release. *T. chilonis* and *T. achaeae* have been promoted to use in controlling cabbage leaf eating caterpillars (Krishnamoorthy, 2012). Among the *Trichogramma* species the egg parasitoid, *T. chilonis* is the dominant species in India (Nagarkatti and Nagaraja, 1979). It is a very aggressive parasitoid and has the ability to increase their capability, sometimes gives near to 100% parasitism depending upon the availability of favorable condition. It is a natural enemy of many harmful lepidopterous insect pests of crops and vegetables (Jalali and Singh, 1993). *Trichogramma* has been used against the lepidopterous pests of cotton, cabbage, apple and tomato (Smith, 1996). They parasitize the eggs of more than 400 lepidopteran pest species (Khan *et al.*, 2004 and Doyon and Boivin, 2005).

Mass rearing of bioagents is a prerequisite of biocontrol programme; this needs a regular and sufficient production of easily culturable factitious insect hosts for mass culturing of any bioagent (Wadaskar *et al.*, 2015).*Trichogramma* species is one of the most widely used biological control agent due to its easy rearing in insectaries and vigorous parasitism on eggs of target hosts. Thus, good quality egg parasitoid, *T. chilonis* could be utilized through inundative release for the management of many lepidopterous insect pest (Bhushan *et al.,* 2012 and Fand *et al.,* 2013).

Cold storage technique must ensure the availability of sufficient numbers (Tezze and Botto, 2004) and quality of egg parasitoids (Bigler, 1994) at the time of release. Therefore, the development of storage techniques for bio-control agents is considered of utmost importance to provide flexibility and efficiency in mass production, to synchronize a desired stage of development for peak release, and to make available standardized stocks for use in research (Greenberg *et al.,* 1996; Leopold, 1998 and Ravensberg, 1992). Besides, cold storage can permit a more cost-effective production schedule (Glenister and Hoffmann, 1998) providing a means to conserve biological control agents when not immediately needed (Pitcher *et al.,* 2002).

To get high rate of emergence in laboratory in hot summer, artificial manipulation in temperature is necessary for successful rearing (Rajendran, 1999). As stated above, there is a demand for information in relation to the mass rearing, emergence and parasitization ability of *T. chilonis* for successful implementation of bio control programmes. Hence, in this report, hypothesis can be drawn to evaluate the effect of a range of cold storage periods on the subsequent performance of *T. chilonis* to assess the effects of such storage on the emergence and parasitization efficiency of laboratory reared *T. chilonis* on eggs of its factitious host *Corcyra cephalonica*.

Materials and Methods

The present investigations on influence of storage on the emergence and parasitization efficiency of laboratory reared *Trichogramma* was carried out at Biocontrol Research Laboratory, JAU, Junagadh.

Experiment was conducted to find out the optimum storage temperature and duration for the parasitoid, *T. chilonis* at pupal stage in the BOD incubators. 200 eggs of *Corcyra* were pasted on the 17 × 11 cm paper card strips and exposed for 24 hrs to the one day age old parasitoids confined in test tubes. A drop of 50% pure honey was provided as adult feed through sterilized absorbent cotton which was placed inside the test tube. Host eggs on strips after exposure of 24 hrs to the parasitoids were taken out from test tube and kept under standard laboratory conditions *i.e.*, 28 ± 1^0 C, and 65 ± 5% RH (Nadeem *et al.*, 2010).

These parasitized cards in the pupal stage were stored at six different temperature regimes *viz*., 6, 8, 10, 12, 14 and 16°C each at 5, 10, 15, 20, 25, 30, 40 and 50 days in biological oxygen demand (BOD) incubators with complete darkness. After completing the respective storage duration, the parasitoid strips were taken out from the BOD incubator and placed at the standard conditions, where they were emerged after 1-3 days.

Observations recorded

The storage period was recorded precisely. At each temperature and storage period, per cent emergence, parasitism and longevity of adults were observed with meticulous care. Percentage of parasitism which was calculated by number of blackened eggs/number of total eggs × 100 and longevity was calculated as days from the day of emergence to day of death.

Results and Discussion

Per cent emergence of *T. chilonis*

The percentage emergence of *Trichogramma* recorded after 5 days of storage at different temperature revealed that 10^0C gave significantly highest emergence of parasitoid (96.20%) and lowest (76.13%) from 6^0C (Table 1). At ten days storage, the highest (94.10%) emergence was observed from *Trichogramma* parasitoids after held at 10^0C storage. emergence percentage. At 10^0C the highest (92.23%) emergence percentage after storage for fifteen days and at 6^0C (55.30%) the least emergence was noted. At twenty days, pupal storage emphatically discloses the significant results on emergence percentage. The highest emergence percentage of *Trichogramma* obtained from 12^0C (66.06%) and the lowest (43.63%) from 6^0C.

The delineated results emphasize significant emergence percentage of *Trichogramma* after stored for twenty five days. The highest emergence percentage of *Trichogramma* obtained from 8^0C (43.33%) was statistically at par with 10 (41.00%) and 12^0C (41.70%). The lowest emergence percentage was observed at 14 and 16^0C emergence was utterly occluded (Table 1). At 6^0C emergence percentage seemed moderately (32.86%). The results revealed significant demarcation on emergence percentage of *Trichogramma* after thirty days storage. The highest emergence percentage of *Trichogramma* obtained from 10^0C (36.66%). The second highest emergence percentage of *Trichogramma* evaluated at 12^0C (32.93%) which was statistically at par with 6^0C (30.60%) and 8^0C (31.93%). Apparently the emergence percentage of *Trichogramma* after storage for forty days obviously divulged significant results. The highest emergence percentage recorded at 6^0C (26.96%). Lowest emergence percentage recorded, at 8^0C (18.76%). Adult emergence was entirely thwarted at 10, 12, 14 and 16^0C. After fifty days of storage the highest emergence percentage recorded at 6^0C (20.63%). Lowest emergence percentage recorded, at 8^0C (10.43%). Adult emergence was entirely thwarted at 10, 12, 14 and 16^0C. The present result indicated that the parasitized *Corcyra* eggs could be stored for 50 days at low temperature (6^0C) for long duration storage. It was evidenced from the above results that the emergence of *Trichogramma* was decreased with increasing storage temperatures and days intervals for storage.

The present results enunciated the considerable emergence of adults up to fifty days at 6^0C and it pertinent with findings of Gharbi (2014) who reported that the emergence rate was 87.56 percent at 15°C but significantly decreased to 45.32 percent at 35°C.

Per cent parasitism of *T. chilonis*

The result on per cent parasitism of *Trichogramma* revealed that after five days storage, the parasitism percentage was differed according to different low temperatures. At 10^0C, the highest parasitism percentage (96.00%) was observed (Table 2). However, the lowest (90.03%) parasitism percentage of *Trichogramma* observed from 16^0C. The empirical data showed significant difference in results on parasitization percentage of *T. chilonis*, after stored for ten days at different low temperatures. The highest parasitism percentage of *Trichogramma* obtained, when it was reared at 10^0C (94.30%) which was statistically at par with 8^0C (93.70%). The lowest parasitism percentage of *Trichogramma* assessed, when it was reared at 16 (89.83%) and 6^0C (90.03%), which was statistically at par with each other.

Table.1 Emergence percentage of *T. chilonis* after stored at various low temperatures

	Emergence (%) at different storage duration (Days)							
Temp.	**5**	**10**	**15**	**20**	**25**	**30**	**40**	**50**
6^0C	60.77(76.13)	50.32(59.23)	48.04(55.30)	41.34(43.63)	34.97(32.86)	33.58(30.60)	31.28(26.96)	27.01(20.63)
8^0C	76.79 (94.53)	73.55 (91.93)	71.37 (89.70)	53.67 (64.86)	41.16 (43.33)	34.40 (31.93)	25.67 (18.76)	18.84 (10.43)
10^0C	79.08 (96.20)	76.18 (94.10)	73.90 (92.23)	53.98 (65.40)	39.81 (41.00)	37.26 (36.66)	4.05 (0.50)	4.05 (0.50)
12^0C	72.57 (90.90)	69.98 (88.20)	67.14 (84.90)	54.37 (66.06)	40.22 (41.70)	35.02 (32.93)	4.05 (0.50)	4.05 (0.50)
14^0C	71.16 (89.43)	70.19 (88.46)	66.43 (84.00)	53.43 (64.50)	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)
16^0C	70.03 (88.33)	68.44 (86.50)	66.01 (83.46)	52.72 (63.30)	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)
Control	80.98 (97.40)							
S.Em ±	2.20	1.67	1.31	1.40	0.62	0.50	0.16	0.10
C.D. at 5 %	4.72	3.64	2.85	3.04	1.35	1.08	0.35	0.21
C.V.%	3.69	3.01	2.45	3.32	2.79	2.48	1.64	1.15

Table.2 Parasitization percentage of *T. chilonis* after stored at various low temperatures

	Parasitism(%) at different storage duration (Days)							
Temp.	5	10	15	20	25	30	40	50
6^0C	73.07*(91.46)	71.64(90.03)	65.27(82.46)	63.87(80.60)	63.02(79.40)	60.51(75.76)	55.53(67.96)	40.87(42.83)
8^0C	76.41 (94.36)	75.48 (93.70)	68.39 (86.40)	60.42 (75.63)	54.15 (65.70)	51.21(60.76)	40.99 (43.03)	33.12 (29.86)
10^0C	78.59 (96.00)	76.21 (94.30)	70.23 (88.53)	57.57 (71.23)	51.06 (60.50)	47.10 (53.66)	4.05 (0.50)	4.05 (0.50)
12^0C	73.85 (92.20)	73.17 (91.60)	72.00 (90.36)	54.07 (65.56)	45.32 (50.56)	41.90 (44.60)	4.05 (0.50)	4.05 (0.50)
14^0C	73.97 (92.30)	73.26 (91.70)	67.56 (85.40)	51.14 (60.63)	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)
16^0C	71.68 (90.03)	71.43 (89.83)	63.71 (80.36)	48.15 (55.50)	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)
Control	80.71 (97.23)							
S.Em ±	1.89	0.95	1.38	0.58	0.56	0.44	0.28	0.12
C.D. at 5 %	4.04	2.05	3.01	1.27	1.22	0.96	0.61	0.26
C.V.%	3.06	1.57	2.50	1.28	1.87	1.56	1.85	1.00

Table.3 Adult longevity (days) of *T. chilonis* after stored at various low temperatures

	Adult longevity (%) at different storage duration (Days)							
Temp.	5	10	15	20	25	30	40	50
6^0C	5.26	5.10	4.50	4.00	3.70	3.60	3.00	2.03
8^0C	5.60	4.63	4.10	3.70	3.00	2.80	2.00	1.03
10^0C	4.26	3.83	3.50	3.53	3.20	3.00	0.50	0.50
12^0C	4.40	3.83	3.20	3.10	2.20	2.00	0.50	0.50
14^0C	4.06	3.60	3.10	2.50	0.50	0.50	0.50	0.50
16^0C	3.76	3.33	3.00	2.00	0.50	0.50	0.50	0.50
Control	6.10							
S.Em ±	0.13	0.11	0.11	0.10	0.07	0.07	0.05	0.03
C.D. at 5%	0.27	0.24	0.24	0.22	0.14	0.14	0.10	0.05
C.V.%	3.29	3.39	3.80	3.97	3.74	3.95	4.95	3.95

The pragmatic data of *Trichogramma* revealed significant difference in results, when it was stored for fifteen days at different low temperatures. The utmost level of parasitization percentage assessed, while it was stored at 12^0C (90.36%). Similar trend of parasitism was observed at 10^0C (88.53%). The least parasitization percentage of *Trichogramma* noted from 16^0C (80.36%), while it was statistically at par with 6^0C (82.46%). At twenty five and thirty days, *Trichogramma* pupal storage fetched significant results on parasitization by parasitoids (Table 2). The uppermost parasitization percentage of *Trichogramma* recorded at 6^0C (79.40%, 75.76%) followed by at 8^0C (65.70%, 60.76%), respectively. The highest parasitization percentage recorded after forty days storage at 6^0C (67.96%), while it was found lowest at 8^0C (43.03%). Emergence was totally thwarted at 10, 12, 14 and 16^0C so, there was no parasitization occurred. *Trichogramma* pupal storage up to fifty days brought significant results on parasitization of adults. The greatest level of parasitization recorded at 6^0C (42.83%) followed by at 8^0C (29.86%). The present result indicated that the parasitized *C. cephalonica* eggs could be stored for 50 days at low temperature (6^0C) for long duration storage. It was evidenced from the above results that the parasitism of *Trichogramma* was decreased with increasing storage temperatures and days intervals for storage.

The present findings indicated that the highest parasitism was obtained from the *T. chilonis* held at 10^0C after five days storage, which was close to control values. This statement utterly supported the Nadeem *et al.*, (2010) who reported 97.4% parasitism after five days storage at 10^0C. Kosha and Brar (2000) elucidated the *Trichogramma* could be stored in the refrigerator and successfully utilized for 23 days without adversely affecting their parasitization efficiency.

Adult longevity (days) of *T. chilonis*

The result on adult longevity revealed that the highest longevity of *Trichogramma* observed from 8^0C (5.60 days) whereas, 5.26 days of adult longevity was perceived when the parasitized card stored at 6^0C which was found next in order (Table 3). At 10 and 12^0C, moderate longevity (4.26 and 4.40 days) obtained, which was statistically at par with each other. The shortest longevity period assessed at 16^0C (3.76 days) and 14^0C (4.06 days). Ten days storage of *Trichogramma* on adult longevity emphatically gave significant results. After stored for ten days, the lengthiest longevity recorded, when *Trichogramma* stored at 6^0C (5.10 days) followed by 8^0C (4.63 days). The shortest longevity of parasitoids evaluated from 16^0C (3.33 days). The moderate longevity and statistically similar results were obtained at 10, 12 and 14^0C such as 3.83, 3.83 and 3.60 days, respectively.

The maximum adult longevity measured, while it was stored at 6^0C (4.50 days) followed by 8^0C (4.10 days), whereas shortest adult longevity noted at 16^0C (3.00 days). The 16^0C was statistically at par with 12 (3.20 days) and 14^0C (3.1 days).The moderate longevity seemed at 10^0C (3.50 days). The present data revealed the significant results on adult longevity of *T. chilonis* after twenty days storage (Table 3). The utmost level of adult longevity obtained from 6^0C (4.00 days) followed by at 8^0C (3.70 days) which was statistically at par with 10^0C (3.53 days). The shortest longevity assessed from 16^0C (2.00 days). Perspicuous data expressed significant results on adult longevity of *Trichogramma* after twenty five and thirty days of storage. At 6^0C, the maximum adult longevity of 3.70 and 3.60 days was assessed, respectively. At 14 and 16^0C, adult emergence was completely occluded in twenty five and thirty days of storage. Longevity of *Trichogramma* after storage of forty and fifty days provided significant results. The adult longevity ranged from 3.00 to 1.03 at 6^0C and 8^0C.

Our findings on decreased adult longevity at 8^0C for prolonging storage are pertinent with the study reported by Ozder (2004), where adult longevity of egg parasitoid *T. cacoeciae* was decreased after 31 days storage at 8^0C. Rundel *et al.,* (2004) exemplified the storage temperatures lower than 10^0C and storage times 3 week or longer had a negative impact on longevity.

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