

Original Research Article

Effect of cold storage of trichocard on adult emergence and parasitization of *Trichogramma chilonis*

Abstract

The present study was carried out to evaluate the temperature for proper storage of trichocards prepared from host egg *Corcyra cephalonica* parasitized by *T. chilonis* (Ishii) prepared from host egg *Corcyra cephalonica*. The effect of differential temperature treatment on parameters like adult emergence of *T. chilonis* and parasitization of stored *Corcyra* eggs was assessed. Parasitized trichocards as well as non-irradiated eggs of *C. cephalonica* were kept maintained at 4±1°C, 8±1°C, 10±1°C and 12±1°C. The trichocards maintained at 10±1°C for 15 days showed highest per cent adult emergence of (73.00 percent %) for 15 days of storage which declined to 57.5 percent by 35 days of storage. (57.50%) when stored for 35 days. By s. For 45 days of storage, the temperatures of 4±1°C and 8±1°C recorded the highest adult emergence of 50.50 and 51.80 per cent adult emergence of *T. chilonis* respectively. Highest efficiency of parasitization (92.3%) was achieved by the parasitoids emerged from trichocards maintained at Treatment with storage temperature of 4±1°C showed maximum percent parasitization (92.30%) by emerged *Trichogramma* adult from stored trichocard which was decreasing significantly decreased with increase in temperature and duration of storage days. Influence of storage of *C. cephalonica* eggs was observed to be significant with highest Per cent parasitization on by *Trichogramma* on stored *Corcyra* eggs recorded stored at highest per cent parasitization at 4±1°C for 15 days which declined thereafter later it was declining with increase in storage days. The temperatures of Storage was listed that at 4±1°C and 10±1°C were found conducive for storage of parasitoid trichocard and eggs of *C. cephalonica* and 12±1°C is not suitable for storage as very low (9%) adult emergence recorded for 15 days with nil percent parasitization.

Keywords: Adult emergence, Parasitization, *Trichogramma chilonis*, Storage, *Corcyra cephalonica*

Introduction

Chemical pesticides are widely used in Agriculture because of their immediate and tangible effect. However due to heavy use of pesticides cause ~~high heavy~~ damage to the living organisms and ~~both~~ environment (Gupta and Dikshit 2010). ~~An~~ ~~ese~~ environment friendly ~~an~~ alternative to solve ~~the~~ pressing ~~the~~ need of pest management is ~~Biological~~ ~~biological~~ control (Ghosh and Ballal 2018). *Trichogramma* species are mostly used in biological control as egg parasitoids for different insect pests ~~of different~~ ~~many~~ crops through ~~augmentation and inundative~~ releases. Trichogrammatids ~~have great potential as egg parasitoids and insect~~ can be easily reared in ~~labs~~ and have a great parasitizing potential against the egg of target host (Nadeem *etal.* 2010).

Trichogrammatids are a group of minute wasps of great importance to biological control. ~~The~~ ~~In~~ ~~India,~~ among the trichogrammatids egg parasitoid *Trichogramma chilonis* ~~the most~~ dominant species in ~~India.~~ *Trichogramma chilonis* is dominant species in India which is very aggressive parasitoid ~~that~~ gives near to 100 per cent parasitization ~~according to availability of~~ under favourable conditions ~~as it has~~ ability ~~to~~ increase ~~their~~ capability (Nagarkata and Nagaraja 1979). ~~Among~~ *Trichogramma* species *Trichogramma chilonis* (Ishii) ~~the~~ egg parasitoid (Hymenoptera; Trichogrammatidae) is ~~and~~ world-over is considered an important in IPM of lepidopteran insect pest of crop ~~and~~ ~~vegetables~~ ~~likes~~ ~~such~~ ~~as~~ cotton, tomato, cabbage and apple (Smith 1996). The ~~trichogramma~~ *Trichogramma* species have proved to be effective when ~~it followed by it~~ ~~used~~ ~~inundative~~ ~~as~~ ~~inundative~~ releases against the several pests especially when their development is not interrupted by different chemical pesticides (Garcia *etal* 1995b; Briere *et al* 1999).

Generally, ~~the issue~~ ~~problem~~ is that with the applied biocontrol is the mismatch between the demand and supply. The parasitoid wasps ~~is~~ ~~are~~ not available when farmer ~~demands~~ ~~for~~ ~~requires~~ ~~it~~ ~~also~~ ~~them~~ ~~and~~ ~~further~~ if it is available at the time of field release, the price is ~~high~~ ~~which~~ ~~is~~ ~~not~~ ~~un~~affordable ~~by~~ ~~to~~ farmers (Lu *et al* 2019; Tezze and Botto 2004; Ayvazet *al.* 2008). ~~The~~ ~~developmental~~ ~~period~~ ~~of~~ ~~parasitoid~~ ~~is~~ ~~directly~~ ~~influenced~~ ~~by~~ ~~the~~ ~~temperature~~ (Nadeem *etal.* 2010) ~~and~~ ~~artificial~~ ~~manipulation~~ ~~of~~ ~~temperature~~ ~~is~~ ~~necessary~~ ~~for~~ ~~successful~~ ~~rearing~~ ~~during~~ ~~summers~~ (Rajendran 1999). These problems ~~is~~ ~~solved~~ ~~could~~ ~~be~~ ~~managed~~ by following ~~the~~ ~~technique~~ ~~of~~ ~~storage~~ ~~proper~~ ~~storage~~ ~~of~~ ~~trichocard~~ ~~at~~ ~~cold~~ ~~temperature~~ ~~helps~~ ~~in~~ ~~mass~~ ~~production~~ ~~as~~ ~~and~~ ~~when~~ ~~required~~ ~~in~~ ~~required~~ ~~(quantity~~ ~~(Tezze~~ ~~and~~ ~~Botto~~ ~~2004).~~ ~~Technique~~ ~~of~~ ~~cold~~ ~~storage~~ ~~must~~ ~~which~~ ~~would~~ ensure the availability good parasitized trichocard at ~~the~~ ~~time~~ ~~of~~ ~~release~~ ~~in~~ ~~sufficient~~ ~~number~~ ~~right~~ ~~time~~ ~~at~~ ~~desired~~ ~~numbers~~ (Bigler 1994) ~~thus~~ ~~playing~~ ~~a~~ ~~major~~ ~~role~~ ~~in~~ ~~effective~~ ~~mass~~ ~~production~~ ~~for~~ ~~field~~ ~~releases~~ (Ravensberg, 1992 and Greenberg *etal.* 1996).

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The storage of parasitoid pupae retards their development so as to harmonize the emergence with preserve of vulnerable stages of host in the field. Therefore, the cold storage technique development is most important for biocontrol for helping efficient in mass production to obtain a desired stage of development for field release of agents and for research used standardized stocks will be available (Ravensberg, 1992 and Greenberg *et al.* 1996).

Thus low temperature

storage is considered an indispensable prerequisite in biological control (Nadeem *et al.* 2010) suggested that the developmental period of parasitoid is indirectly influenced by the temperature. To get high rate emergence in laboratory in summer season, artificial manipulation in temperature is necessary for successful rearing (Rajendran 1999). Long term storage through storability can improve time availability for field release of endoparasitoid also leading to decrease in production cost. If we are able to enhance shelf life of *T. chilonis* and trichocard will be available in off season thus helpful to entrepreneurs and biological laboratories.

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Hence the present experiment was conducted to estimate the effects of storing the pupae of egg parasitoid *T. chilonis* Ishii at low temperature on its emergence and parasitizing potential of laboratory reared *T. chilonis* on egg of its facultitious host *Corcyra cephalonica*.

Material and Method

The present study on the effect of cold storage on the adult emergence and parasitization of *T. chilonis* and *Corcyra cephalonica* was carried out at Biocontrol laboratory, College of Agriculture, Nagpur. The experiments were carried out for host eggs *C. cephalonica* with a completely randomized experimental design with four different temperatures for different durations as a treatment, replicating 5 times and with five replications for each of the temperatures as well as durations (Table).

Comment [GN1]: Explain in detail about how you could set up the different temperatures of storage and how the sampling was done. Say totally how many samples were involved for each sample date at each of the temperature tested.

Rearing of *Corcyra cephalonica*

Culture of *C. cephalonica* was obtained from the Biocontrol lab College of Agriculture, Nagpur and maintained in controlled condition 27-30°C temperature 70±1% humidity (RH) with complete darkness in laboratory for successive generations on sorghum grains in Biocontrol laboratory College of Agriculture Nagpur. The moth emerged from

40th days ~~after inoculation of *Corcyra* eggs in the medium of sorghum grains~~ were collected daily and transferred into ~~specially design egg laying oviposition chamber having iron mesh both externally and internally on top and bottom respectively~~ and fresh eggs were collected from egg laying chamber and used for making trichocards.

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Culturing of *Trichogramma chilonis*

Experiment was conducted to find out the best storage temperature and duration for the parasitoid. Nucleus culture of *T. chilonis* was procured from Biocontrol lab College of Agriculture, Nagpur. Eggs obtained from *C. cephalonica* were treated with UV rays (30 WUV tube for 40-45 min) to prevent hatching. ~~In century cards (14×20cm), manually counted 2000 eggs of *Coreyra*~~ *Corcyra* eggs were pasted at the rate of 2000 eggs per card and ~~counted manually~~ were ~~pasted on the 14×20cm century card and~~ exposed to the parasitoid for 5 days. Such cards had the parasitoid in pupal stage which after parasitization of this parasitoid card in pupal stage were stored at ~~4~~ four different temperature regimes viz. 4±1°C, 8±1°C, 10±1°C and 12±1°C each for 15, 25, 35 and 45 days in refrigerator and Biological oxygen demand incubators with complete darkness.

Comment [GN2]:

Comment [GN3]: How was it possible to adjust the temperature in a fridge to such variations? Did you use four different refrigerators? And did you draw the samples from the same lot for different periods? Explain.

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Percentage Rate of adult emergence of *T. chilonis*

After ~~fixed each storage specific storage days parasitized period, parasitized strips cards (Trichocards) were taken retrieved out~~ from the refrigerator and BOD incubators. ~~Observations on adult emergence was made under stereo zoom microscope by counting the host eggs with adult emergence holes. Per cent adult emergence of *Trichogramma* adult was calculated was calculated using the formula: from *Coreyra* eggs by counting host egg in which there was an orifice intended for exit of adults under stereo zoom microscope.~~

Comment [GN4]: Was the experiment conducted in the refrigerator or incubator?

$$\% \text{ Emergence} = \frac{\text{Total no. of adult emergence numbers of eggs with emergence holes}}{\text{Total no. number of eggs}} \times 100$$

Comment [GN5]: Instead, it can be mentioned as 2000 as it was the original number of eggs used for the experiment

Percentage parasitization by emerged of Parasitization efficacy of *T. chilonis*

The adults emerged from ~~stored~~ trichocards ~~stored~~ at 4±1°C, 8±1°C, 10±1°C and 12±1°C ~~each for for different durations of~~ 15, 25, 35 and 45 days. The ~~emerged adults~~ of *Trichogramma* were exposed to irradiated *Corcyra* eggs ~~pasted at the rate of @8000 eggs/strips card.~~ Percentage parasitization were recalculated by using formula:-

Comment [GN6]: How many parasitoids were used? Please mention the parasitoid: host ratio.

$$\% \text{ Parasitization} = \frac{\text{Total no. of parasitized eggs}}{\text{Total no. of eggs exposed}} \times 100$$

Comment [GN7]: This could be mentioned as 8000 eggs.

Statistical analysis

Data recorded during present study on percent adult emergence and parasitization by *T. chilonis* stored *Corcyra* eggs were statistically analyzed by using OPSTAT software which is available online on Hissar Agricultural University, Hissar.

Comment [GN8]: Were the *Corcyra* eggs stored? If so, for how long? Please mention in the Material and Method.

Comment [GN9]: Was the data subjected to transformation?

Comment [GN10]:

Comment [GN11]: Mention the name of the analysis.

Results

Percent emergence of *T. chilonis*

~~The result on per cent~~ The rate of adult emergence of *T. chilonis* observed ~~that after~~ storage of trichocards ~~at different temperature for storage a duration~~ of 15 days ~~recorded were~~ significantly different at different temperatures with the maximum adult emergence of 73.00 per cent and lowest of 9.00 per cent ~~from at~~ 10±1°C and 12±1°C respectively (Table 1). After twenty-five days of ~~stored storage~~ trichocard gives maximum 68.00 per cent adult emergence ~~when it stored~~ at 10±1°C. Corresponding reductions in adult emergence was observed with the increase in the duration of storage and temperature tested. The moderate and lowest adult emergence recorded at 10±1°C and 12±1°C is 57.50 and 3.20 per cent respectively. ~~When trichocard stored upto thirty five days significantly maximum adult emergence obtained from different temperature. It was evidenced that results mentioned from present study *T. chilonis* adult emergence per cent was reduced as increase in temperature and number of storage days.~~ The present study ~~enunciated that finding of appreciable~~ adult emergence upto 35 days at 10±1°C ~~is pertinent agrees with the study of Mohamed et al. (2020) who elucidated pupal stage of *Trichogrammatoidaeba* could be stored at 10°C upto 28-30 days giving maximum performance of adult emergence 93.80 per cent. However, at 45 days of storability rate of release of trichocards are to be doubled for field release to achieve maximum emergence if the trichocards are under cold storage for 45 days. Thus similar results have been reported by the present study is in collaborated with the studies of Singh et al. (1997) who reported showing that *Trichogramma T. chilonis* Ishii can be stored at 5°C upto 3 weeks without hampering their per cent adult emergence and. Further Ayvazet al. (2008) who investigated showed that pupae of *Trichogramma evanescens* can be stored at 4°C for upto 3 weeks without affecting performance of *T. chilonis*.~~

Comment [GN12]: 1. Did you refer to the publication in this field: Geetha, N., 2010. Appraisal of quality parameters of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) as affected by prolonged cold storage of parasitoid cards. Entomol., 35: 51-54. You can discuss your findings along this line.

Comment [GN13]: *chilonis evanescens*? Verify.

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Percent parasitization rate by of emerged *T. chilonis* adults

Adult emergence and parasitization by *T. chilonis* adult emergence per cent was were found to decrease with reduced as increase in temperature and number of storage days duration of the storage. The result

on-per cent parasitization of *Trichogramma T. chilonis* adults emerged from the trichocards revealed that after fifteen days storage, the parasitization percent was differed in accord with different low temperatures significantly varied with temperature. At 4±1°C, the highest parasitization (85.24%) was observed (Table 2) followed by 8±1°C. However, the lowest parasitization (76%) of *T. chilonis* observed from 10±1°C when trichocard stored for 15 days. Storage of The highest parasitization percentage of *T. chilonis* obtain when it was reared at 4±1°C (70.24%) followed by 10±1°C (60.00%). The lowest percent parasitization of *T. Chilonis* assessed, when it was reared at 8±1°C (56.75%) for thirty-five days of storage. Trichocards storage at forty-five days for 45 days obtained results on parasitization were remarkable by parasitoid. The topmost percent parasitization of *T. Chilonis* listed highest parasitization at 4±1°C (60.87%) followed by 8±1°C (51.37%) when trichocard stored for forty-five days. No adult emergence was lowest and totally nil at 12±1°C after 45 days of storage could be noted and thus there was no parasitization occurred could be observed. It was evidenced that results mentioned from present study *T. chilonis* adult emergence per cent was reduced as increase in temperature and number of storage days. The present study is in collaborated with the studies of Singh *et al.* (1997) who reported that found no decrease in parasitization efficiency if *Trichogramma T. chilonis* Ishii can be stored was stored at 5°C upto 3 weeks, without hampering their percent efficiency of parasitization. Similarly Tezze However, Teze and Botto (2004) reported indicated that storage of trichocards upto 50 days at 4±1°C without affecting did not affect the parasitization by performance of *T. chilonis* on parasitization.

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Conclusions

Obtained results proved that Storage of pupae of *T. chilonis* trichocards stored at 4±1° and 8±1°C upto 35 and 45 days were was found to be the effective for maximum adult emergence and percent parasitization after storage of trichocard upto 35 and 45 days. The treatment 10±1°C found significant upto 15 days for storage of trichocard for adult emergence of *T. chilonis* followed by 25 and 35 days. However, treatment 12±1°C recorded non-significant at 15 to 45 days of storage. The data recorded during present study may be The findings would be useful to utilized for improving improve the availability of trichocards in during off seasons of production for field release.

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Comment [GN14]: Some journals are given as full name and some in abbreviated form. Please modify.

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Comment [GN15]: Alignment

(Hymenoptera:Trichogrammatidae).JournalofBiologicalControl.30:1.

Table1: Effect ofstorageof*T.chilonis*onadultemergence(%)

Noofstoragedays	Percentadultemergenceatdifferenttemperature			
	4±1°C	8±1°C	10±1°C	12±1°C
15	66.72 (54.78)	69.90 (56.72)	73.00 (58.72)	9.00 (17.41)
25	62.00 (51.98)	66.50 (54.74)	68.00 (55.57)	5.30 (13.23)

35	55.80 (48.37)	56.30 (48.66)	57.50 (49.33)	3.20 (10.26)
45	50.50 (44.94)	51.80 (46.04)	0.00 (0.32)	0.00 (0.32)
Ftest	Sig	Sig	Sig	NS
SD(\pm m)	1.67	2.05	0.97	0.55
CD@1%	5.06	6.22	2.93	1.69

*Fig in parentheses are arcsin transformed values.

Comment [GN16]: Either the table or figure would do. Using both is redundant.

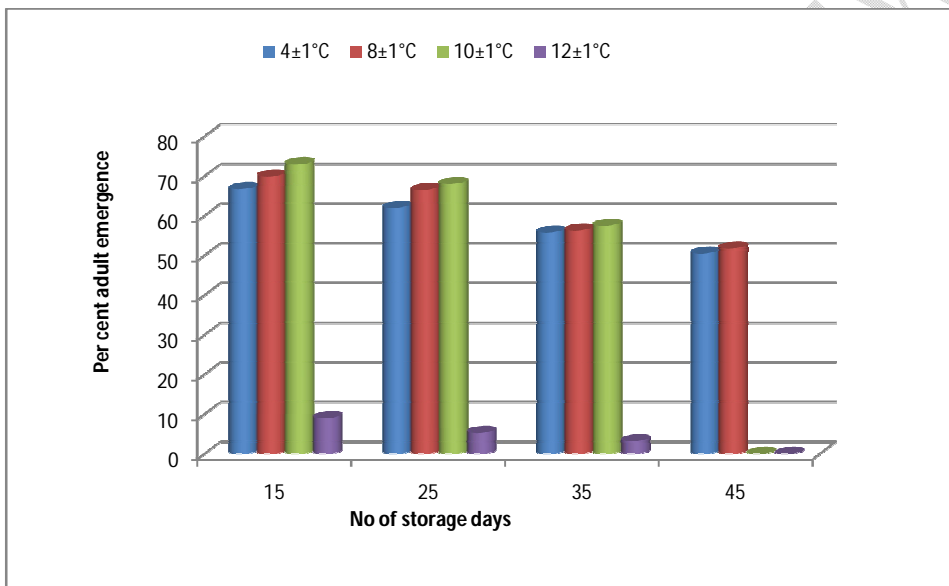


Fig 1: Percent adult emergence of *T. chilonis*

Table 2: Effect of storage on percent parasitization by emerged *T. chilonis* from stored trichocard (%)

No of storage days	Percent parasitization by emerged <i>T. chilonis</i> at different temperature			
	4±1°C	8±1°C	10±1°C	12±1°C
15	85.24 (68.21)	80.82 (65.19)	76.00 (61.67)	0.00 (0.32)
25	73.63 (59.16)	69.00 (56.30)	64.87 (53.74)	0.00 (0.32)
35	70.24 (57.24)	56.75 (48.89)	60.00 (52.48)	0.00 (0.32)
45	60.87 (51.35)	51.37 (45.79)	0.00 (0.32)	0.00 (0.32)
Ftest	Sig	Sig	Sig	NS
SD(±m)	2.78	2.74	3.06	
CD@1%	8.43	8.30	9.27	

*Fig in parentheses are arcsin transformed value

Comment [GN17]: Table or figure would do. Please delete one.

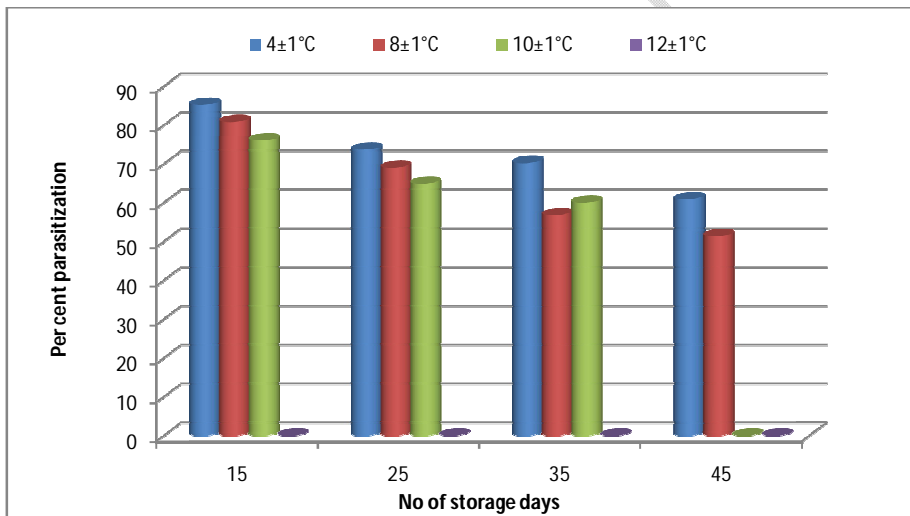


Fig 2: Percent parasitization by emerged *T. chilonis* from stored trichocard