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Evaluation of Best Method for Sterilization of Eri silkworm Eggs Under U.V. Radiation and Refrigerator Storage for Trichocard Production

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ABSTRACT

Effect of U.V. radiation and refrigerator storage on hatching of Eri silkworm eggs was studied at the regional centre of All India coordinated Research project on Biological control of crop pests, Regional Agricultural Research Station, Anakapalle, Visakhapatnam Andhra Pradesh, during the year 2016-17 to evaluate the best method for sterilization of Eri silkworm eggs. The eggs of eri silkworm were subjected to different periods of U.V radiation treatment ranging from 30 minutes, 45 minutes, 60 minutes, 75 minutes, 90 minutes, 120 minutes and 150 minutes for two generations, the data revealed that none of the U.V radiation treatments prevented hatching of eri silkworm eggs. Mean Percentage of hatching in U.V irradiated eggs ranged from 52.99 to 86.83.

A progressive reduction was observed in trend of hatching of eri silkworm eggs with increase of storage period upon refrigeration. Complete reduction in hatching of eri silkworm eggs was observed when stored for 10 days at temperatures (4 ^{0}C and 6 ^{0}C) tested, likewise hatching of eri silkworm eggs was completely prevented when stored for a period of 15 days at 9 ^{0}C. The studies showed that refrigeration of eri silkworm eggs is the best method to prevent hatching completely when compared to U.V radiation to sterilize the eggs of eri silkworm and these Eri silkworm eggs can be inturn used in *Trichogramma* production.

Keywords: *Eri silkworm , Rearing, Rice moth, Trichogramma chilonis.*

Trichogrammatids are important egg parasitoids used against lepidopteran pests-especially since 1926 when a suitable mass rearing strategy was developed (Flanders, 1929). The biology of *Trichogramma* spp. has been studied extensively (Nagaraja, 1973; Strand, 1986). Different species of *Trichogramma* proved to be effective against different pests infesting various crops in different ecosystems. Among all the identified species, *Trichogramma chilonis* (Ishii) is extensively used in India, China, Japan, Nepal, Pakistan, Taiwan, Reunion Island, Kenya, Australia and South Africa due to its wide host acceptability and high survival capacity (Jalali *et al.,* 2006). This minute parasitoid is exclusively used as egg parasitoid, therefore, play important role in pest suppression programme by destroying the early stage of the pest thereby, curtailing the use of pesticides and contribute in preventing environmental pollution. (Dileep *et al.,*2015).

Corcyra cephalonica (Stainton) (Lepidoptera Pyralidae) is economically an important stored grain pest in Asia, Africa,North America and Europe (Atwal and Dhaliwal, 2008). Ayyar (1919) made the first record of *C. cephalonica.* It is a major pest of not only rice but also feed on wheat, maize, sorghum, groundnut, cotton seeds, coffee, spices and cocoa beans, and millet (Kumar and Kumar, 2001). Besides being a pest, it is the factitious host for 75 natural enemies of which 60 of parasitoids and 15 of predators including a few that are host-specific in nature. It also serves as a host for nematodes and mites (Manjunath, 2013). Its wide acceptability is unique and turned out to be a boon for mass production of bio-control agents.

Recent studies indicated that the production of *T. chilonis* on the eggs of eri silkworm *Samia cynthia ricini* was a farmer friendly system and it could potentially yield trichogrammatids with superior biological attributes. Among the commercially exploited silkworms, eri silkworm is completely domesticated multi voltine, poly-phagous species under non-mulberry sector which is reared throughout the year.

MATERIAL AND METHODS

The initial population of the host insect, Eri silkworm (ESW) with national accession number NBAII-MP-SAT-01, Eri trichocard *i.e.,* egg parasitoid *T. chilonis* reared on eri silkworm with National accession number NBAII- MP –TRI-26, host insect rice moth, *C. cephalonica* National accession number: NBAII-MP-PYR-O1 and egg parasitoid *T. chilonis* reared on *C. cephalonica* with National accession number NBAII-MP-TRI-13 were collected from National Bureau of Agriculture Insect Resources (NBAIR) Bangalore and was maintained at AICRP on biological control laboratory, Regional Agricultural Research Station, Anakapalle, and utilized for conducting the study. The culture of eri silkworm was reared as per the mass rearing protocols (methodology) developed at NBAIR Bangalore, during the experimental period.

Rearing of *C. cephalonica*

Rice moth, *C. cephalonica* was cultured on broken grains of maize in plastic basins. Heat sterilized broken grains were added @ 2.5 kg per basin to which 100 g of groundnut meal and 5 g of yeast powder was added. To prevent bacterial contamination 1 vial of streptomycin sulphate (0.05%) was incorporated in the basins, half capsule (tablet) of vitamin E, 2 grams of proteinex was used per basin. The *C. cephalonica* eggs were deposited @ 0.25 cc/basin. After uniform mixing of the contents, the basin was covered with muslin cloth. The adults of *C. cephalonica* moths started emerging from the medium after 30-35 days and continued upto 90 days. The emerged adults that rested on the inner sides of the muslin cloth were collected during morning hours in glass specimen tubes (15 x 2.5 cm). Adult moths were then transferred to a mating drum from which the eggs were collected on daily basis for four days from each drum.

Rearing of *T. chilonis* on *C. cephalonica*

C.cephalonica eggs which were collected from the mating drum were cleaned and taken in glass petriplates which were then exposed to U.V light (30 watt) in a closed chamber so that the embryo gets killed without damaging the egg yolk contents. The U.V sterilized eggs were sprinkled on drawing card boards smeared with thin layer of diluted gum and finally the cards were pasted with eggs of *C. cephalonica.* Eggs were dried under the fan and then were put into polythene bags containing nucleus parasitized cards at a ratio of 1:6 (parasitized eggs: fresh eggs) for exposure. The parasitoids (Plate 1) emerging from the parasitized nucleus card has parasitized the fresh eggs.

Rearing of *Samia cynthia ricini*

Egg masses of Eri silkworm were laid on the inner walls of the cages. Egg masses were collected manually by dislodging the eggs from the walls of the cages by using test tubes. Collected egg masses were kept at room temperature for continuing the host culture. The eggs hatched in 6 to 7 days, after hatching, tender castor leaves were fed to the freshly hatched larvae or neonates of ESW in plastic trays covered with black cloth. Jars were cleaned and fresh food was provided daily. Cocoons were formed within fed leaf bits in the corners of the trays in 25 days and cocoons were kept in cages for adult emergence. A rectangular cage covered with black cloth inside nylon mesh and was used for releasing the moths. The freshly emerged adults of ESW were released into the cage for egg laying.

Rearing of *T.chilonis* on Eri silkworm eggs

Reared ESW egg masses were stored in refrigerator for a period of 15 days (9 ^{0}C),10 days (4 ^{0}C and 6 ^{0}C) and the refrigerated egg masses were washed under tap water and left at room temperature for 5 minutes to dry followed by treatment with KOH solution (0.1%) and allowed to dry at room temperature for 5 minutes Finally KOH treated eggs were washed under tap water and kept at room temperature for 5 minutes to dry, individual eggs were separated manually from the egg masses and dried loose eggs were utilized for making eri trichocards. The loose eggs were pasted manually onto a card with glue and allowed for drying. The ESW egg card was introduced into polythene bags containing nucleus parasitized cards in 1:6 ratio provided with fine streaks of 50% diluted honey as feed for the adults of *T. chilonis* (Plate 1)**.** Mated females of *T. chilonis* parasitize the eggs of ESW and the parasitized eggs turn black in colour.

RESULTS AND DISCUSSION

The eggs of Eri silkworm were exposed to different periods of U.V radiation ranging from 30 minutes, 45 minutes, 1:0 hour, 1hour15minutes, 1 hour 30 minutes, 2:00 hours and 2 hours 30 minutes and for two generations, The data revealed that there was reduction in percent hatching of eri

silkworm eggs, as the duration of exposing the eggs to U.V treatment increased. In case of first generation exposure of eggs to U.V irradiation for 30 minutes has resulted in 87% egg hatching and it gradually decreased to 50.66% on exposing the eggs to a period of 2hour 30 minutes (Table1; Fig 1).

Similar trend of reduction in per cent egg hatching was observed in second generation where 86.66% of egg hatching was observed at 30 minutes and it gradually decreased to 55.33% at 2 hour 30 minutes when compared to control (98%) (Table 1; Fig 1) (*i.e*., eggs were kept at room temperature without subjecting to U.V radiation) at standard laboratory conditions.

None of the U.V radiation treatments prevented hatching of Eri silkworm eggs. Percentage of hatching in U.V irradiates eggs ranged from 52.99 to 86.83. In case of first generation, U.V radiation exposure treatments viz., 2 hour 30 minutes and 2 hour were found to be effective treatments in reducing per cent egg hatching of eri silkworm eggs (50.66% and 54.33% respectively) compared to U.V unexposed eggs (control) which were statistically on par with each other and were statistically different from 1hour 30 minutes (69.66%), 1hour 15 minutes (72.66%) and 1hr (73.33%). Highest per cent egg hatching of eri silkworm eggs was noticed in 45 minutes (83.33%) and 30 minutes (89%) U.V radiation exposure treatments over the control which are statistically on par with each other. In second generation, at 2 hour 30 minutes U.V radiation exposure treatment resulted in highest reduction in eri silkworm egg hatching 55.33 per cent and was found to be statistically on par with 2 hours (56.33%) and 1hour 30 minutes (62.00%). The lowest reduction was observed in 1hour 15 minutes and 1hour exposure to U.V treatment respectively followed by 45 minutes (84.66 %) and 30 minutes (86.66%) which were found to be statistically on par with each other.

These results are in accordance with Lalitha *et al*. (2013) who reported 58-78 per cent hatching of Eri silkworm eggs on U.V radiation for 30 minutes, 1 hour, 1hour30 minutes, 2 hour and 2hour30 minutes. This was probably because eri silkworm has a relatively thicker chorion compared to chorion of *Corcyra cephalonica* egg and chorion of the egg is known to be responsible for maintaining the shape of the eggs by providing air tightness.

The perusal of data in tables 2 and 3 revealed a progressive reduction in trend of hatching of Eri silkworm eggs with increase of storage period upon refrigeration. Complete reduction in hatching of eri silkworm eggs was observed when eggs were stored for 10 days at temperatures (4 ^{0}C and 6 ^{0}C) tested (Table 2; Fig 2), likewise hatching of Eri silkworm eggs were completely prevented when stored for a period of 15 days at 9 ^{0}C (Table 3; Fig 3). A drastic reduction was observed on the second day of storage at 4^{0}C compared to refrigeration at 6 ^{0}C and 9 ^{0}C , This reveals that with increase in duration of incubation , per cent egg hatching decreased both at 4 ^{0}C and 6 ^{0}C.

These results are in accordance with Lalitha *et al*. (2010) reported that storage of eri silkworm eggs at 9^{0}C for two weeks completely prevented the hatching of eri silkworm eggs as compared to U.V radiation to prevent hatching in *C. cephalonica* eggs. In the current study, extended storage, probably because of refrigeration at the right age/stage might have helped to retain the natural rigidity of the chorion further promoting optimum parasitism.

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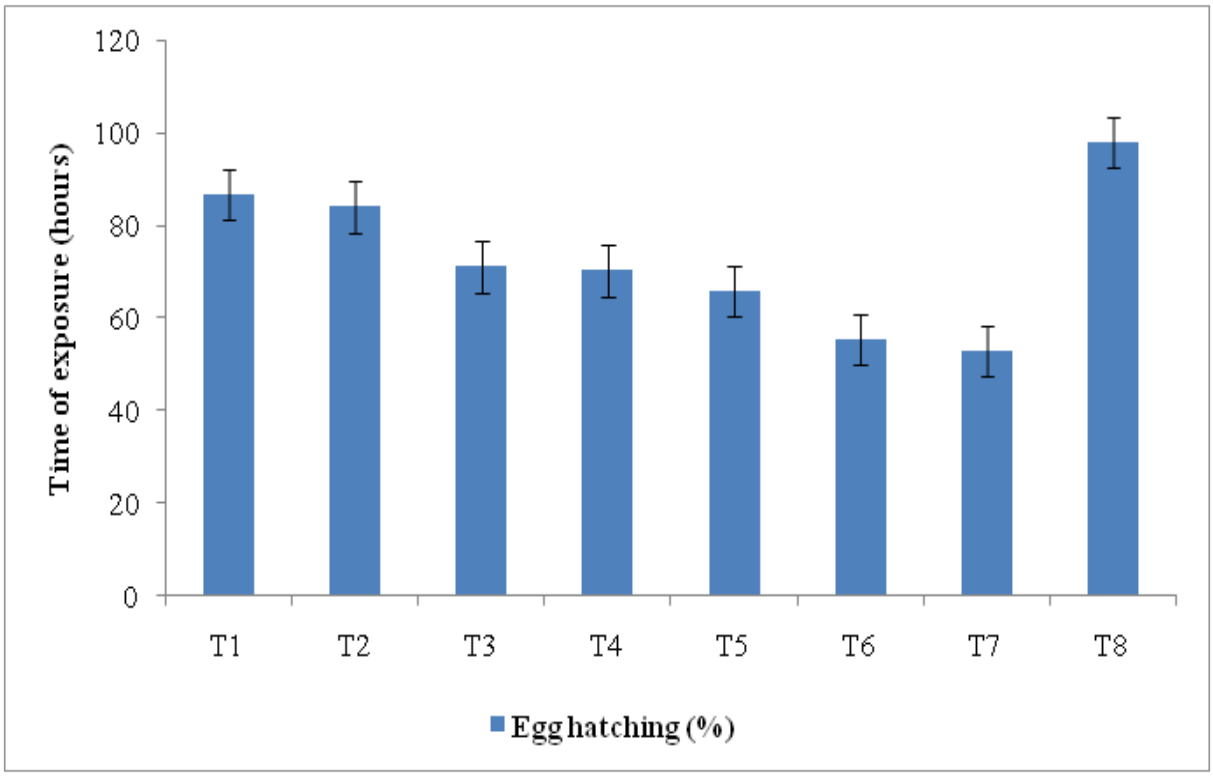
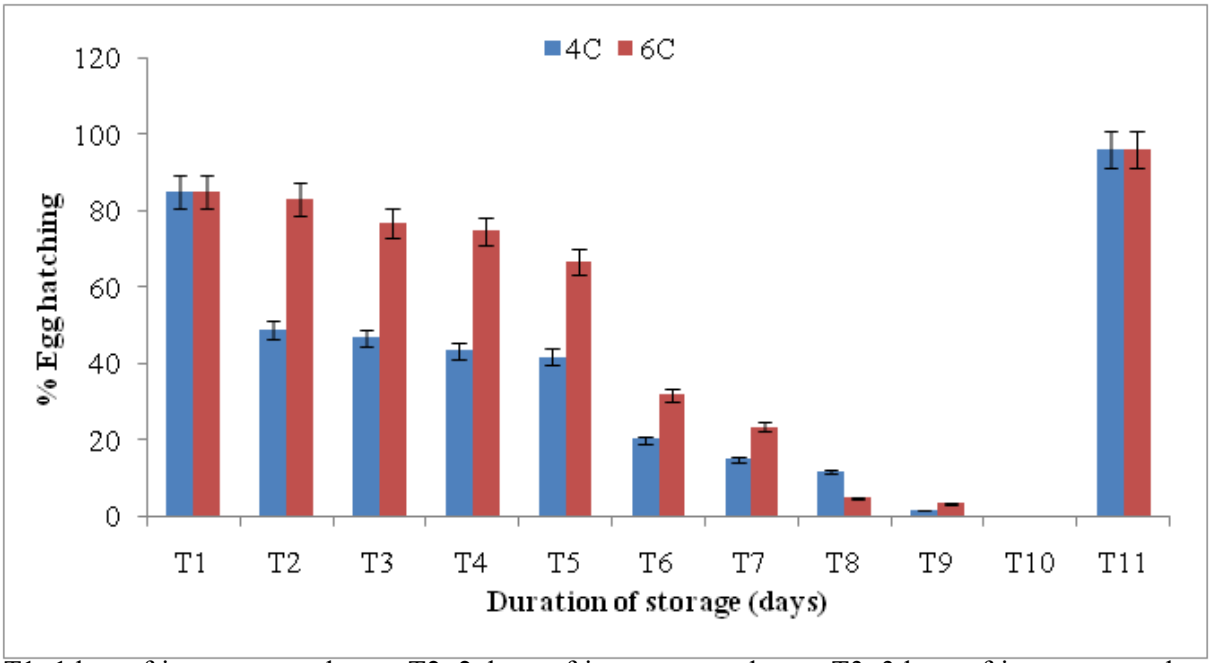


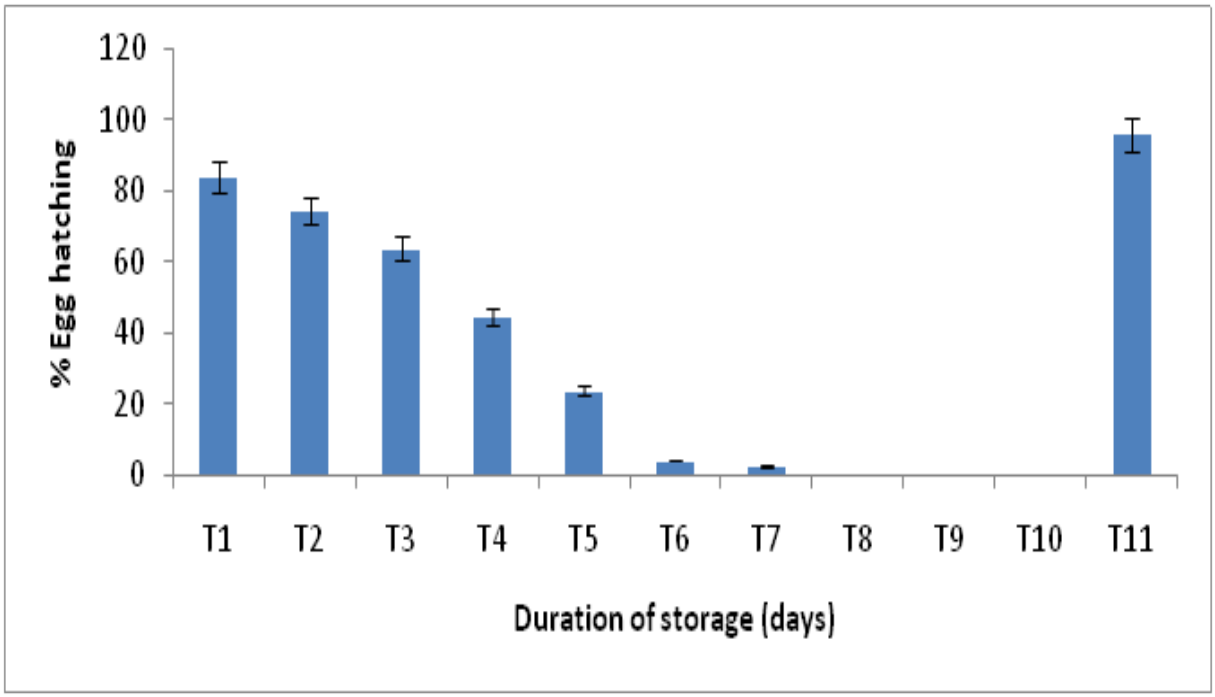
T1=30min,T2=45min,T3=1hr,T4=1hr15min,T5=1hr30min,T6=2hr,T7=2hr30min, T8=control

Fig.1 . Effect of U.V radiation on hatching of eri silkworm eggs.



T1=1day refrigerator stored eggs, T2=2 days refrigerator stored eggs, T3=3days refrigerator stored eggs, T4=4days refrigerator stored eggs, T5=5days refrigerator stored eggs, T6=6days refrigerator stored eggs, T7=7days refrigerator stored eggs, T8=8days refrigerator stored eggs, T9=9days refrigerator stored eggs, T10=10days refrigerator stored eggs, T11=control

Fig .2. Effect of refrigeration storage on hatching of eri silkworm eggs at 4^0Cand 6^0C.



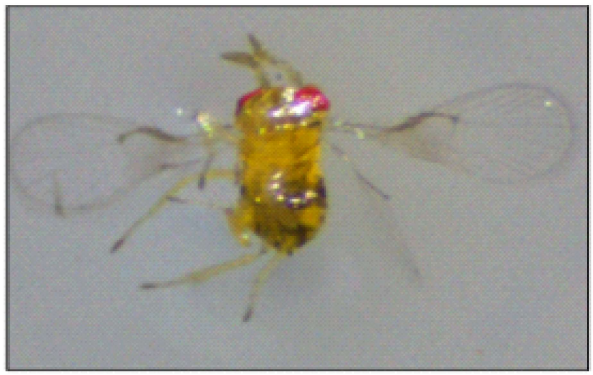
T1=1day refrigerator stored eggs, T2=2 days refrigerator stored eggs, T3=4 days refrigerator stored eggs, T4=6days refrigerator stored eggs, T5=7days refrigerator stored eggs, T6=8days refrigerator stored eggs, T7=10days refrigerator stored eggs, T8=15days refrigerator stored eggs, T9= 3 weeks refrigerator stored eggs, T10=4 weeks refrigerator stored eggs, T11=control

Fig.3. Effect of refrigeration (9 ^{0}C) on hatching of eri silkworm eggs.

Male *T.chilonis* reared on eri silkworm

Female *T.chilonis* reared on eri silkworm.

Male *T.chilonis* reared on rice moth



Female *T.chilonis* reared on rice moth

Plate 1. *T.chilonis* reared on eri silkworm and rice moth.

Table 1 Effect of U.V radiation on hatching of eri silkworm eggs.

Duration (or0 Time of Exposure	Egg Hatching (%)**		
	I gen	II gen	Mean
T_1-30minutes	87.00	86.66	86.83
	(69.90)^{e}	(68.57)^{d}	(68.69)
T_2-45 minutes	83.33	84.66	83.99
	(65.89)^{de}	(66.98)^{cd}	(66.38)
T_3-1hour	73.33	69.00	71.16
	(58.91)^{c}	(56.18)^{b}	(57.49)
T_4-1hour 15minutes	72.66	68.00	70.33
	(58.46)^{c}	(55.52)^{b}	(56.97)
T_5-1hour 30 minutes	69.66	62.00	65.83
	(56.56)^{bc}	(51.92)^{ab}	(54.20)
T_6-2hours	54.33	56.33	55.33
	(47.47)^{a}	(48.62)^{a}	(48.04)
T_7-2hour 30 minutes	50.66	55.33	52.99
	(45.36)^{a}	(48.05)^{a}	(46.69)
T_8-control (No U.V. Exposure)	98.00	98.00	98.00
	(82.01)^{f}	(82.01)^{e}	(82.01)
SEm ±	2.28	1.64	1.75
CD (P=0.05)	6.83	4.92	5.68
CV %	3.81	2.79	3.67

** Values in parentheses are arc sine transformed values.
Numbers followed by same superscript are not statistically different.

SEm : Standard Error of Mean
CD : CriticalDifference
CV : Coefficient of variation

Duration of storage	Hatching of eri silkworm egg (%)** at	
	4^0C	6^0C
T_1- 1 day storage of eggs	85.00 (67.21)^f	85.00 (67.21)^j
T_2-2 days storage of eggs	48.66 (44.21)^e	83.00 (65.67)^i
T_3-3 days storage of eggs	46.66 (43.06)^e	76.66 (61.11)^h
T_4-4 days storage of eggs	43.33 (41.14)^e	74.66 (59.76)^g
T_5-5 days storage of eggs	41.66 (40.18)^e	66.66 (54.71)^f
T_6-6 days storage of eggs	20.00 (26.52)^d	31.66 (34.22)^e
T_7-7 days storage of eggs	15.00 (22.77)^c	23.33 (28.86)^d
T_8-8 days storage of eggs	11.66 (19.93)^c	4.66 (12.12)^c
T_9-9days storage of eggs	1.66 (7.33)^b	3.33 (10.33)^b
T_{10}-10 days storage of eggs	0 (0)^a	0 (0)^a
T_{11}-control (storage of eggs at room temperature).	96.00 (78.68)^g	96.00 (78.68)^k
SEm ±	1.07	1.11
CD (P=0.05)	3.16	3.25
CV %	6.58	5.37

** Values in parentheses are arc sine transformed values.
Numbers followed by same superscript are not statistically different.

SEm : Standard Error of Mean
CD : CriticalDifference
CV : Coefficient of variation

Table 3. Effect of refrigeration on hatching of eri silkworm eggs at 9 ^{0}C

Duration of storage at 9^{o}C	Hatching of eri silkworm eggs (%)** Refrigerator temperature (9^{o}C)
T_1- 1 day storage of eggs	84.00 (66.42)^{h}
T_2-2 days storage of eggs	74.33 (59.57)^{g}
T_3-4 days storage of eggs	64.00 (53.11)^{f}
T_4-6 days storage of eggs	44.66 (41.91)^{e}
T_5-7days storage of eggs	24.00 (29.31)^{d}
T_6-8 days storage of eggs	4.33 (11.89)^{c}
T_7-10 days storage of eggs	2.66 (9.26)^{b}
T_8- 15 days storage of eggs	0.00 (0.00)^{a}
T_9- 3 weeks storage of eggs	0.00 (0.00)^{a}
T_{10}- 4 weeks storage of eggs	0.00 (0.00)^{a}
T_{11}-control (storage of eggs at room temperature)	96.00 (78.68)^{i}
SEm ±	0.87
CD (P=0.05)	2.57
CV %	6.14

** Values in parentheses are arc sine transformed values.
Numbers followed by same superscript are not statistically different.

SEm : Standard Error of Mean
CD : CriticalDifference
CV : Coefficient of variation

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